Clinical and histopathologic predictors of therapeutic response to bronchial thermoplasty in severe refractory asthma

Maha Zohra Ladjemi, PhD,[a,b]*‡ Leonarda Di Candia, PhD,[a,b]*§ Nicolas Heddebaut, MS,[a,b] Camille Techoueyres, MD,[a,b] Eloise Airaud, BTS,[a,b] David Soussan, MS,[a,b] Marie-Christine Dombret, MD,[a,b,c,d] Fatima Hamidi, MS,[a,b] Noëlline Guillou, MS,[a,b]‖ Pierre Mordant, MD, PhD,[a,b,d,e] Yves Castier, MD, PhD,[a,b,d,e] Séverine Létuvé, PhD,[a,b] Camille Taillé, MD, PhD,[a,b,c,d] Michel Aubier, MD,[a,b] and Marina Pretolani, PharmD, PhD[a,b] *Paris, France*

Background: Phenotypes and endotypes predicting optimal response to bronchial thermoplasty (BT) in patients with severe asthma remain elusive.

Objective: Our aim was to compare the clinical characteristics and hallmarks of airway inflammation and remodeling before and after BT in responder and partial responder patients with severe asthma refractory to oral steroids and to omalizumab.

Methods: In all, 23 patients with severe refractory asthma were divided into BT responders (n = 15) and BT partial responders (n = 8), according to the decrease in asthma exacerbations at 12 months after BT. Clinical parameters were compared at baseline and 12 months after BT, and hallmarks of airway inflammation and remodeling were analyzed by immunohistochemistry in bronchial biopsy specimens before and 3 months after BT.

Results: At baseline, the BT responders were around 8 years younger than the BT partial responders ($P = .02$) and they had a greater incidence of atopy, higher numbers of blood eosinophils (both $P = .03$) and IgE levels, higher epithelial IFN-α expression, and higher numbers of mucosal eosinophils and IL-33–positive cells ($P \leq .05$). A reduction in blood eosinophil count, serum IgE level, type 2 airway inflammation, and numbers of mucosal IL-33–positive cells and mast cells associated with augmented epithelial MUC5AC and IFN-α/β immunostaining was noted after BT in responders, whereas the numbers of mucosal IL-33–positive cells were augmented in BT partial responders. Most of these changes were correlated with clinical parameters. Subepithelial membrane thickening and airway smooth muscle area were similar in the 2 patient groups at baseline and after BT.

Conclusion: By reducing allergic type 2 inflammation and increasing epithelial MUC5AC and anti-viral IFN-α/β expression, BT may enhance host immune responses and thus attenuate exacerbations and symptoms in BT responders. Instead, targeting IL-33 may provide a clinical benefit in BT partial responders. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: *Bronchial epithelium, T2-type inflammation, MUC5AC, IL-33, mast cells, IFN- α/β exacerbations, asthma control, bronchial thermoplasty*

Bronchial thermoplasty (BT) is a nonpharmacologic treatment option for patients with severe asthma who remain symptomatic despite maximal medication. The initial purpose of this treatment was to reduce airway smooth muscle (ASM) mass in proximal airways through the delivery of thermal energy via a dedicated catheter.[1,2] Previous investigations by our and other groups have shown that BT markedly decreased not only ASM mass but also other structural abnormalities involved in airway narrowing and bronchial (hyper)reactivity, such as the number of neuroendocrine epithelial cells and nerve endings.[3-5] These effects were correlated with a reduction in the number of severe exacerbations, hospitalizations for asthma, and emergency department visits, as well as with improved quality of life.[1-3,6-9]

Other studies showed that BT decreased the epithelial expression of MUC5AC and its driver IL-13,[10,11] reduced tissue fibrosis (ie, subepithelial basement membrane [SBM] thickening), decreased deposition of collagen type I,[3,5,12] decreased alveolar levels of transforming growth factor-β,[13] and downregulated the production of heat shock protein 60 by the bronchial epithelium, which promotes fibroblast-mediated remodeling.[14] BT may also target airway inflammation, as shown by a decrease in the proportion of alveolar eosinophils and levels of the leukocyte-chemotactic chemokine CCL5.[13]

In the aforementioned studies, however, correlations between histopathologic/biologic alterations and clinical parameters were not investigated.

Importantly, patients with severe asthma do not respond homogeneously to BT, and some of them show partial or no

From [a]Inserm UMR1152, Physiopathologie et Epidémiologie des Maladies Respiratoires, Université de Paris, Faculté de Médicine; [b]the Laboratory of Excellence, INFLAMEX, Université Sorbonne Paris Cité and DHU FIRE; [c]the Département de Pneumologie A and [e] the Département de Chirurgie Thoracique, Hôpital Bichat-Claude Bernard, Paris; and [d]the Assistance Publique des Hôpitaux de Paris.

*These authors contributed equally to this work.

‡Maha Zohra Ladjemi, PhD, is currently at Institut Cochin, U1016 Inserm/CNRS UMR8104, Université de Paris, Paris, France.

§Leonarda Di Candia, PhD, is currently at Scinopsis, Fréjus, France.

‖Noëlline Guillou, MS, is currently at Centre d'Immunologie et des Maladies Infectieuses (CIMI), Inserm UMR1135/CNRS ERL8255, Université Pierre et Marie Curie, Paris, France.

Supported by Boston Scientific, Marlborough, Mass; AstraZeneca, Gaithersburg, Md; and Legs Poix, Chancellerie des Universités, Paris, France.

Disclosure of potential conflict of interest: M. Pretolani's institution has received funding from AstraZeneca; S. Létuvé has received funding from AstraZeneca and GSK; and C. Taillé reports grants and personal fees from Sanofi, GSK, AstraZeneca, and Novartis outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication October 21, 2019; revised December 8, 2020; accepted for publication December 17, 2020.

Corresponding author: Marina Pretolani, PharmD, PhD, Inserm UMR1152, Université de Paris, Faculté de Médecine, 16 rue Henri Huchard, F-75018, Paris, France. E-mail: marina.pretolani@inserm.fr.

0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2020.12.642

Abbreviations used
ACT: Asthma Control Test
AQLQ: Asthma Quality of Life Questionnaire
ASM: Airway smooth muscle
BT: Bronchial thermoplasty
OCS: Oral corticosteroid
T2: Type 2
SBM: Subepithelial basement membrane

clinical improvement.[3,5,15] Therefore, the major current challenge in clinical practice is to define the eligibility criteria for BT, which implies identification of specific biomarkers for responders.[5,15]

To answer these questions, we compared demographic and clinical parameters with the nature of airway inflammation and remodeling in 23 patients with severe refractory asthma. These patients were defined as BT responders or BT partial responders in accordance with changes in the rate of exacerbations 12 months after the procedure as the primary efficacy end point.

We compared the epithelial expression of MUC5AC and MUC5B and the ratio thereof, which have been associated with an epithelial type 2 (T2)-high signature and airway eosinophilia.[16-18] We also determined the epithelial levels of the IL-13Rα2 receptor, which binds the T2 cytokine IL-13[19] of the alarmin IL-33, which propagates T2 signals through the activation of innate immune cells,[20,21] and the levels of type I (α/β) interferons.[22] Moreover, we investigated hallmarks of airway inflammation, including mucosal eosinophils, neutrophils, IL-13– and IL-17A–positive cells,[10,23,24] and mucosal and intramuscular mast cells.[23,25] Finally, we quantified ASM area and SBM thickening, as validated targets of BT.[3,4,12]

METHODS

Patients and BT procedure

In all, 23 adults with severe uncontrolled asthma were recruited in the Respiratory Diseases Department of the Bichat Hospital (Paris, France) (see Table E1 in the Online Repository at www.jacionline.org).[3,4] Spirometry results, number of exacerbations, and scores on the Asthma Control Test (ACT) were recorded before and 3 and 12 months after BT.[3,4] BT was performed by using the ALAIR System (Boston Scientific, Marlborough, Mass) for a total of 3 sessions separated by 1-month intervals.[3,4]

A bronchoscopy was performed 2 weeks before the first BT session and 3 months after the last session[3,4] to collect 4 bronchial biopsy specimens from the lower right lung lobe for immunohistochemical studies. Control biopsy specimens were obtained from surgically resected bronchial specimens of 10 nonsmoking donors for lung transplantation.[26]

The 23 patients with severe asthma were classified as BT responders (n = 15) or BT partial responders (n = 8) by using a cutoff of fewer than 3 exacerbations for responders and 3 or more exacerbations for partial responders during the 12 months following BT. Indeed, this cutoff value was 1 of the eligibility criteria for including patients with severe refractory asthma in this study, and therefore, all patients had at least 3 severe exacerbations requiring oral corticosteroid (OCS) bursts during the year before inclusion. The 15 responders had suboptimally controlled asthma (a mean ACT score of 16.6), whereas the 8 partial responders remained uncontrolled (a mean ACT score of 8.6) (Table I).[27,28]

This protocol was approved by the Comité de Protection des Personnes Ile-de-France I Ethics Committee (No. 2012-Sept-13003), and all subjects gave their written informed consent (ClinicalTrials.gov identifier NCT01777360).

Histopathologic studies

Epithelial immunolocalization of MUC5AC, MUC5B, IL-13Rα2, IL-33, and IFN-α/β, as well as the number of mucosal cells positive for major basic protein (eosinophils), IL-13, IL-17A, elastase (neutrophils), tryptase (mast cells), and IL-33, were determined on sections from 4 paraffin-embedded bronchial biopsy specimens per patient (for details, see Table E2 in the Online Repository at www.jacionline.org). We also quantified intramuscular mast cells, ASM area, and SBM thickening, as described previously.[3,4]

Statistical analysis

Qualitative clinical variables were reported as numbers and percentages and compared by using the *t* test, chi-square test, or Fisher exact test (2 tailed). Nonnormal variables were reported as medians and 25%-to-75% interquartile ranges and compared by using the Kruskal-Wallis analysis followed by the Mann-Whitney *U* test or by the Wilcoxon matched pairs rank test.

