

# The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma

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**Background:** Allergen-specific T<sub>H</sub>2 responses contribute to the development of allergic asthma. Their increase may be due to a reduced early exposure to environmental pathogens, which induces a T<sub>H</sub>1 response, and thereby suppresses the allergic T<sub>H</sub>2 response. QbG10 (bacteriophage Qbeta-derived virus-like particle with CpG-motif G10 inside), a novel Toll-like receptor 9 agonist packaged into virus-like particles, was designed to stimulate the immune system toward a T<sub>H</sub>1-mediated protective response.

**Objective:** We examined clinical efficacy, safety, and tolerability of QbG10 with patient-reported and objective clinical outcome parameters in patients with mild-to-moderate persistent allergic asthma.

**Methods:** In this proof-of-concept parallel-group, double-blind, randomized trial, 63 asthmatic patients followed conversion to a standardized inhaled steroid and were treated with 7 injections of either QbG10 or placebo. Incorporating a controlled steroid withdrawal, the effects on patient-reported (day- and nighttime asthma symptoms, salbutamol usage, and 7-item-Asthma Control Questionnaire scores) and objective clinical outcome measures (FEV<sub>1</sub>, fraction of exhaled nitric oxide, and blood eosinophils) were assessed over 12 weeks (ClinicalTrials.gov number, NCT00890734).

**Results:** All patient-reported parameters improved overall between week 0 and 12 in QbG10-treated patients (n = 33) despite steroid withdrawal, compared with deteriorations observed under placebo (n = 30, *P* < .05). At week 12, two thirds of the QbG10-treated patients had their asthma “well controlled” (Asthma Control Questionnaire score ≤ 0.75) compared with one third under placebo. FEV<sub>1</sub> had worsened to a clinically significant extent in patients on placebo, while it remained stable in QbG10 patients. Adverse events were mostly injection site reactions occurring after QbG10 administration.

**Conclusion:** Treatment with QbG10 may contribute to continued asthma control during steroid reduction in patients on moderate or high-dose inhaled steroids. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

**Key words:** Persistent allergic asthma, Toll-like receptor 9, virus-like particle, immune modulator, clinical trial

Achieving asthma control is an important goal in the long-term management of asthma, and anti-inflammatory therapy with inhaled corticosteroids (ICSs) is the cornerstone of treatment. Despite treatment with ICSs or combined therapy with ICSs plus long-acting beta-agonists, many subjects with asthma remain symptomatic and fail to achieve asthma control. At this stage, options for patients include increase in the ICS dose, add-on therapy with leukotriene antagonists, and anti-IgE therapy for a subgroup of allergic asthma patients with severe disease and recurrent exacerbations. Besides issues regarding adherence to ICSs,<sup>1,2</sup> the dose-response curve at medium-to-high doses of ICSs is flat, meaning that because of little potential for clinical improvement, systemic effects of ICSs become of greater concern.<sup>3</sup> Hence, there remains an unmet therapeutic need for asthmatic patients who fail to achieve symptom control on ICSs alone, and multiple treatment approaches targeting the underlying inflammation are currently under evaluation.

Allergy is a predominant risk factor for developing asthma, and the pathogenesis is largely promoted by T<sub>H</sub>2 cells found in the airways of asthmatic patients. Upregulation of T<sub>H</sub>2-mediated immune responses provokes an increase in IgE levels and secretion of T<sub>H</sub>2 cytokines, which inhibit the differentiation of counterbalancing T<sub>H</sub>1 cells. Moreover, cytokines secreted by T<sub>H</sub>2 cells can directly be linked to the underlying allergic inflammation (eg, IL-5 and eotaxin)<sup>4</sup> or to excessive mucus production in airways (IL-13). Allergen-specific immunotherapy (SIT) aims at therapeutically interfering with T<sub>H</sub>2 responses. However, SIT is limited to certain allergens, the clinical benefit in asthma is modest, and SIT is contraindicated in those patients suffering from severe asthma.<sup>5,6</sup> An immune-modifying treatment without administration of allergen-redirecting allergic T<sub>H</sub>2 responses to a more balanced mixed T<sub>H</sub>1/T<sub>H</sub>2 response may therefore be an attractive addition to conventional asthma therapies. Such an immunological response may be achieved by mimicking infections with natural pathogens.<sup>7</sup> Stimulating Toll-like receptors that recognize pathogen-associated molecular patterns may be one way to achieve this goal. To this end, a virus-like nanoparticle called QbG10 (bacteriophage Qbeta-derived virus-like particle with CpG-motif G10 inside) has been developed that exhibits many properties of infectious agents without the ability to replicate and cause disease. QbG10 consists of a protein shell derived from the bacteriophage Qbeta. It is filled with the DNA oligomer G10 (bacterial

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**Abbreviations used**

|        |                                                                           |
|--------|---------------------------------------------------------------------------|
| ACQ:   | Asthma Control Questionnaire                                              |
| AEs:   | Adverse events                                                            |
| ASMS:  | Average daily (asthma) symptom and medication score                       |
| BDP:   | Beclomethasone dipropionate                                               |
| CpG:   | Cytosine linked to a guanine by a phosphate bond                          |
| FENO:  | Exhaled nitric oxide                                                      |
| G10:   | Bacterial oligonucleotide with CpG motif                                  |
| ICSs:  | Inhaled corticosteroids                                                   |
| pDCs:  | Plasmacytoid dendritic cells                                              |
| QbG10: | Bacteriophage Qbeta-derived virus-like particle with CpG-motif G10 inside |
| SIT:   | Allergen-specific immunotherapy                                           |
| TLR-9: | Toll-like receptor 9                                                      |

oligonucleotide with CpG motif), which is rich in nonmethylated CG motifs (cytosines linked to a guanine by a phosphate bond [CpGs])<sup>8</sup> and is a potent ligand for Toll-like receptor 9 (TLR-9).<sup>9</sup> In contrast to chemically stabilized B-type CpGs, which have previously been used in the clinic,<sup>5,10-15</sup> G10 is an A-type CpG and induces production of IFN- $\alpha$  by plasmacytoid dendritic cells (pDCs) rather than IL-12.<sup>16</sup> Packaging of G10 into the protein shell of the virus-like particle protects the DNA from degradation and because of nanomolecular size allows efficient targeting of pDCs *in vivo*.<sup>17</sup> Interestingly, in humans but not in mice, IFN- $\alpha$  is a potent inhibitor of existing T<sub>H</sub>2 responses by inducing degradation of the transcription factor GATA-3.<sup>18</sup> In addition, TLR-9-mediated activation of pDCs has been shown to induce regulatory T cells, which may also be able to halt ongoing T<sub>H</sub>2 responses.<sup>19</sup> Findings from trials in allergic diseases indicate that the immunotherapeutic QbG10 results in clinical benefits when used in combination with specific allergen extracts<sup>20</sup> or alone.<sup>21</sup>

To assess the efficacy of QbG10 in patients with mild-to-moderate persistent allergic asthma (corresponding to treatment steps 3 and 4 according to Global Initiative of Asthma 2010), we performed a proof-of-concept clinical trial in patients well controlled on ICSs alone incorporating a controlled steroid withdrawal period to study the effect of QbG10 treatment on patient-reported and objective clinical outcome measures. Further objectives were safety and tolerability of QbG10.

**METHODS****Patient population and trial design**

Sixty-three patients whose allergic asthma symptoms required long-term treatment with ICS doses of 500  $\mu$ g or more of beclomethasone dipropionate (BDP) equivalent were enrolled in this double-blind, parallel-group trial at 5 German study sites. Randomized patients were allocated with sequentially numbered patient boxes (computer-generated randomization list with a block size of 4) to receive either 7 injections of 0.9 mg QbG10 ( $n = 33$ ) or placebo ( $n = 30$ ), respectively. The intention was to stay with the accumulating dose of 6.3 mg of QbG10 close to the previous clinical trial with QbG10.<sup>21</sup> The trial design is shown in Fig 1, giving details about the incorporated controlled steroid withdrawal and the assessments performed. In this article's Online Repository at [www.jacionline.org](http://www.jacionline.org), a detailed description of inclusion and exclusion criteria is given.

The trial was conducted in accordance with the ICH-GCP Guidelines (Directive CPMP/ICH/153/95) and the Declaration of Helsinki (1964) and the subsequent revisions. The protocol was reviewed and approved by local independent ethics committees and the concerned German national health authority (Paul-Ehrlich-Institute). All patients provided informed consent before any study procedures were conducted.

**Outcome measures**

Throughout the trial, patients completed daily an asthma diary on special paper (dotforms, PharmaForms GmbH, Schwerte, Germany) and with a digital pen with an integrated electronic data capture solution (AMEDON GmbH, Luebeck, Germany) facilitating compliance monitoring and ensuring adherence to its completion. The following items were documented: day- and nighttime asthma symptoms, morning peak expiratory flow measured with a portable peak flowmeter, puffs of relief medication salbutamol per 24 hours, and dosage of morning/evening inhalation of BDP (Cyclocaps BDP, single dose-dry-powder inhaler, PB Pharma GmbH, Meerbusch, Germany, 100, 200, or 400- $\mu$ g capsules).

The average daily (asthma) symptom and medication score (ASMS; score 0-7) calculated as the mean value of average (asthma) symptom score (score 0-8) and average (asthma) medication score (score 0-6) was determined throughout the trial. Average (asthma) symptom score was defined as the sum of the day- and nighttime asthma scores, each with a score range of 0 (no symptoms) to 4 (asthma very bad; bad night).<sup>22</sup> Average (asthma) medication score (score 0-6) was based on the number of puffs of salbutamol per 24 hours, using the same scoring as used in the Asthma Control Questionnaire (ACQ).<sup>23</sup> The ACQ (score 0-6) represents the mean of responses to 7 items including frequency and severity of daytime and nighttime symptoms, limitations of activities within the preceding 7 days, a measure for salbutamol use, and measured FEV<sub>1</sub>. This validated questionnaire was indispensable because of safety reasons during the steroid-reduction phase. Patients were subjected to next lower BDP levels only upon predefined safety criteria on asthma control and the investigator's clinical judgment. Furthermore, pulmonary function was measured by means of spirometry according to American Thoracic Society/European Respiratory Society guidelines. Additional exploratory outcomes were fraction of exhaled nitric oxide (FENO), measurement of blood eosinophils, and airway-hyperresponsiveness to methacholine challenge recorded as PC20.

With regard to immunogenicity, serum concentrations of total IgE, IgG, and anti-Qb IgG were assessed by using ELISA.

Safety and tolerability were assessed by recording the incidence and severity of adverse events (AEs), vital signs, standard laboratory, urinalysis, as well as physical and injection site examinations. Local reactions were captured in a patient diary for 3 days after injection and followed-up by the investigators.