Associations between histopathologic and clinical variables were assessed by using the nonparametric Spearman rank correlation coefficient (ρ) and the Benjamini and Hochberg correction with a familywise error rate of 0.05 for multiple comparisons. Statistical analyses were performed by using Prism (GraphPad Software, San Diego, Calif) and R 2.15.2 software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Clinical characteristics of the recruited patients with severe asthma overall

The 23 recruited patients with severe asthma had uncontrolled asthma at baseline despite optimal management and maximal medications (see Table E1). They also showed airflow obstruction and at least 3 annualized exacerbations requiring OCS bursts.[3,4] Of the 23 patients with severe asthma, 22 were treated regularly with an OCS, with a mean dose of oral prednisone of 29.4 mg per day (see Table E1).

In agreement with our previous study,[3] treatment with BT significantly improved scores on the ACT and on Asthma Quality of Life Questionnaire (AQLQ) and decreased the annualized rate of severe exacerbations and hospitalizations for asthma at 3 and 12 months (see Table E1). In addition, the proportion of patients with severe asthma requiring OCS was significantly lower 3 and 12 months after BT, with a reduction in the mean daily dose of oral prednisone at both time points (see Table E1).

Clinical characteristics of BT responders and BT partial responders

At baseline, the 15 BT responders were approximately 8 years younger ($P = .02$) and had a higher incidence of atopy ($P = .03$), higher numbers of peripheral blood eosinophils ($P = .04$), and higher levels of total serum IgE ($P = .04$) than the BT partial responders had (Table I).

Baseline exacerbation rates and ACT and AQLQ scores were not statistically different between BT responders and BT partial responders (Table I). The proportions of patients treated with long-lasting β_2-agonists, leukotriene modifiers, OCSs, and long-acting muscarinic antagonists, as well as the daily doses of an inhaled corticosteroid and oral prednisone, were comparable between the 2 groups (Table I).

Twelve months after BT treatment, the responders had reduced numbers of peripheral blood eosinophils ($P = .02$) and lower levels of total IgE ($P = .04$), whereas no changes were observed in the partial responders (Table I). The numbers of neutrophils were not statistically different across groups ($P = .65$). As

TABLE I. Characteristics of patients with severe asthma who were classified as BT responders and BT partial responders before and 12 months after BT

Parameter	Patients with severe asthma classified as BT responders		Patients with severe asthma classified as BT partial responders		*P* value*
	Before BT	After BT	Before BT	After BT	
Subjects (no.)	15		8		—
Women, no. (%)	7 (47)	—	6 (75)	—	.38
White, no. (%)	13 (87)	—	6 (75)	—	.59
Age (y), mean ± SD	46.7 ± 7.9	—	54.9 ± 6.2	—	**.02**
Body mass index (kg/m^2), mean ± SD	31.5 ± 9.4	—	26.0 ± 4.1	—	.13
Smoking history, never smokers/former smokers (%/%)	9/6 (60/40)	—	5/3 (63/37)	—	1.00
Age of asthma onset (y), mean ± SD	20.3 ± 17.7	—	18.8 ± 18.3	—	.84
Asthma duration (y), mean ± SD	26.3 ± 18.2	—	30.4 ±17.1	—	.60
With history of atopy, no. (%)	11 (73)	—	2 (25)	—	**.03**
Blood eosinophils/mm^3, median (25%-75% IQR)	270 (153-415)	108 (63-243)†	145 (88-192)‡	135 (72-315)	**.03**
Blood neutrophils/mm^3, median (25%-75% IQR)	10,700 (5,670-15,900)	5,940 (4,710-8,410)	8,585 (8,108-9,062)	8240 (7,535-9,775)	.65
Total serum IgE (IU/mL), median (25%-75% IQR)	330 (144-497)	129 (62-168)†	180 (133-317)‡	177 (58-203)	**.04**
Respiratory function, mean ± SD					
Prebronchodilator FEV_1 (L)	2.11 ± 0.81	2.20 ± 0.74	1.92 ± 0.60	1.56 ± 0.64	.23
Postbronchodilator FEV_1 (L)	2.23 ± 0.76	2.60 ± 1.14	2.27 ± 0.70	1.76 ± 0.76	.23
Prebronchodilator FEV_1 (% of predicted)	65.1 ± 18.5	68.5 ± 16.1	66.3 ± 20.0	53.9 ± 23.7	.37
Postbronchodilator FEV_1 (% of predicted)	73.7 ± 20.3	76.6 ± 16.5	74.1 ± 18.3	57.8 ± 22.0	.17
Prebronchodilator FEV_1/FVC (% of predicted)	60.9 ± 13.3	64.2 ± 10.4	60.2 ± 10.8	52.3 ± 14.8	.25
Postbronchodilator FEV_1/FVC (% of predicted)	63.0 ± 13.0	63.5 ± 10.1	62.2 ± 11.2	56.6 ± 19.8	.77
Reversibility to β2-agonists (mL)	228 ± 226	245 ± 174	373 ± 121	176 ± 108	.37
Treatment					
Long-acting β_2-agonist, no. (%)	15 (100)	15 (100)	8 (100)	8 (100)	1.00
Daily dose of ICS (μg of beclomethasone equivalents), mean ± SD	2553 ± 256	2000 ± 0	2000 ± 0	2000 ± 0	1.00
Maintenance use of OCS, no. (%)	14 (93)	7 (47)†	8 (100)	8 (100)§	**.001**
Daily dose of oral prednisone (mg), mean ± SD	28.6 ± 13.9	14.7 ± 12.7†	30.9 ± 23.2	20.3 ± 20.6	**.04**
Antileukotriene, no. (%)	6 (43)	7 (47)	3 (38)	3 (38)	.96
Long-acting muscarinic antagonist, no. (%)	4 (27)	3 (20)	2 (25)	5 (63)	.18
Asthma control					
With uncontrolled asthma, no. (%)	15 (100)	4 (27)†	8 (100)	7 (88)§	**<.0001**
Score on ACT, mean ± SD	6.4 ± 2.0	16.6 ± 5.0†	6.1 ± 1.6	8.6 ± 3.2§	**<.0001**
Score on AQLQ, mean ± SD	1.9 ± 9.7	4.4 ± 1.6†	1.7 ± 0.8	2.3 ± 1.4§	**<.0001**
Annual no. of severe exacerbations, mean ± SE	10.7 ± 1.5	0.9 ± 0.2†	10.0 ± 2.3	3.4 ± 0.3†,§	**<.0001**
Annual rate of hospitalization for asthma, mean ± SE	1.8 ± 0.7	0.1 ± 0.1†	2.9 ± 1.4	0.8 ± 0.4†,§	**.003**

BMI, Body mass index; *FVC*, forced vital capacity; *ICS*, inhaled corticosteroid.
Boldface denotes statistical significance.
*One-way ANOVA, chi-square test, or Kruskal-Wallis test.
†$P < .05$, as compared with values obtained before BT in each group.
‡$P < .05$, between patients with severe asthma classified as BT responders and those classified as BT partial responders before BT.
§$P < .05$, between patients with severe asthma classified as BT responders and those classified as BT partial responders after BT (Wilcoxon matched-pairs test, Student *t* test, Fisher exact test [2 tailed], or Poisson test).

expected, BT also decreased the number of exacerbations by 99% and increased the ACT score by more than 2.5-fold in responders ($P < .0001$ for both comparisons) (Table I). The annual exacerbation rate was also reduced after BT in partial responders (66% [$P = .02$]), but score on the ACT was not augmented significantly (a 1.4-fold increase, as compared with before BT [$P = .19$]). However, the reduction in exacerbations was more marked in BT responders than in BT partial responders ($P < .0001$). A similar difference was found for annualized hospitalizations for asthma, for which rates were reduced by BT in both patient groups, although less markedly in partial responders than in responders (72% and 94%, respectively [$P = .02$]) (Table I).

Scores on the AQLQ were higher 12 months after BT than before BT in responders ($P = .0002$) but not in partial responders ($P = .35$). This difference was statistically significant ($P = .005$) (Table I).

The proportion of BT responders necessitating a long-term OCS and the daily dose of oral prednisone were reduced at 12 months ($P = .01$ and $P = .007$, respectively) (Table I). In contrast, BT partial responders required doses of an OCS similar to those taken before BT ($P = .35$) (Table I). The use of leukotriene modifiers or long-acting muscarinic antagonists and the daily doses of inhaled corticosteroids were similar in BT responders and BT partial responders (Table I).

The overall baseline respiratory function parameters did not differ between BT responders and BT partial responders, although a nonsignificant trend toward lower values of prebronchodilator and postbronchodilator FEV_1 values ($P = .37$ and $P = .17$,

respectively) and a worse reversibility to β_2-agonists ($P = .37$) were observed in BT partial responders (Table I).

Histopathology of the recruited patients with severe asthma overall

Before BT, the overall group of 23 patients with severe asthma showed higher epithelial expression of MUC5AC (but not MUC5B), higher levels of IL-13Rα2 and IFN-α/β, and a higher MUC5AC-to-MUC5B ratio than shown by the controls (see Table E3 in the Online Repository at www.jacionline.org). These patients also had elevated numbers of mucosal eosinophils and IL-13–, IL-17A–, and IL-33–positive cells, as well as higher numbers of intramuscular (but not mucosal) mast cells (see Table E3). The epithelial expression of IL-33 and the number of mucosal neutrophils were not statistically different across the study groups, although a trend toward an increase in epithelial IL-33 expression was noted in patients with severe asthma before and after BT as compared with expression in the controls (see Table E3). SBM thickening and ASM area were also higher in the patients with severe asthma before BT than in the controls (see Table E3).[3,23]

Treatment with BT was followed by a significant increase in the epithelial expression of MUC5AC and MUC5B ($P = .05$ and $P = .009$, respectively) and IFN-α ($P = .04$), as compared with the values measured before BT (see Table E3). The number of mucosal IL-13–positive cells and the numbers of mucosal and intramuscular mast cells were reduced after BT ($P = .008$ and $P = .04$, respectively), whereas the numbers of mucosal IL-17A–positive cells were not significantly modified (see Table E3). ASM area and SBM thickening were significantly lower 3 months after BT than before BT ($P < .0001$, for both comparisons) (see Table E3).[3,4]

Histopathology in BT responders and BT partial responders

At baseline, the BT responders showed higher epithelial expression of IFN-α1 ($P = .01$, [Fig 1, *C* and *D*]) and elevated numbers of mucosal eosinophils ($P = .02$, Fig 2, *C* and *D*) and IL-33–positive cells ($P = .04$ [Fig 2, *A* and *B*]) than shown by the BT partial responders (Figs 1 and 2). No other hallmarks of airway inflammation or remodeling differentiated the BT responders from the BT partial responders at baseline (Table II).