**Power and sample size**

The sample size was determined on the reduction of endpoint FENO (ppb) from the phase of ICS stable dose to the phase of ICS reduced dose (maximum reduction was considered) based on already published data (Belda et al<sup>24</sup>). Assuming a difference of 12 ppb between QbG10 and placebo with an SD of 15, a necessary group size of 26 was estimated to achieve a power of 80% and an  $\alpha$  value of 0.05 for a 2-sided test. Taking potential dropouts during the study into consideration, a total sample size of 60 patients (30 per group) was deemed necessary.

**Statistical analysis**

**Intention-to-treat analysis.** The data from all randomized subjects were analyzed on an intention-to-treat basis. For intention-to-treat analyses, all randomized subjects were included who were exposed to the study drug and for whom at least 1 postbaseline documentation of efficacy was available. For all efficacy parameters, last observations were carried forward for dropouts. In the case of deviations to the planned steroid-reduction scheme, values of the last visit with steroid dose on plan were carried forward as well.

**Inferential methods.** For ACQ, ASMS, average (asthma) symptom score, and average (asthma) medication score as well as for FEV<sub>1</sub>, mixed-model repeated-measures analyses of variance were fitted. Respective baseline values, treatment, visit, and time by treatment interaction were included into the models as fixed effects. Time by treatment interaction was included to allow the calculation of contrasts for every visit. Patient was included as random effect. An autoregressive covariance structure was used for analysis. Contrasts were calculated to assess the change from baseline to each time point between treatment groups. For those contrasts, *P* values were adjusted between week 6 and 12 according to Bonferroni-Holm.

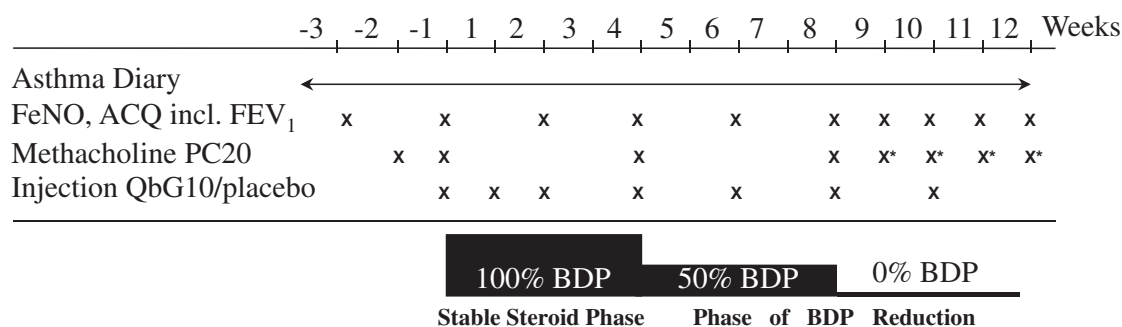


**FIG 1.** Outline of trial design and interventions performed. Prior to randomization, patients were converted to a run-in daily dosage of either 400  $\mu$ g (for patients on 500-800  $\mu$ g BDP equivalent) or 800  $\mu$ g of BDP (for patients on >800  $\mu$ g BDP equivalent) inhaled morning and evening. The first 3 subcutaneous injections of study drug were given weekly and then every second week on opposite upper arms, alternatively. During the first 4 weeks, patients were maintained on 100% BDP. Then, the dose was reduced to 50% for the following 4 weeks. At the end of week 8, BDP was completely withdrawn for the last 4 weeks, if clinically justifiable and following predefined safety criteria (see this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Site visits took place every 1 or 2 weeks. \*Airway hyperresponsiveness was assessed at the time of maximum BDP reduction or at week 12.

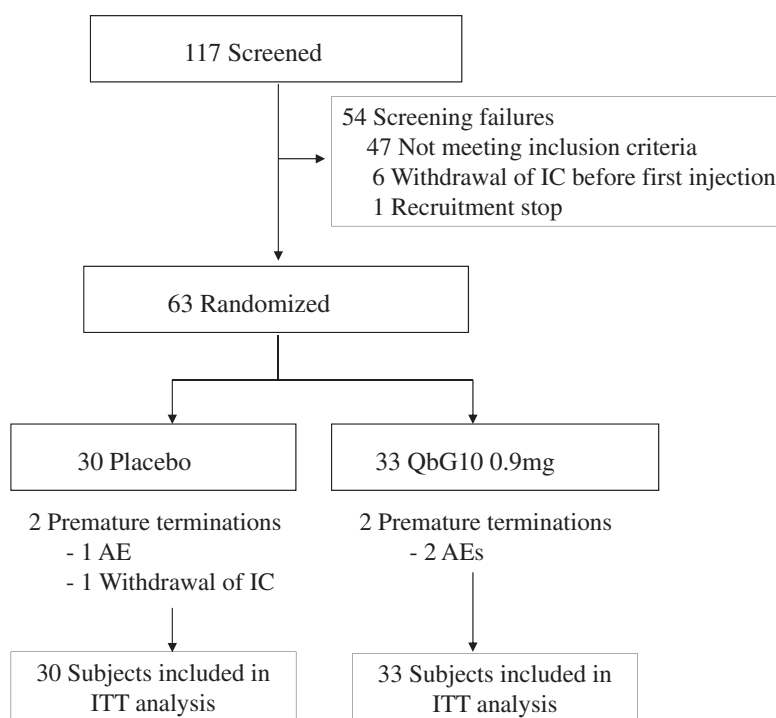


**FIG 2.** CONSORT diagram. ITT, Intention to treat.

For scores derived from the asthma diary, the average of daily values between 2 visits was calculated.

Results on ACQ were additionally evaluated using a responder determination. A score of less than 0.75 on the ACQ was defined as response. Response was analyzed by using generalized estimating equation methodology. Baseline values for response, treatment, time, and time by treatment interaction were included into this model. Time by treatment interaction was included to allow the calculation of contrasts for every visit. Patient was included as random effect. Contrasts were calculated to evaluate odds ratios of well-controlled asthma (response) at each time point between treatment groups. For those contrasts, *P* values were adjusted between week 6 and 12 according to Bonferroni-Holm.

Proc mixed and proc genmod of the software package SAS 9.2 (SAS Institute, Inc, Cary, NC) were used for data analysis.

## RESULTS

A total of 63 asthmatic patients were assigned to treatment: 33 to 0.9 mg of QbG10 and 30 to placebo. The first subject was randomized in May 2009 and the last one in January 2010. Two subjects of each treatment group discontinued the clinical trial prematurely (see Safety section and Fig 2). The treatment groups were well matched with respect to demographic and clinical characteristics (Tables I and II). Patients of both treatment groups followed the BDP reduction scheme in a comparable manner as displayed in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). Patients treated with 800 or 400  $\mu$ g of BDP at baseline followed the steroid reduction in a comparable manner as well.

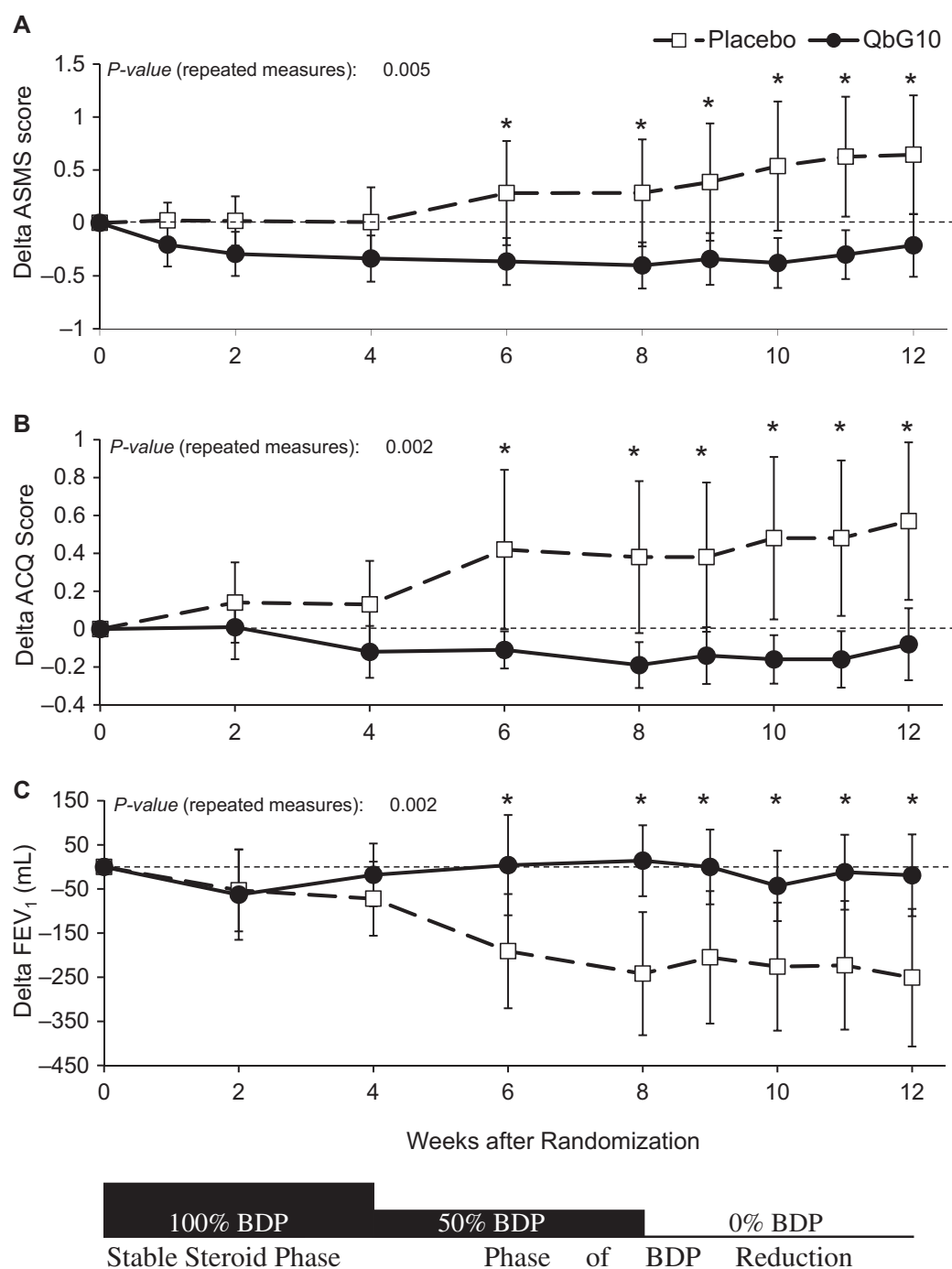


**FIG 3.** Change in ASMS and ACQ score and in  $FEV_1$  from baseline. **A**, Changes in ASMS given as delta scores between baseline and the respective visits. Absolute ASMS baseline and week 12 scores: Placebo 0.91 and 1.52; QbG10 1.22 and 1.01, respectively. **B**, Changes in reported scores on the 7-item-ACQ. Absolute baseline and week 12 scores: Placebo 0.92 and 1.48; QbG10 0.85 and 0.77, respectively. **C**, Changes in  $FEV_1$  of the 2 groups. Patients' morning  $FEV_1$  was measured always at the same time ( $\pm 1$  hour), and the best (highest)  $FEV_1$  per time point was documented. Absolute  $FEV_1$  baseline and week 12 scores: Placebo 2992 and 2741 mL; QbG10 3044 and 3025 mL, respectively.  $P$  values (repeated measures) were calculated by using SAS Proc mixed. Error bars represent 95% confidence intervals. \* $P < .05$  calculated for the mean difference between placebo and QbG10 at the respective week after randomization.