BT increased the epithelial expression of MUC5AC, but not the expression of MUC5B, in responders (Fig 1, *A* and *B* and Table II) and reduced the MUC5AC-to-MUC5B ratio by 58% ($P = .06$ [Table II]). In these patients, BT also downregulated the extent of epithelium area positive for IL-13Rα2 ($P = .03$ [Table II]), the number of mucosal eosinophils ($P = .02$ [Fig 2, *C* and *D*]), and the numbers of IL-13– ($P = .04$ [Table II]) and IL-33–positive cells ($P = .004$ [Fig 2, *A* and *B*]), as well as the numbers of mucosal and intramuscular mast cells ($P < .001$ and $P = .04$, respectively [Fig 2, *E* and *F*, and Table II]). This was concomitant with a higher epithelial expression of IFN-α/β after BT than before BT in this group of patients ($P = .04$ and $P = .007$, respectively [Fig 1, *C* and *F*]). In contrast, these histopathologic parameters were not modified in the partial responders (Table II and Figs 1 and 2).

Other histopathologic differences between the 2 patients groups included numbers of mucosal and intramuscular mast cells ($P = .05$ [Fig 2, *E* and *F* and Table II]) and levels of epithelial expression of IFN- α/β that remained persistently low ($P = .04$) after BT in the partial responders (Fig 1, *C-F*). Overall, there were no changes in the epithelial expression of IL-33 across the study groups (Table II).

Finally, SBM thickening and ASM area were reduced to a similar extent following BT in both responders and partial responders ($P \leq .003$ for all comparisons) (Table II).

Correlation analyses in BT responders and BT partial responders

We next examined potential correlations between clinical parameters at 12 months and hallmarks of airway inflammation and remodeling at 3 months after BT in the BT responders and BT partial responders (Table III and see Table E4 in the Online Repository at www.jacionline.org).

In BT responders, the number of exacerbations was negatively correlated with epithelial MUC5AC and IFN-α expression, whereas a positive association was found with the numbers of mucosal eosinophils, IL-13–positive cells, and mucosal and intramuscular mast cells; SBM thickening; and ASM area (Table III). Scores on the ACT and AQLQ were positively correlated with epithelial MUC5AC expression and negatively associated with the number of mucosal mast cells and parameters of airway remodeling (Table III).

In contrast, the only correlation found in BT partial responders was a positive association of the number of exacerbations with SBM thickening and ASM area (see Table E4).

Finally, BT responders showed positive correlations between epithelial IFN-α expression ($\rho = 0.53$; $P = .006$) and MUC5AC and MUC5B expression ($\rho = 0.49$; $P = .01$), as well as between numbers of MUC5AC and mucosal IL-17A–positive cells ($\rho = 0.43$; $P = .03$) and numbers of neutrophils ($\rho = 0.50$; $P = .005$). In addition, the number of IL-13–positive cells was associated with the number of mucosal mast cells ($\rho = 0.43$; $P = .02$), and the number of eosinophils was correlated with epithelial IL-33 expression ($\rho = 0.45$; $P = .03$). These correlations were not observed in BT partial responders (data not shown).

No significant correlations were found between any of the histopathologic parameters and the daily doses of OCS (Table III), respiratory function, or the number of heat activations in these patients overall (Tables III and E4 and data not shown).

DISCUSSION

This study aimed at characterizing phenotypes and endotypes of patients with severe refractory asthma associated with optimal response to BT. Indeed, patients with severe asthma do not respond homogeneously to BT, and the major current challenge in clinical practice is to define the eligibility criteria for BT. To this end, we studied hallmarks of epithelial and mucosal inflammation that have never been explored before, including epithelial and mucosal IL-33, epithelial expression of IL-13 receptor α2 and type I (α/β) interferon, and numbers of mucosal and intramuscular mast cells. Our study provides new insights on the characteristics of patients who are more likely to benefit from BT and adds novel findings regarding BT effects on mucosal inflammation and epithelial inflammatory and antiviral responses in relation with clinical parameters.

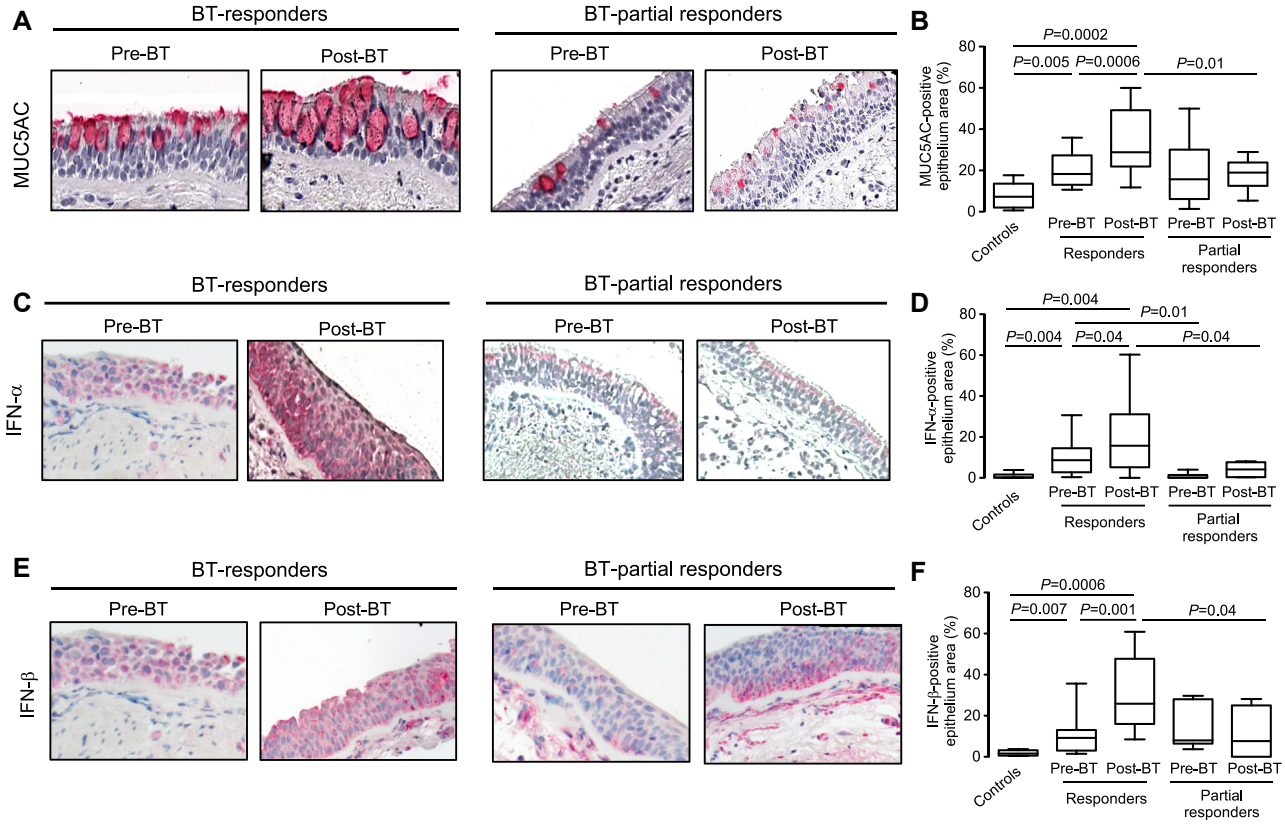

FIG 1. Epithelial alterations and mucosal inflammation before and after BT in patients with severe asthma who were classified as BT responders and BT partial responders. Exemplary photographs showing the epithelial immunolocalization of MUC5AC (**A**), IFN-α (**C**), and IFN-β (**E**) before and 3 months after BT in patients with severe asthma who were responders and those who were partial responders. After BT, the epithelial immunolocalization of these markers was higher in patients with severe asthma who were responders as opposed to partial responders. Original magnifications, ×200. **B, D,** and **F,** Immunostaining of each marker was quantified by image analysis, with data expressed as medians (interquartile range = 25%-75%) for 15 BT responders and 8 BT partial responders. $P = .0003$ (**B**); $P = .02$ (**D**); and $P = .01$ (**F**) (Kruskal-Wallis test). Significance was calculated by using the Mann-Whitney *U* test between controls and patients with severe asthma before BT or after BT, as well as by using Wilcoxon matched pairs rank test between patients with severe asthma before and after BT.

We showed that at baseline, patients with severe asthma who had an optimal response to BT (BT responders) were younger, had a higher incidence of atopy, higher numbers of peripheral blood eosinophils, and a higher total serum IgE level than the BT partial responders had.

Twelve months after BT, blood eosinophil and IgE levels were reduced exclusively in responders despite a decrease in OCS use, and this reduction was associated with a significant improvement in asthma control and quality of life. These findings support the hypothesis that the clinical benefit of BT in responders involved a downregulation of allergic/T2-type responses. Notably, 14 of these 15 BT responders failed to improve with omalizumab, suggesting that BT may provide an additional beneficial effect for atopic patients with severe asthma who are refractory to this biologic.

The bronchial epithelium is a major driver of airway inflammation and remodeling in asthma, and recent studies have shown that BT causes alterations in its morphology and functions.[10,14,29] The degree of patients' response to BT in our study was mirrored by differences in epithelial characteristics at baseline, with BT responders showing higher numbers of mucosal eosinophils and IL-33–expressing cells and greater epithelial expression of the antiviral cytokine IFN-α.

Both BT responders and BT partial responders had elevated numbers of mucosal IL-17A–positive cells before BT as compared with the numbers in the controls, suggesting that these patients exhibited a dual T2/T17 endotype.

After BT, T2 markers such as numbers of eosinophils, IL-13– and IL-33–positive cells, and epithelial IL-13 receptor α2 expression, as well as the numbers of mucosal and intramuscular mast cells, were reduced in responders but not in partial responders. Although these changes cannot prove cause and effect, they support the hypothesis that an attenuation of T2 responses in the airways and attenuation of mast cell accumulation and attraction within the ASM may participate in the clinical benefit of BT in responders. In agreement with this, lower mucosal T2 marker levels and mast cell numbers were correlated with fewer exacerbations in this group, and reduced mucosal mast cells were also significantly associated with improved ACT and AQLQ scores. These data suggest that BT may dampen the

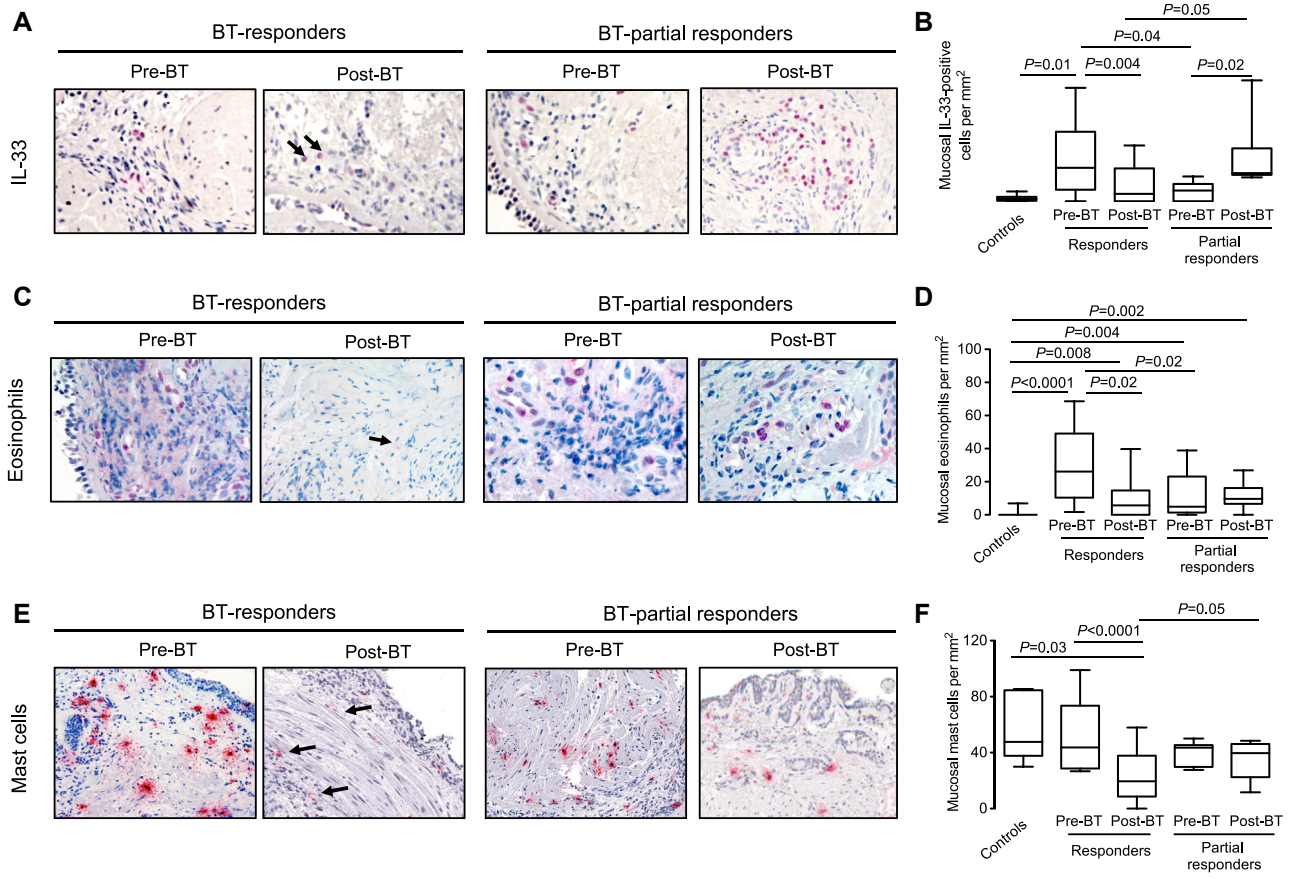

FIG 2. Mucosal inflammation before and after BT in patients with severe asthma who were classified as BT responders and BT partial responders. Exemplary photographs showing the distribution of mucosal cells expressing IL-33, major basic protein (eosinophils), and tryptase (mast cells) (*red deposit*) before and 3 months after BT in patients with severe asthma who were responders and partial responders. After BT, the densities of mucosal of IL-33–positive cells, eosinophils, and mast cells was lower (*arrows*) in responders than in patients with severe asthma who were partially responsive to BT. The frequency of mucosal cells expressing IL-33 after BT was higher in partial responders than in responders. Original magnifications, ×200 and ×400 for IL-33 immunostaining. **B, D,** and **F,** Immunostaining of each marker was quantified by image analysis, and data are expressed as medians (interquartile range = 25%-75%) of 15 BT responders and 8 BT partial responders. $P = .009$ (**B**); $P = .02003$ (**D**); and $P = .03$ (**F**) (Kruskal-Wallis test). Significance was calculated by using the Mann-Whitney *U* test between controls and patients with severe asthma before BT and after BT and by using the Wilcoxon matched pairs rank test between patients with severe asthma before and after BT.

production of mast cell chemotactic factors from different cell types present in the airways, including the ASM itself, leading to a reduction of the overall inflammatory response.

Conversely, the persistence of intramuscular mast cells after BT in partial responders could explain the nonsignificant decrease in prebronchodilator and postbronchodilator FEV_1 values and the worse reversibility to β_2-agonists observed in these patients. Indeed, mast cell–derived mediators play an important role in ASM contraction,[30] which may lead to airway narrowing and lower FEV_1 values. This hypothesis, however, should be tested in a larger number of patients.

Epithelial MUC5AC expression increased after BT in responders, but not in partial responders, and its levels were negatively correlated with exacerbations. Our findings seem to contradict the general notion that MUC5AC overexpression contributes to airway obstruction but not to poor asthma control,[31] but they agree with data in mice showing that upregulated *Muc5ac* expression in the lung epithelium is associated with protection against influenza virus–mediated infection.[32] Also of note, MUC5AC expression is upregulated in response to allergic inflammatory challenge or viral infections.[33,34]

This increase in MUC5AC after BT contradicts a recent report showing epithelial MUC5AC reduction in patients with severe asthma following BT.[10] This discrepancy may originate from differences in the clinical characteristics of the patients included in these 2 trials, in their medication, and in the time point selected after BT at which to study epithelium alterations. Furthermore, no correlations between decrease in MUC5AC and clinical outcomes were investigated in the latter study. Of interest, however, a recent proteomic analysis showed that BT treatment of 8 patients with severe asthma was followed by an increase in their alveolar levels of MUC5AC.[14] These and our current observations indicate that BT may differentially regulate the signals involved in the expression of this mucin. Although IL-13 is considered a major driver of MUC5AC production,[10,11] IL-17A may also stimulate MUC5AC production in human airway

TABLE II. Histopathologic parameters in controls and patients with severe asthma who were classified as BT responders and BT partial responders

Parameter	Controls	Patients with severe asthma classified as BT responders		Patients with severe asthma classified as BT partial responders		*P* value*
		Before BT	After BT	Before BT	After BT	
Patients (no.)	10	15		8		
Epithelium activation and remodeling, median (25%-75% IQR)						
MUC5B-positive area (%)	3.0 (1.6-5.4)	4.1 (2.6-5.4)	5.8 (4.6-12.2)	2.2 (1.0-6.0)	3.2 (2.3-8.7)	.10
MUC5AC/MUC5B ratio	1.5 (0.7-1.7)	5.5 (2.8-7.1)†	2.3 (1.3-7.1)	6.1 (4.3-17.2)†	4.7 (2.0-8.4)†	**.003**
IL-13Rα2–positive area (%)	1.5 (0.5-1.9)	19.6 (16.4-29.1)†	9.5 (8.1-13.7)†,‡	17.8 (5.6-23.3)†	17.6 (5.0-28.9)†	**.008**
IL-33–positive cells/mm^2	225.4 (0.0-410.8)	716.0 (266.7-964.8)	759.0 (475.3-1206.3)	658.1 (423.9-1302.6)	679.3 (176.6-986.6)	.18
Mucosal inflammation, median (25%-75% IQR)						
Neutrophils/mm^2	26.6 (18.9-40.1)	30.6 (22.7-38.6)	30.8 (18.5-51.5)	40.0 (16.4-109)	23.3 (15.9-37.1)	.44
IL-13–positive cells/mm^2	2.8 (1.0-4.4)	8.9 (5.4-11.0)†	3.9 (2.9-6.5)‡	9.1 (3.7-14.5)†	8.8 (3.6-12.7)	**.01**
IL-17A–positive cells/mm^2	20.5 (13.5-37.6)	66.3 (59.8-115.4)†	63.4 (44.5-124.0)†	87.4 (74.2-111.1)†	77.7 (58.2-107.4)†	**.03**
Intramuscular mast cells/mm^2	0.0 (0.0-0.0)	47.1 (25.8-74.8)†	25.2 (6.3-50.2)†,‡	51.3 (32.2-88.4)†	48.4 (30.4-52.2)†,§	**.0002**
Airway remodeling, median (25%-75% IQR)						
SBM thickening (μm)	2.9 (2.4-3.0)	5.4 (3.7-6.8)†	3.1 (2.4-4.0)‡	5.6 (5.2-6.5)†	3.6 (2.5-4.4)‡	**<.0001**
ASM area (% of total biopsy area)	6.6 (4.6-7.0)	18.0 (16.0-19.0)†	6.1 (4.2-8.0)‡	20.3 (17.9-22.8)†	6.6 (5.2-9.5)‡	**.0002**

MUC, Mucin.
Boldface denotes statistical significance.
*Kruskal-Wallis test.
†$P < .05$, as compared with controls.
‡$P < .05$, between the pre-BT and post-BT values in each group.
§$P < .05$, between patients with severe asthma classified as BT-responsive and BT–partially responsive after BT (Mann-Whitney U-test or Wilcoxon matched-pairs test).