Patient-reported outcome measures improved significantly versus placebo from week 6 onward until study end. The ASMS worsened by 67% in the placebo group until study end, while it improved by 17% in patients treated with QbG10 (Fig 3,

A,  $P = .001$ ). The differences in ASMS were a result of significant changes in both, use of salbutamol and the symptom scores (Fig 4). Asthma control as assessed with the mean ACQ score improved in the patients treated with QbG10 (change from 0.85 to

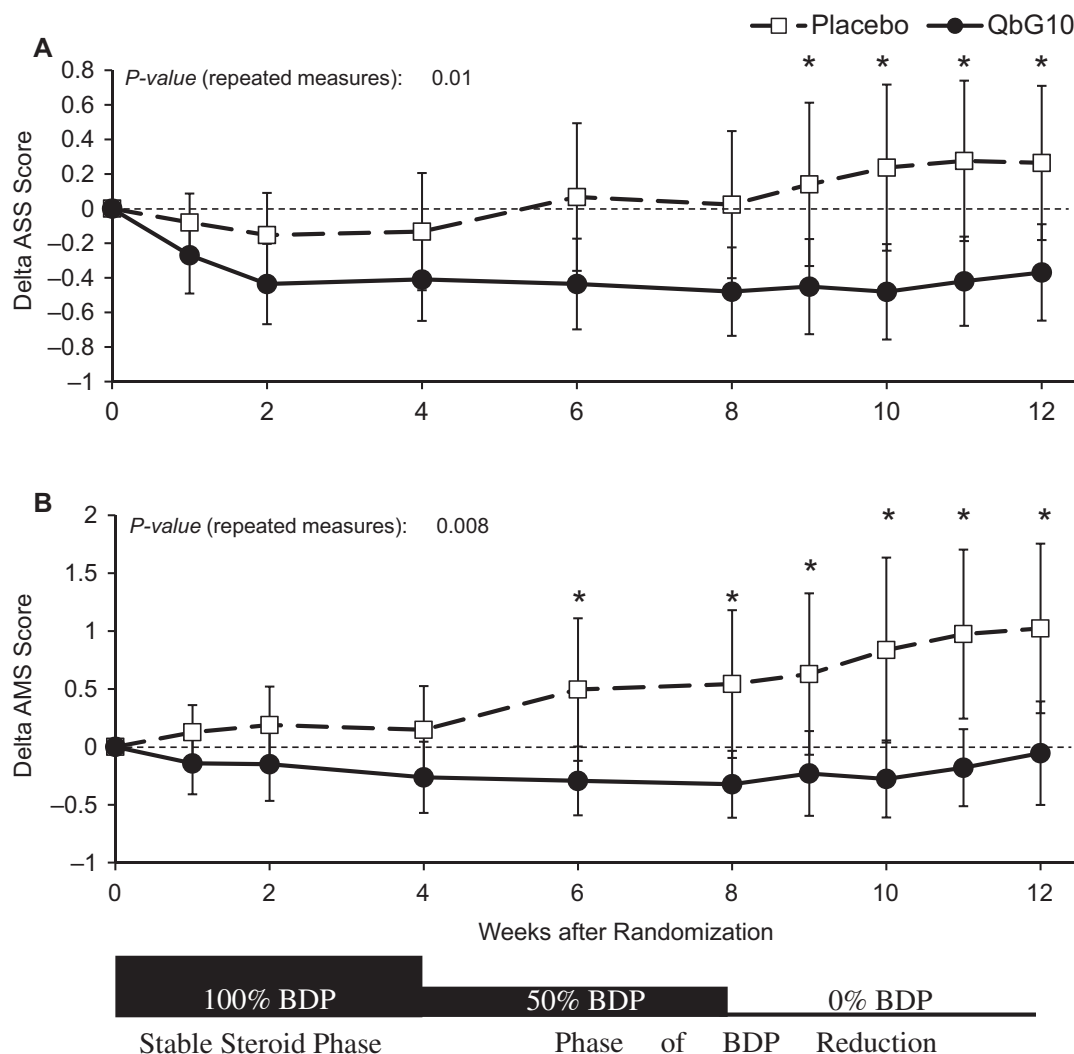


**FIG 4.** Change in average (asthma) symptom score (ASS) and average (asthma) medication score (AMS) from baseline. Changes in ASS (**A**) and AMS (**B**) determined with the patients' daily recordings in the asthma diaries are presented as delta scores between baseline and the respective visits. Both scores were calculated as the average between 2 visits.  $P$  values (repeated measures) were calculated by using SAS Proc mixed. Error bars represent 95% confidence intervals. \* $P < .05$  calculated for the mean difference between placebo and QbG10 at the respective week after randomization. Absolute ASS baseline and week 12 scores: Placebo 0.91 and 1.18; QbG10 1.11 and 0.74, respectively. Absolute AMS baseline and week 12 scores: Placebo 0.91 and 1.86; QbG10 1.34 and 1.29, respectively.