TABLE III. Correlation analyses between clinical and histopathologic parameters at 12 months in patients with severe asthma classified as BT responders

Parameter	Exacerbations		Score on the ACT		Score on the AQLQ		Daily dose of OCS	
	ρ	*P* value	ρ	*P* value	ρ	*P* value	ρ	*P* value
Epithelium MUC5AC-positive area	–0.47	**.009**	0.54	**.002**	0.43	**.02**	–0.17	.45
Epithelium MUC5B-positive area	–0.27	.15	0.28	.13	0.21	.27	–0.22	.34
Epithelium IL-13Rα2–positive area (%)	0.21	.27	–0.22	.25	–0.29	.12	0.24	.29
Epithelium IL-33–positive area (%)	0.00	.99	0.12	.56	0.02	.93	0.08	.75
Epithelium IFN-α–positive area (%)	–0.39	**.03**	0.13	.53	0.13	.52	–0.27	.28
Epithelium IFN-β–positive area (%)	–0.31	.07	0.27	.21	0.15	.48	–0.31	.24
Mucosal eosinophils/mm^2	0.36	**.03**	–0.16	.44	–0.29	.15	0.09	.73
Mucosal neutrophils/mm^2	–0.14	.48	0.14	.45	–0.03	.88	0.06	.80
Mucosal IL-13–positive cells/mm^2	0.35	**.04**	–0.14	.45	–0.09	.65	0.09	.70
Mucosal IL-17A–positive cells/mm^2	–0.03	.86	0.09	.62	–0.20	.28	0.16	.56
Mucosal mast cells/mm^2	0.47	**.008**	–0.38	**.02**	–0.39	**.04**	0.07	.77
Intramuscular mast cells/mm^2 ASM	0.37	**.03**	–0.12	.52	–0.16	.41	–0.15	.52
Mucosal IL-33–positive cells/mm^2	0.16	.39	–0.19	.32	–0.21	.27	–0.18	.44
SBM thickening (μm)	0.43	**.02**	–0.57	**.001**	–0.56	**.001**	0.32	.12
ASM area (% of total biopsy area)	0.82	**<.0001**	–0.76	**<.0001**	–0.70	**<.0001**	0.15	.53

MUC, Mucin.
The Spearman rank order method with Benjamini and Hochberg correction (n = 15 patients with severe asthma classified as BT responders, before and 12 months after BT).
Boldface denotes statistical significance.

epithelial cells via IL-17RA, IL-17RC, and the Act1/MAPK pathway.[35] This is supported by our findings showing that MUC5AC epithelial expression was positively correlated with the numbers of mucosal IL-17A–positive cells in BT responders but not in partial responders, despite a lack of modulatory effects of BT on the overall T_H17-type response.

Previous studies have shown that impaired clearance of viruses in patients with uncontrolled asthma was associated with a deficient induction of IFN-α/β, with worsening of airway symptoms and a higher frequency of exacerbations.[22,36] Furthermore, a recent study demonstrated elevated expression of interferons and T2 cytokines, as analyzed from bronchosorption and nasosorption in patients with asthma who were infected with rhinovirus.[37] Although we have no direct clinical evidence that respiratory viruses precipitated the exacerbations in our patients, these observations suggest that epithelial IFN-α/β overexpression in BT responders may act as a protective factor against virus-induced exacerbations. Further studies should be conducted to address this specific point.

Interestingly, treatment with BT led to a rise in the number of mucosal IL-33–positive cells in partial responders. Different cell types, including mast cells and monocytes/macrophages, may express this cytokine.[20] Given the persistence of mucosal and intramuscular mast cells observed after BT in this group of

patients, it can be hypothesized that these cells sustain airway inflammation and ASM activation through IL-33 expression.[38]

Previous studies have demonstrated that the release of this alarmin is triggered by mechanisms commonly associated with asthma pathology, particularly viral infections,[39] and evidence has supported the ability of IL-33 to dampen interferon-driven antiviral immunity.[40,41] Together, these and our current observations suggest that repeated viral infections promote sustained IL-33 overexpression by mucosal cells, which in turn participates in the persistence of severe exacerbations and symptoms in BT partial responders.

We also found that in BT responders, the proportion of patients requiring OCS after BT and the doses of oral prednisone taken were lower than those observed for partial responders. Because corticosteroids have been shown to downregulate MUC5AC in bronchial epithelial cells[42] as well as IFN-α1 in monocytes,[43] there is the possibility that a reduction in OCS use in BT responders contributed to the increased expression of these molecules. However, this hypothesis is not supported by our data showing a lack of correlation between daily use of an OCS and levels of epithelial MUC5AC, IFN-α, and inflammatory cells. In contrast, IL-33 is considered to be a relatively steroid-insensitive target,[21] and this mechanism involves the IL-33–dependent inhibition of glucocorticosteroid receptor nuclear translocation via its phosphorylation.[44] This and the lack of correlation between number of mucosal IL-33–positive cells and OCS use reported here suggest that a direct effect of steroid therapy on the elevation of numbers of IL-33–positive cells in BT partial responders is unlikely.

Most of the patients in this study would fall into the classification of having steroid-resistant asthma, which is characterized by persistent airway inflammation despite treatment with high doses of corticosteroids. Although patients with steroid-resistant asthma showed increased numbers of circulating and mucosal neutrophils,[45] we found no difference in airway and peripheral neutrophils between BT responders and partial responders. These data suggest that mechanisms other than steroid resistance may be involved in the different response to BT in these patients.

We acknowledge that our current study has certain limitations. The relative low number of patients with severe asthma included in the study may have reduced the power of some statistical analyses. In addition, immunohistochemical studies at later time points after BT would allow determination of the persistence of the observed tissue alterations. Also, the detection of MUC5AC was performed to find only stainable mucin stored in the goblet cells of the bronchial epithelium and not to find a secreted form, because our protocol did not include the collection of sputum or bronchoalveolar lavage samples. A quantification of secreted mucins would strengthen the link between MUC5AC and the formation of airway mucus gels during asthma exacerbations.[17] The association between increased levels of mucins and clinical indicators of sputum and cough could also be investigated by adding a validated cough and sputum questionnaire (eg, Cough and Sputum Assessment Questionnaire

score).[46] Finally, the determination of viral load in the airways would be useful for investigation of potential correlations between epithelial IFN-α/β expression and exacerbations.[34]

In conclusion, the current study has demonstrated that BT improved clinical outcomes in patients with severe atopic eosinophilic steroid-dependent asthma in association with a reduction in T2-type inflammation. On the other hand, mucosal IL-33 overexpression in BT partial responders may dampen antiviral responses and sustain eosinophilic- and mast cell–type inflammation. Thus, the use of anti–IL-33–blocking antibodies may represent a novel therapeutic option for these patients.

We thank the patients who participated in the study, Olivier Thibaudeau and Laure Wingertsmann (Morphology Platform, INSERM UMR1152) for the preparation of bronchial tissue sections and haematoxylin and eosin staining, and the Investissements d'Avenir programme ANR-11-IDEX-0005-02, Sorbonne Paris Cité, Laboratoire d'Excellence INFLAMEX.

Key messages

- **BT may provide various degrees of clinical benefit for individuals with severe refractory asthma, depending on their phenotype and endotype. At baseline, those patients with BT-responsive severe asthma were younger and had a higher incidence of atopy, higher circulating eosinophil and IgE levels, and greater expression of mucosal T2 markers and IL-33 than BT partial responders had.**
- **Treatment with BT reduced systemic and mucosal allergic/T2 inflammation, whereas augmented epithelial MUC5AC and IFN-α/β expression in BT responders, but not in BT partial responders. In contrast, BT partial responders had greater numbers of mucosal IL-33–expressing cells after BT than the BT responders had. Most of these changes were correlated with the main clinical parameters of asthma control and symptoms.**

REFERENCES

1. Mainardi AS, Castro M, Chupp G. Bronchial thermoplasty. Clin Chest Med 2019; 40:193-207.
2. Tan LD, Yoneda KY, Louie S, Hogarth DK, Castro M. Bronchial thermoplasty: a decade of experience: state of the art. J Allergy Clin Immunol Pract 2019;7:71-80.
3. Pretolani M, Bergqvist A, Thabut G, Dombret MC, Knapp D, Hamidi F, et al. Effectiveness of bronchial thermoplasty in patients with severe refractory asthma: clinical and histopathologic correlations. J Allergy Clin Immunol 2017;139: 1176-85.
4. Pretolani M, Dombret MC, Thabut G, Knapp D, Hamidi F, Debray MP, et al. Reduction of airway smooth muscle mass by bronchial thermoplasty in patients with severe asthma. Am J Respir Crit Care Med 2014;190:1452-4.
5. Thomson NC. Recent developments in bronchial thermoplasty for severe asthma. J Asthma Allergy 2019;12:375-87.
6. Pavord ID, Cox G, Thomson NC, Rubin AS, Corris PA, Niven RM, et al. Safety and efficacy of bronchial thermoplasty in symptomatic, severe asthma. Am J Respir Crit Care Med 2007;176:1185-91.
7. Cox G, Thomson NC, Rubin AS, Niven RM, Corris PA, Siersted HC, et al. Asthma control during the year after bronchial thermoplasty. N Engl J Med 2007;356: 1327-37.
8. Castro M, Rubin AS, Laviolette M, Fiterman J, De Andrade Lima M, Shah PL, et al. AIR2 Trial Study Group. Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. Am J Respir Crit Care Med 2010;181:116-24.
9. Wechsler ME, Laviolette M, Rubin AS, Fiterman J, Lapa e Silva JR, Shah PL, et al. Asthma Intervention Research 2 Trial Study Group. Bronchial thermoplasty: long-term safety and effectiveness in patients with severe persistent asthma. J Allergy Clin Immunol 2013;132:1295-302.
10. Haj Salem I, Gras D, Joubert P, Boulet LP, Lampron N, Martel S, et al. Persistent reduction of mucin production following bronchial thermoplasty in severe Asthma. Am J Respir Crit Care Med 2019;199:536-8.
11. Seibold MA. Interleukin-13 stimulation reveals the cellular and functional plasticity of the airway epithelium. Ann Am Thorac Soc 2018;15(suppl 2):S98-102.

12. Chakir J, Haj-Salem I, Gras D, Joubert P, Beaudoin ÈL, Biardel S, et al. Effects of bronchial thermoplasty on airway smooth muscle and collagen deposition in asthma. Ann Am Thorac Soc 2015;12:1612-8.
13. Denner DR, Doeing DC, Hogarth DK, Dugan K, Naureckas ET, White SR. Airway inflammation after bronchial thermoplasty for severe asthma. Ann Am Thorac Soc 2015;12:1302-9.
14. Sun Q, Fang L, Roth M, Tang X, Papakonstantinou E, Zhai W, et al. Bronchial thermoplasty decreases airway remodelling by blocking epithelium-derived heat shock protein 60 (HSP60) secretion and protein arginine methyltransferase 1 (PRMT1) in fibroblasts. Eur Respir J. Eur Respir J 2019;54:1900300.
15. d'Hooghe JNS, Ten Hacken NHT, Weersink EJM, Sterk PJ, Annema JT, Bonta PI. Emerging understanding of the mechanism of action of bronchial thermoplasty in asthma. Pharmacol Ther 2018;181:101-7.
16. Evans CM, Kim K, Tuvim MJ, Dickey BF. Mucus hypersecretion in asthma: causes and effects. Curr Opin Pulm Med 2009;15:4-11.
17. Martínez-Rivera C, Crespo A, Pinedo-Sierra C, García-Rivero JL, Pallarés-Sanmartín A, Marina-Malanda N, et al. Mucus hypersecretion in asthma is associated with rhinosinusitis, polyps and exacerbations. Respir Med 2018;135:22-8.
18. Lachowicz-Scroggins ME, Yuan S, Kerr SC, Dunican EM, Yu M, Carrington SD, et al. Abnormalities in MUC5AC and MUC5B protein in airway mucus in asthma. Am J Respir Crit Care Med 2016;194:1296-9.
19. Yang SJ, Allahverdian S, Saunders ADR, Liu E, Dorscheid DR. IL-13 signaling through IL-13 receptor α2 mediates airway epithelial wound repair. FASEB J 2019;33:3746-57.
20. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell-derived cytokines: more than just signaling the alarm. J Clin Invest 2019;1291441-51.
21. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. J Allergy Clin Immunol 2013;132:676-85.
22. Denney L, Ho LP. The role of respiratory epithelium in host defence against influenza virus infection. Biomed J 2018;41:218-33.
23. Aubier M, Thabut G, Hamidi F, Guillou N, Brard J, Dombret MC, et al. Airway smooth muscle enlargement is associated with protease-activated receptor 2/ligand overexpression in patients with difficult-to-control severe asthma. J Allergy Clin Immunol 2016;138:729-39.
24. Andersson CK, Adams A, Nagakumar P, Bossley C, Gupta A, De Vries D, et al. Intraepithelial neutrophils in pediatric severe asthma are associated with better lung function. J Allergy Clin Immunol 2017;139:1819-29.
25. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med 2002;346:1699-705.
26. Fernandes J, Hamidi F, Leborgne R, Beau R, Castier Y, Mordant P, et al. Penetration of the human pulmonary epithelium by *Aspergillus fumigatus* hyphae. J Infect Dis 2018;218:1306-13.
27. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol 2004;113:59-65.
28. Schatz M, Sorknesss CA, Li JT, Marcus P, Murray JJ, Nathan RA, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. J Allergy Clin Immunol 2006;117:549-56.
29. Bonser LR, Erle DJ. The airway epithelium in asthma. Adv Immunol 2019;142:1-34.
30. Säfholm J, Manson ML, Bood J, Al-Ameri M, Orre AC, Raud J, et al. Mannitol triggers mast cell-dependent contractions of human small bronchi and prostacyclin bronchoprotection. J Allergy Clin Immunol 2019;144:984-92.
31. Dunican EM, Elicker BM, Gierada DS, Nagle SK, Schiebler ML, Newell JD, et al. National Heart Lung and Blood Institute (NHLBI) Severe Asthma Research Program (SARP). Mucus plugs in patients with asthma linked to eosinophilia and airflow obstruction. J Clin Invest 2018;128:997-1009.
32. Ehre C, Worthington EN, Liesman RM, Grubb BR, Barbier D, O'Neal WK, et al. Overexpressing mouse model demonstrates the protective role of Muc5ac in the lungs. Proc Natl Acad Sci USA 2012;109:16528-33.
33. Bonser LR, Erle DJ. Airway mucus and asthma: the role of MUC5AC and MUC5B. J Clin Med 2017;6:112.
34. Ridley C, Thornton DJ. Mucins: the frontline defence of the lung. Biochem Soc Trans 2018;46:1099-106.
35. Xia W, Bai J, Wu X, Wei Y, Feng S, Li L, Zhang J, Xiong G, Fan Y, Shi J, Li H. Interleukin-17A promotes MUC5AC expression and goblet cell hyperplasia in nasal polyps via the Act1-mediated pathway. PLoS One 2014;9:e98915.
36. Zhu J, Message SD, Mallia P, Kebadze T, Contoli M, Ward CK, et al. Bronchial mucosal IFN-α/β and pattern recognition receptor expression in patients with experimental rhinovirus-induced asthma exacerbations. J Allergy Clin Immunol 2019;143:114-25.
37. Hansel TT, Tunstall T, Trujillo-Torralbo MB, Shamji B, Del-Rosario A, Dhariwal J, et al. A comprehensive evaluation of nasal and bronchial cytokines and chemokines following experimental rhinovirus infection in allergic asthma: increased interferons (IFN-γ and IFN-λ) and type 2 inflammation (IL-5 and IL-13). EBioMedicine 2017;19:128-38.
38. Kaur D, Gomez E, Doe C, Berair R, Woodman L, Saunders R, et al. IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. Allergy 2015;70:556-67.
39. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. Am J Respir Crit Care Med 2014;190:1373-82.
40. Werder RB, Zhang V, Lynch JP, Snape N, Upham JW, Spann K, et al. Chronic IL-33 expression predisposes to virus-induced asthma exacerbations by increasing type 2 inflammation and dampening antiviral immunity. J Allergy Clin Immunol 2018;141:1607-19.
41. Ravanetti L, Dijkhuis A, Dekker T, Sabolgal Pineros YS, Ravi A, et al. IL-33 drives influenza-induced asthma exacerbations by halting innate and adaptive antiviral immunity. J Allergy Clin Immunol 2019;143:1355-70.
42. Chen Y, Nickola TJ, DiFronzo NL, Colberg-Poley AM, Rose MC. Dexamethasone-mediated repression of MUC5AC gene expression in human lung epithelial cells. Am J Respir Cell Mol Biol 2006;34:338-47.
43. Norbiato G, Bevilacqua M, Vago T. Glucocorticoids and the immune system in AIDS. Psychoneuroendocrinology 1997;22(Suppl 1):S19-25.
44. Hirahara K, Mato N, Hagiwara K, Nakayama T. The pathogenicity of IL-33 on steroid-resistant eosinophilic inflammation via the activation of memory-type ST2+ CD4+ T cells. J Leukoc Biol 2018;104:895-901.
45. Macdowell AL, Peters SP. Neutrophils in asthma. Curr Allergy Asthma Rep 2007;7:464-8.
46. Crawford B, Monz B, Hohlfeld J, Roche N, Rubin B, Magnussen H, et al. Development and validation of a cough and sputum assessment questionnaire. Respir Med 2008;102:1545-55.

METHODS

Patients

Between October 2013 and November 2016, a total of 23 adults aged 29 to 64 years with severe refractory asthma[E1,E2] were recruited at the Pneumology A Department of the Bichat Hospital (Paris, France) (Table E1). Of these 23 adults with severe asthma, 2 have been included in previous studies.[E3,E4] All patients gave their written consent, and the protocol was approved by the Comité de Protection des Personnes Ile-de-France I Ethics Committee (No. 2012-Sept-13003). This trial (the ASMATHERM protocol) is registered at ClinicalTrials.gov under the identifier NCT01777360.

Half of these patients were female and had a history of atopy, as established by positive results of a skin prick test to common allergens, serum levels of specific IgE of at least 0.35 IU/mL, and mild blood eosinophilia. The key inclusion criteria were (1) subjects with severe uncontrolled asthma despite optimal management and maximal medications for at least 12 months before entry[E3-E5]; (2) at least 3 exacerbations, defined as a worsening of asthma symptoms requiring OCS bursts during the previous year; and (3) a prebronchodilator FEV_1 value between 30% and 70% of predicted.

We defined asthma exacerbations according to the need for OCS for more than 3 days and/or hospital admission. This corresponds to the severe exacerbation classification by the European Respiratory Society/American Thoracic Society guidelines on severe asthma.[E6]

Asthma control was assessed by using score on the Asthma Control Test (ACT), which is based on a response scale from 1 to 25, according to which a score higher than 21 over the past 4 weeks indicates complete control, a response between 16 to 20 indicates asthma that is somewhat controlled, a score lower than 15 indicates asthma that is poorly controlled, and a score lower than 7.5 indicates asthma that is not controlled.[E5,E7] In keeping with these criteria, the 23 adults with severe asthma included in this study showed a mean number of annualized exacerbations of 10.5 and a mean ACT score of 6.3 at entry. In all, 22 patients required maintenance with an OCS, with mean daily doses of 29.4 mg of prednisone. At the time of inclusion, they also received add-on therapies, including leukotriene modifiers (9 of 23 patients) and oral long-lasting anti-antimuscarinics (21 of 23 patients) (Table E1).

Of the 23 patients, 19 met the criteria for receiving omalizumab for 6 months during the year before entry in the protocol without a successful clinical outcome. Omalizumab was stopped at least 6 months before the first BT session (Table E1).[E3,E4] None of the patients were treated with mAbs targeting IL-5, IL-4/IL-13, or IL-17 or their receptors.