0.77 points,  $-9\%$ ). In contrast, the mean ACQ score increased from 0.92 to 1.48 by 0.56 points ( $+61\%$ ) in the placebo group (Fig 3, B,  $P = .0007$ ), resulting in a difference of 0.64 points between the 2 treatment groups at study end, exceeding the threshold of a clinically important difference of 0.5 points.<sup>25</sup> Indeed, while at enrolment, 42% and 40% of the patients had fully controlled asthma (defined ACQ scores  $\leq 0.75$ ) in the active and placebo group, respectively, 67% of QbG10-treated patients had their asthma controlled at study end, in contrast to the 33% in the placebo group ( $P = .0008$ , Fig 5). From week 8 onward until study end, the percentage of patients with fully controlled asthma was significantly higher on QbG10 treatment than on placebo.

Objective clinical measurements demonstrated that on QbG10 treatment FEV<sub>1</sub> remained unchanged, with a decrease of only 19 mL ( $-0.6\%$ ), in contrast to placebo patients who showed worsening of lung function by 251 mL until study end ( $-8\%$ ,

$P = .002$ ) during the BDP reduction phase (Fig 3, C). Subanalyses with patients treated with 400 or 800  $\mu\text{g}$  of BDP at baseline and with mild versus moderate persistent asthma showed no differences in results on the clinical outcome measures.

Eosinophils in peripheral blood over the 2 reduction phases were significantly lower in QbG10 than in placebo patients ( $P = .01$ , repeated measures, data not shown). Measurement on FENO showed a trend in favor of QbG10 although not statistically significant (change from baseline placebo  $+15.4$  ppb vs QbG10  $+10.0$  ppb;  $P = .166$ , repeated measures). Together, these parameters may be suggestive of an anti-inflammatory effect of QbG10 treatment.

Self-measured peak expiratory flow decreased during BDP reduction and withdrawal, but the difference between placebo and QbG10 patients was not statistically significant. Improvement in airway hyperreactivity, assessed as mean PC20, was numerically

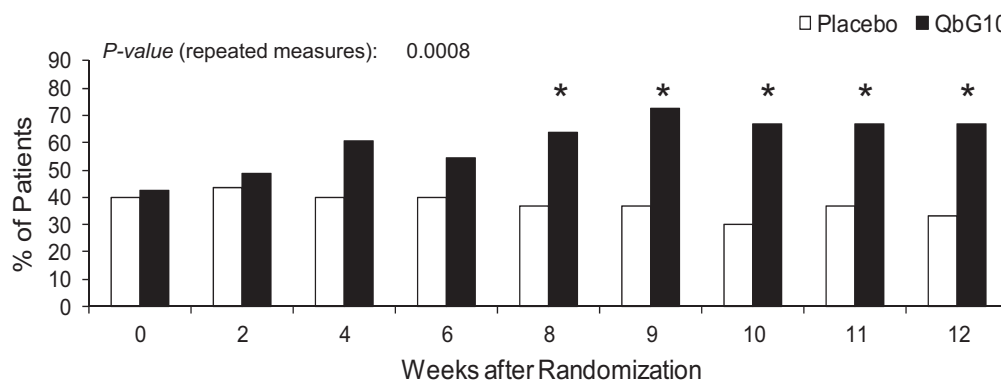


**FIG 5.** Percentage of patients with well-controlled asthma. The bars represent the percentage of patients with a 7-item-ACQ score of  $\leq 0.75$  points. *P* value (repeated measures) was calculated by using the Proc Genmod procedure. \**P* < .05 calculated for the mean difference between placebo and QbG10 at the respective week after randomization.

**TABLE I.** Demographics at screening

| Characteristics  | Placebo (n = 30) | QbG10 (n = 33) | <i>P</i> value |
|------------------|------------------|----------------|----------------|
| Sex, n (%)       |                  |                | .56*           |
| Male             | 14 (47)          | 13 (39)        |                |
| Female           | 16 (53)          | 20 (61)        |                |
| Age (y)          |                  |                | .77            |
| Mean†            | 43.8 ± 12.5      | 42.8 ± 13.8    |                |
| Range            | 18-65            | 19-69          |                |
| Body mass index† | 26.6 ± 4.6       | 26.0 ± 4.1     | .60            |

*P* values are based on *t* tests or on \*Pearson  $\chi^2$  tests.

†Values are means ± SD.

larger in QbG10-treated than in placebo-treated patients, however, not to a statistically significant extent.

Total IgE and IgG levels were measured 3 times during the study and remained largely unchanged in both treatment arms. Qb-specific IgG was not measurable in placebo patients, while it was clearly induced on QbG10 treatment.

## Safety

Thirty-five subjects (56%; placebo *n* = 17 and QbG10 *n* = 18) experienced a total of 109 AEs: 35 events (32%) occurred in the placebo group, whereas 74 events (68%) were reported in the QbG10 group. In both treatment groups, more than half of the AEs were of mild intensity, followed by moderate AEs and a small number of severe AEs, which are described in connection with premature discontinuations. Table III shows all AEs that occurred in more than 1 patient. A total of 51% of all AEs were of suspected relationship to the study drug, with a vast majority occurring in patients treated with QbG10, which was due to the higher occurrence of injection site reactions (injection site erythema, pruritus, and swelling). Two events of headache in QbG10 patients were the only systemic reactions rated as suspected AE occurring in more than 1 patient.