We used the values fewer than 3 exacerbations (mean = 0.5) and 3 or more exacerbations (mean = 3.4), measured 12 months after BT, as a primary standard to define these 23 adults with severe asthma as BT responders (n = 15) or BT partial responders (n = 8), respectively.[E8] This cutoff value was among the criteria for including these patients in the current protocol (see earlier). Although a decrease in exacerbation frequency was observed in both groups, the reduction in exacerbations was more marked in the BT responders than in the BT partial responders ($P < .0001$).

The 15 patients with severe asthma classified as BT responders were deemed as having an optimal clinical response and partially or completely controlled asthma 12 months after BT because their mean ACT score was 16.6. In contrast, the 8 patients with severe asthma classified as BT partial responders had poorly or uncontrolled asthma, with a mean ACT score of 8.6.[E5,E7]

BT procedure and biopsy sample collection

In all, 3 sessions of BT were performed at 1-month intervals by using the ALAIR System (Boston Scientific).[E3,E4] Medians of 51 (interquartile range [IQR] = 39-62), 58 (IQR = 49-64), and 70 (IQR = 55-77) heat activations were administered during the first, second, and third BT sessions, respectively (administered in the right lower lobe, the left lower lobe, and the 2 upper lobes, respectively). No heat activation was delivered in the middle lobe. A bronchoscopy was performed 15 days before the first BT procedure and 3 months after the last proceudre.[E3,E4] A total of 4 bronchial biopsy samples were collected from 3 locations in the right lower lobe (B7-B8, B8-B9, and B9-B10).

In previous studies, we established that ASM area measured before the first BT session in the biopsy samples collected in the right lower lobe was not statistically different from that determined in the biopsy samples obtained from the left lower lobe or from the right and left upper lobes.[E3] In addition, BT reduced the ASM area to a similar extent in all treated lobes at 3 months.[E3] This was also observed for several other immunohistochemical parameters (ie, blood and lymphatic vessels, ASM, neuroendocrine cells, nerve endings, eosinophils, neutrophils, and collagen) and morphologic parameters (glands and epithelium area, SBM thickening) across the 10 bronchial biopsy samples.[E4] Therefore, we considered the data originating from the 4 biopsy samples obtained from the right lower lung lobe to be representative of the rest of the lung lobes. In parallel, because BT also significantly decreased the ASM area in the middle untreated lobe (which was originally defined as the internal control),[E3] in the current study we have included a separate group of surgically resected bronchial specimens from 10 nonsmoking asthma-free lung transplantation donors (6 males and 4 females with a mean age of 47.7 ± 19.3 years).[E9] We could not include a group of healthy subjects because bronchoscopies are not ethically justified for research studies by our institutions.

Morphometry and immunohistochemistry

Approximately 40 serial sections (4 μm each) were obtained for each biopsy sample, and morphology was assessed every 10 sections after staining with Mayer hematoxylin. Bronchial tissue sections were deparaffinized in xylene and dehydrated in ethanol, after which antigens were retrieved under citrate buffer solution at pH 6.0 in a steam heat device at 97°C for 40 minutes.

For the immunohistochemical analyses, tissue sections were incubated with the primary antibodies that are listed in Table E2. We examined the epithelium expression of MUC5AC, MUC5B,[E10] the innate immune cytokine IL-33,[E11] IL-13Rα2,[E12] and the antiviral cytokines IFN-α and IFN-γ.[E13,E14]

We also enumerated mucosal eosinophils (major basic protein–positive cells); neutrophils (elastase-positive cells); mast cells (tryptase-positive cells); and IL-33–, IL-13–, and IL-17A–positive cells.[E15,E16] Mast cells were also quantified in the ASM, as described elsewhere.[E16] For each antigen, immunohistochemical staining and analysis were performed on 4 separate areas of the bronchial biopsy samples (ie, 2 sections before BT and 2 sections after BT per patient).

Epithelium and mucosal markers were visualized at ×20 and ×400 magnification, respectively, by using a slide scanner coupled to an image analyzer (Calopix, TRIBVN, Chatillon, France). Biopsy area (in mm^2) and the proportion of intact epithelium area over the total epithelium area were determined by morphometry on Mayer hematoxylin–stained tissue sections at ×60 magnification. The median values of the biopsy areas in the controls and in the patients with severe asthma before and after BT were 0.87 mm^2 (IQR = 0.51-1.29 mm^2), 1.17 mm^2 (IQR = 0.92-1.45 mm^2), and 0.99 mm^2 (IQR = 0.72-1.18 mm^2), respectively ($P = .47$ [Kruskal-Wallis test]). The respective median proportions of intact epithelium area over the total epithelium area in the controls and in the patients with severe asthma before BT and after BT were as follows: 38.6% (IQR = 21.5%-47.2%), 30.5% (IQR =13.2%-46.2%), and 33.2% (IQR =16.8%-39.8%) (overall $P = .63$ [Kurskal-Wallis test]).

Immunohistochemical data were expressed as a percentage of intact epithelium-stained area over the total epithelium area for MUC5AC, MUC5B, IL-13Rα2, IFN-α1, and IFN-β; as number of IL-33–positive cells per mm^2 of epithelium area; and as numbers of eosinophils, neutrophils, IL-13–, IL-33–, and IL-17A–positive cells per mm^2 of the bronchial submucosa.[E15,E16] ASM area and SBM thickening were assessed by morphometry and computer-assisted image analysis. The area of the bronchial submucosa occupied by the ASM was determined on α-actin–stained tissue sections, and the final results were expressed as the percentage ASM area to total biopsy area.[E2,E3,E15] SBM thickening (in μm) was determined in tissue sections stained with Mayer haematoxylin, from the base of the bronchial epithelium to the outer limit of the reticular lamina of the basement membrane. Measurements (between 20 and 50) were made at regular intervals of 50 μm along the length of the SBM at × 400 magnification.[E15]

Antigen detection was assessed by using the Avidin Biotin Complex from the Vectastain'ABC-Alkaline Phosphatase (Vector Laboratories, Eurobio/Abcys, Les Ulis, France), and the immunostaining was visualized with the liquid permanent chromogen Fast Red (DakoCytomation, Les Ulis, France), followed by light nuclear Mayer hematoxylin counterstaining. All parameters were examined in 2 serial sections from the same biopsy sample and in sections from the 4 biopsy samples obtained before and after BT. Sections were analyzed in blind fashion, and the final value was the mean of all the measurements obtained for each patient. The coefficients of variation between biopsy samples from the same patient and between each parameter of the same biopsy sample were 8% to 13 % and 6% to 9 %, respectively.

REFERENCES

E1. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014;43:343-73.

E2. American Thoracic Society. Proceedings of the ATS workshop on refractory asthma. Am J Respir Crit Care Med 2000;162:2341-51.

E3. Pretolani M, Dombret MC, Thabut G, Knap D, Hamidi F, Debray MP, et al. Reduction of airway smooth muscle mass by bronchial thermoplasty in patients with severe asthma. Am J Respir Crit Care Med 2014;190:1452-4.

E4. Pretolani M, Bergqvist A, Thabut G, Dombret MC, Knapp D, Hamidi F, et al. Effectiveness of bronchial thermoplasty in patients with severe refractory asthma: clinical and histopathologic correlations. J Allergy Clin Immunol 2017;139: 1176-85.

E5. Schatz M, Sorknesss CA, Li JT, Marcus P, Murray JJ, Nathan RA, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. J Allergy Clin Immunol 2006;117:549-56.

E6. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014;43:343-73.

E7. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol 2004;113:59-65.

E8. Kroes JA, Zielhuis SW, van Roon EN, Ten Brinke A. Prediction of response to biological treatment with monoclonal antibodies in severe asthma. Biochem Pharmacol 2020;179:113978.

E9. Fernandes J, Hamidi F, Leborgne R, Beau R, Castier Y, Mordant P, et al. Penetration of the human pulmonary epithelium by *Aspergillus fumigatus* hyphae. J Infect Dis 2018;218:1306-13.

E10. Haj Salem I, Gras D, Joubert P, Boulet LP, Lampron N, Martel S, et al. Persistent reduction of mucin production following bronchial thermoplasty in severe asthma. Am J Respir Crit Care Med 2019;199:536-8.

E11. Castanhinha S, Sherburn R, Walker S, Gupta A, Bossley CJ, et al. Pediatric severe asthma with fungal sensitization is mediated by steroid-resistant IL-33. J Allergy Clin Immunol 2015;136:312-22.

E12. Verstockt B, Perrier C, De Hertogh G, Cremer J, Creyns B, Van Assche G, et al. Effects of epithelial IL-13Rα2 expression in inflammatory bowel disease. Front Immunol 2018;9:2983.

E13. Denney L, Ho LP. The role of respiratory epithelium in host defence against influenza virus infection. Biomed J 2018;41:218-33.

E14. Zhu J, Message SD, Mallia P, Kebadze T, Contoli M, Ward CK, et al. Bronchial mucosal IFN-α/β and pattern recognition receptor expression in patients with experimental rhinovirus-induced asthma exacerbations. J Allergy Clin Immunol 2019;143:114-25.

E15. Aubier M, Thabut G, Hamidi F, Guillou N, Brard J, Dombret MC, et al. Airway smooth muscle enlargement is associated with protease-activated receptor 2/ligand overexpression in patients with difficult-to-control severe asthma. J Allergy Clin Immunol 2016;138:729-39.

E16. Andersson CK, Adams A, Nagakumar P, Bossley C, Gupta A, et al. Intraepithelial neutrophils in pediatric severe asthma are associated with better lung function. J Allergy Clin Immunol 2017;139:1819-29.