Local injection site reactions were generally most pronounced 1 to 2 days after injection and rapidly declined thereafter. Maximal reactions occurred after the third injection (the mean diameter for erythema was  $7.2 \pm 5.8$  cm, for swelling  $4.3 \pm 5.5$  cm, and for induration  $3.4 \pm 4.9$  cm, respectively) and diminished after each following injection. Moreover, maximal severity of two thirds of the documented injection site reactions was judged as mild by the patients.

**TABLE II.** Clinical characteristics

| Characteristics                          | Placebo (n = 30) | QbG10 (n = 33) | <i>P</i> value |
|------------------------------------------|------------------|----------------|----------------|
| Asthma status (GINA 2007), n (%)         |                  |                | .98*           |
| Mild persistent†                         | 21 (70)          | 23 (70)        |                |
| Moderate persistent                      | 9 (30)           | 10 (30)        |                |
| Asthma duration (y)                      |                  |                | .05            |
| Mean‡                                    | 19.5 ± 13.7      | 13.5 ± 10.4    |                |
| Range                                    | 1-50             | 1-45           |                |
| Allergen testing (prick positive), n (%) |                  |                | .68*           |
| Seasonal allergy                         | 6 (20)           | 6 (18)         |                |
| Perennial allergy                        | 11 (37)          | 11 (33)        |                |
| Seasonal and perennial allergy           | 13 (43)          | 16 (49)        |                |
| Allergic rhinitis, n (%)                 | 23 (77)          | 27 (82)        | .61*           |
| Atopic dermatitis, n (%)                 | 1 (3)            | 4 (12)         | .20*           |
| IgE (U/mL)                               |                  |                | .58            |
| Geometric mean                           | 183              | 154            |                |
| Range                                    | 16-3697          | 100-3457       |                |
| Asthma symptom score‡                    |                  |                |                |
| Daytime                                  | 0.57 ± 0.50      | 0.60 ± 0.60    | .82            |
| Nighttime                                | 0.37 ± 0.46      | 0.53 ± 0.36    | .2             |
| Asthma medication score‡                 | 0.91 ± 1.19      | 1.34 ± 1.73    | .25            |
| ASMS‡                                    | 0.91 ± 0.90      | 1.22 ± 1.19    | .25            |
| 7-Item ACQ‡                              | 0.92 ± 0.44      | 0.85 ± 0.50    | .58            |
| BDP starting dose, n (%)                 |                  |                | .40*           |
| 800 µg                                   | 15 (50)          | 20 (61)        |                |
| 400 µg                                   | 15 (50)          | 13 (39)        |                |
| Mean (µg)‡                               | 588 ± 201        | 631 ± 201      | .41            |
| Morning PEF (mL)‡                        | 423 ± 127        | 435 ± 101      | .67            |
| FEV <sub>1</sub> ‡                       |                  |                |                |
| Mean (mL)                                | 2992 ± 880       | 3044 ± 657     | .79            |
| Percentage of predicted value            | 89.3 ± 14.0      | 92.5 ± 16.2    | .40            |
| Methacholine PC <sub>20</sub> (mg/mL)‡   | 0.7 ± 1.3        | 0.8 ± 1.1      | .69            |
| Eosinophil count in blood (/nL)          | 0.21 ± 0.14      | 0.20 ± 0.16    | .73            |
| FENO (parts per billion)§                | 21.3 ± 11.4      | 20.5 ± 10.7    | .78            |

GINA, Global Initiative of Asthma; PEF, peak expiratory flow.

Note. Baseline for clinical characteristics was the beginning of the stable steroid phase. *P* values are based on *t* tests or on \*Pearson  $\chi^2$  tests.

†The asthma status of 1 subject was documented as intermittent, although treated with long-term inhaled steroids.

‡Values are means ± SD.

§FENO was measured at a flow of 50 mL/s.

There were 2 unrelated serious AEs, both under QbG10 treatment. One patient was hospitalized because of an inguinal hernia, and another patient was diagnosed with breast cancer.

**TABLE III.** All AEs with an incidence of more than 1 patient

| Preferred term            | Placebo |    | QbG10 |    | P value |
|---------------------------|---------|----|-------|----|---------|
|                           | n       | %  | n     | %  |         |
| Injection site erythema   |         |    | 8     | 24 | .005    |
| Injection site pruritus   |         |    | 4     | 12 | .11     |
| Injection site swelling   |         |    | 4     | 12 | .11     |
| Nasopharyngitis           | 6       | 20 | 4     | 12 | .50     |
| Asthma*                   | 3       | 10 | 2     | 6  | .66     |
| Headache                  | 1       | 3  | 2     | 6  | 1.00    |
| Injection site induration |         |    | 2     | 6  | .49     |
| Injection site edema      |         |    | 2     | 6  | .49     |
| Injection site pain       |         |    | 2     | 6  | .49     |
| Rhinitis                  |         |    | 2     | 6  | .49     |
| Rhinorrhea                |         |    | 2     | 6  | .49     |
| Dyspnea                   | 3       | 10 |       |    | .10     |
| Oropharyngeal pain        | 2       | 7  |       |    | .22     |

P values were calculated by using Fisher exact test.

Local reactions captured in the patient diary that persisted until the following visit or required medical intervention were documented as AEs. For details on injection site reactions, see this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). Empty cells = 0.

\*All 5 AEs of asthma were not of suspected relationship to the study drug and were rated as mild or moderate.