TABLE E1. Characteristics of the patients with severe asthma before and 3 and 12 months after BT

Parameter	Before BT	3 mo after BT	12 mo after BT	*P* value*
Subjects (no.)	23	23	23	
Women, no. (%)	12 (52)	—	—	—
Age (y), mean ± SD	47.8 ± 8.9	—	—	—
White origin	18 (78)	—	—	—
Body mass index (kg/m^2), mean ± SD	30.0 ± 8.3	—	—	—
Never-smokers/former smokers, no. (%)	14/9 (61/39)	—	—	—
History of atopy, no. (%)	13 (56)	—	—	—
Age at asthma onset (y), mean ± SD	19.8 ± 17.5	—	—	—
Asthma duration (y), mean ± SD	27.7 ± 17.5	—	—	—
Blood eosinophils/mm^3, median (IQR)	240 (125-440)	160 (68-320)	125 (63-248)	.20
Blood neutrophils/mm^3, median (IQR)	8205 (5298-13320)	7830 (6850-10310)	7630 (5325-8975)	.79
Total serum IgE (IU/mL), median (IQR)	244 (143-497)	192 (98-345)	134 (56-320)	.40
Respiratory function				
Prebronchodilator FEV_1 (L)	2.04 ± 0.74	2.01 ± 0.71	1.98 ± 0.76	.96
Postbronchodilator FEV_1 (L)	2.24 ± 0.72	2.18 ± 0.68	2.28 ± 1.08	.93
Prebronchodilator FEV_1 (% of predicted)	65.5 ± 18.6	64.5 ± 18.0	63.4 ± 19.9	.93
Postbronchodilator FEV_1 (% of predicted)	71.6 ± 19.5	70.4 ± 18.5	69.9 ± 20.5	.96
Prebronchodilator FEV_1/FVC (% of predicted)	61.3 ± 11.1	62.3 ± 14.0	60.0 ± 13.1	.82
Postbronchodilator FEV_1/FVC (% of predicted)	61.3 ± 11.9	63.8 ± 13.5	60.5 ± 13.9	.71
Reversibility to β_2-agonist (mL)	265 ± 254	191 ± 164	258 ± 273	.67
Treatment				
Long-acting β_2-agonist, no. (%)	23 (100)	21 (91)	23 (100)	.13
Daily dose of an ICS (μg of beclomethasone equivalent), mean ± SD	2261 ± 915	2043 ± 878	1774 ± 338	.11
Maintenance use of an OCS, no. (%)	22 (96)	14 (61)	15 (64)	**.02**
Daily dose of oral prednisone (mg), mean ± SD	29.4 ± 17.3	18.6 ± 12.8†	18.1 ± 19.7†	**.03**
Antileukotriene, no. (%)	9 (39)	11 (48)	10 (43)	.35
Long-acting muscarinic antagonist, no. (%)	7 (30)	6 (26)	8 (35)	.14
Asthma control				
With uncontrolled asthma, no. (%)	23 (100)	10 (43)†	11 (48)†	**<.0001**
Score on ACT, mean ± SD	6.3 ± 1.8	14.1 ± 5.3†	13.8 ± 5.8†	**<.0001**
Score on AQLQ, mean ± SD	1.8 ± 0.7	3.3 ± 1.4†	3.7 ± 1.8†	**<.0001**
Annual rate of severe exacerbations requiring OCS bursts, mean ± SE	10.5 ± 1.2	0.9 ± 0.3†	1.7 ± 0.3†,‡	**<.0001**
Annual rate of hospitalizations for asthma, mean ± SE	2.5 ± 0.7	0.1 ± 0.1†	0.3 ± 0.1†	**<.0001**

FVC, Forced vital capacity; *ICS*, inhaled corticosteroid.
Boldface denotes statistical significance.
*ANOVA or Kruskal-Wallis test of chi-square test.
†$P < .05$ as compared with before BT.
‡$P < .05$ as compared with 3 months after BT (Student *t* test for paired value, Wilcoxon matched pairs rank test, Fisher test [2 tailed] or Poisson test).

TABLE E2. Primary antibodies used for immunohistochemistry

Primary antibody	Isotype	Clone	Dilution	Manufacturer
Anti-MUC5AC	Mouse IgG1κ	45M1	1:500	Invitrogen
Anti-MUC5B	Rabbit IgG	H-300	1:200	Santa Cruz Biotechnology
Anti-eosinophil major basic protein	Rabbit IgG	Polyclonal	1:50	Abcam
Anti-neutrophil elastase	Mouse IgG1κ	NP57	1:100	Dako
Anti–mast cell tryptase	Mouse IgG1κ	AA1	1:50	Dako
Anti–IL-17A	Goat IgG	Polyclonal	1:100	R&D Systems
Anti–IL-13	Rabbit IgG	Polyclonal	1:200	Abcam
Anti–IL-13Rα2	Goat IgG	Polyclonal	1:20	R&D Systems
Anti–IL-33	Goat IgG	Polyclonal	1:100	R&D Systems
Anti–IFN-α	Mouse IgG2bκ	F-7	1:10	Santa Cruz Biotechnology
Anti–IFN-β	Rabbit IgG	Polyclonal	1:2000	Life Technologies
Anti–smooth muscle α-actin	Mouse IgG2a	1A4	1:200	Sigma Aldrich

IL-13Rα2, IL-13 receptor α2.

TABLE E3. Airway inflammation and remodeling in controls and patients with severe asthma before and 3 months after BT

Parameter	Controls	Patients with severe asthma		*P* value*
		Before BT	3 mo after BT	
Subjects (no.)	10	23	23	—
Epithelium alteration, median (25%-75% IQR)				
MUC5AC-positive area (%)	7.2 (2.3-12.2)	18.6 (13.0-27.5)†	24.4 (18.2-33.2)†,‡	**.001**
MUC5B-positive area (%)	3.0 (1.6-5.4)	3.4 (2.2-5.5)	7.6 (3.5-12.0)‡	**.02**
MUC5AC/MUC5B ratio	1.5 (0.7-1.7)	5.0 (1.9-12.4)†	3.0 (1.3-11.2)	**.01**
IL-13Rα2–positive area (%)	1.5 (0.5-1.9)	19.3 (5.4-33.6)†	10.5 (6.5-25.0)†	**.001**
IL-33–positive cells/mm^2	225.4 (0.0-810.8)	682.7 (273.7-1103.7)	743.5 (300.2-1227.3)	.07
IFN-α–positive area (%)	0.2 (0.2-1.6)	6.4 (0.5-13.8)†	10.2 (2.7-25.1)†,‡	**.01**
IFN-β–positive area (%)	1.6 (0.9-2.5)	9.3 (5.7-24.9)†	20.2 (8.5-35.7)†	**.004**
Airway inflammation, median (25%-75% IQR)				
Eosinophils/mm^2	0.5 (0.0-2.7)	11.6 (4.1-30.2)†	7.9 (0.0-16.6)†	**.01**
Neutrophils/mm^2	26.6 (18.9-40.1)	30.6 (20.4-83.2)	27.9 (18.5-57.1)	.47
IL-13–positive cells/mm^2	2.8 (1.0-4.4)	7.1 (5.3-10.4)†	4.9 (2.9-5.8)‡	**.002**
IL-17A–positive cells/mm^2	20.5 (13.5-37.6)	82.1 (64.1-115.4)†	74.9 (44.5-111.0)†	**.006**
Mast cells/mm^2	47.7 (37.7-84.6)	43.8 (27.9-58.7)	21.0 (11.5-45.3)‡	**.004**
Intramuscular mast cells/mm^2	0.0 (0.0-0.0)	51.3 (25.9-77.1)†	33.2 (12.6-55.8)‡	**.004**
IL-33–positive cells/mm^2	0.0 (0.0-0.3)	21.0 (7.5-51.5)†	14.0 (1.9-44.4)†	**.01**
Airway remodeling, median (25%-75% IQR)				
SBM thickening (μm)	2.9 (2.4-3.0)	5.5 (4.4-6.8)†	3.3 (2.4-4.1)‡	**<.0001**
ASM area (% of total biopsy area)	6.6 (4.6-7.0)	18.3 (16.0-23.0)†	5.6 (4.4-8.0)‡	**<.0001**

IL-13Rα2, IL-13 receptor α2.
Boldface denotes statistical significance.
*Kruskal-Wallis test.
†$P < .05$ as compared with controls (Mann-Whitney *U* test).
‡$P < .05$ as compared to patients with severe asthma before BT (Wilcoxon matched pairs rank test).

TABLE E4. Correlation analyses between clinical and histopathologic parameters in patients with severe asthma classified as BT partial responders, as determined before and 12 months after BT

	Exacerbations		Score on ACT		Score on AQLQ		Daily dose of OCS	
Parameter	**ρ**	***P* value**	**ρ**	***P* value**	**ρ**	***P* value**	**ρ**	***P* value**
Epithelium MUC5AC-positive area	–.36	.17	.34	.19	.24	.29	–.31	.24
Epithelium MUC5B-positive area	–.06	.83	.11	.67	–.01	.98	.07	.80
Epithelium IL-13Rα2–positive area (%)	–.18	.50	.23	.39	.20	.47	.29	.28
Epithelium IL-33–positive area (%)	–.06	.83	.20	.50	.41	.15	–.05	.88
Epithelium IFN-α–positive area (%)	–.12	.68	- .19	.51	–.23	.43	.34	.24
Epithelium IFN-β–positive area (%)	.15	.65	.00	.99	–.08	.80	.26	.41
Mucosal eosinophils/mm^2	.34	.23	–.19	.51	–.26	.37	.17	.55
Mucosal neutrophils/mm^2	.22	.42	–.17	.53	–.11	.69	.18	.51
Mucosal IL-13–positive cells/mm^2	.14	.60	.16	.53	.25	.29	–.20	.45
Mucosal IL-17A–positive cells/mm^2	.13	.63	–.05	.85	–.14	.60	.10	.72
Mucosal mast cells/mm^2	.19	.51	.03	.93	–.17	.56	.16	.58
Intramuscular mast cells/mm^2 of ASM	.20	.54	–.28	.38	–.39	.21	.07	.83
Mucosal IL-33–positive cells/mm^2	–.24	.38	.12	.65	.09	.74	–.04	.89
SBM thickening (μm)	.78	**.0003**	–.40	.12	–.31	.24	.16	.58
ASM area (% of total biopsy area)	.75	**.0009**	–.30	.26	–.15	.59	.22	.41

IL-13Rα2, IL-13 receptor α2; *MUC*, mucin.

Values determined by using the Spearman rank order method and Benjamini and Hochberg correction (n = 8 patients with severe asthma classified as BT partial responders, before and 12 months after BT). Boldface denotes statistical significance.