There were 4 premature discontinuations during the trial as displayed in the CONSORT diagram (Fig 2). One subject treated with placebo withdrew informed consent for personal reasons, and another patient on placebo discontinued because of asthma exacerbation. Two subjects on QbG10 discontinued because of AEs, which were rated by the investigator as of possible causal relationship to the study drug: one subject experienced moderate flu-like symptoms 6 days after the third injection; the other subject was withdrawn by the investigator at the time of the planned third injection with severe symptoms of emotional distress and headache, moderate vertigo, mild lip cyanosis, and mild injection site swelling; other reported symptoms such as fever, tachycardia, and tongue swelling were not present at the on-site visit and could therefore not be verified by the investigator. Three days after the on-site visit, the patient confirmed at a telephone call that all symptoms had resolved completely.

Safety blood analyses (hematology, clinical chemistry) were performed at 3 occasions. Mean values of the assessed parameters were all within normal ranges. Clinically significant abnormalities were detected neither at urinalysis and physical examination nor at assessments of vital signs and electrocardiogram performed with all patients pre- and posttreatment.

## DISCUSSION

This first prospective proof-of-concept study in a limited number of patients with allergic asthma shows clinical activity of repeated subcutaneous injections of QbG10, a TLR-9 agonist. Restoring  $T_H2/T_H1$  imbalances in the immune response of asthmatic patients has been considered a therapeutic approach for many years,<sup>26</sup> which led to the development of monoclonal antibodies neutralizing proinflammatory  $T_H2$ -type molecules, for example, anti-IgE, -IL-4, -IL-5, -IL-9, and -IL-13, with some of them demonstrating clinical efficacy in asthma.<sup>27-30</sup> Alternatively, stimulation of  $T_H1$  pathways also has the potential to counterbalance some of the inflammatory features in asthma. Attempts have been made by direct administration of  $T_H1$  cytokines to asthmatic subjects, with inconsistent results,<sup>31,32</sup> or by conjugating

$T_H1$  inducers (immunostimulatory sequences) to specific allergen immunotherapy, where some long-term benefits could be observed.<sup>5</sup> Moreover, it was recently demonstrated that molecules with immunostimulatory sequences containing a CpG motif produce clinical benefits in atopic, allergic disease even without concomitant SIT, indicating a broader anti-inflammatory potency of this approach.<sup>21</sup> Our trial confirms findings from prior studies, and for the first time extends evidence of the feasibility of  $T_H1$  stimulation as a therapeutic approach to an asthmatic population dependent on ICSs.

In our clinical trial, we demonstrated that add-on QbG10 treatment allowed considerable reduction or withdrawal of ICSs while asthma symptoms remained stable or even improved. Indeed, while 40% (placebo) and 42% (QbG10) of the patients showed fully controlled asthma (ACQ score  $\leq 0.75$ ) at study entry, this number increased to 67% in the QbG10-treated population, while it decreased to 33% in the placebo group. Overall, QbG10 had a significant impact on patient-reported asthma symptoms, asthma control, and lung function. Because no allergen was coadministered with QbG10, the drug itself contributed to preserved asthma control during steroid withdrawal.

The clinical relevance of the magnitude of observed effects in our study needs to be set into a broader perspective, but is to a certain extent difficult to comprehend, because we used a steroid withdrawal design. Nevertheless, the major advantage of this approach lies in the possibility to induce a flare up of asthma symptoms and inflammation under controlled circumstances.<sup>33</sup> When administered to a comparable asthma population over 12 weeks, once-daily oral montelukast failed to prevent the fall in  $FEV_1$  during steroid withdrawal.<sup>22</sup> In this study,  $FEV_1$  fell by  $-227$  mL under montelukast compared with  $-194$  mL under placebo, the difference being nonsignificant. This compares with a decrease of  $-19$  mL with QbG10 and  $-251$  mL with placebo in our study. Furthermore, in the study by Kanniess et al,<sup>22</sup> montelukast had no effect on airway hyperresponsiveness, sputum eosinophils, and FENO, while, in line with our findings with QbG10, asthma symptoms and rescue salbutamol use were positively affected by montelukast. Borish et al<sup>34</sup> evaluated the effect of a 12-week-treatment with an inhaled soluble IL-4 receptor in asthmatic patients dependent on ICS, following steroid discontinuation at randomization. In their trial,  $FEV_1$  fell by  $-0.4$  L under placebo, while there was a significantly smaller decrease with active drug ( $-0.1$  L,  $P = .05$ ). Asthma symptoms, measured by a score comparable to ours, increased in the placebo group by  $+1.4$  points on average, while these remained stable with active treatment ( $+0.1$  points on average,  $P = .07$ ). Finally, during a phase III omalizumab trial also incorporating a steroid-reduction phase of 14 weeks,<sup>35</sup> significant differences for omalizumab versus placebo were observed for day- and nighttime asthma symptoms (no data on ACQ) and rescue medication use, although because of different symptom scores used, it is difficult to compare these effects with our results. In this trial, significant differences for  $FEV_1$  during the steroid-reduction phase with omalizumab versus placebo were reported, although these differences were driven by initial improvements by add-on omalizumab rather than a prevention of  $FEV_1$  decline during the steroid-reduction phase.<sup>35</sup> We therefore believe that despite limitations and differences of our study design and methodology used, the results observed for QbG10 here are encouraging from a clinical perspective and comparable to known effects of other investigational or approved anti-inflammatory asthma treatments.

An interesting observation in our trial was that the ASMS as well as ACQ scores improved to a statistically significant extent after QbG10 injection already during the first weeks with stable ICS doses. This may be indicative for a fast onset of action of the QbG10 treatment and demonstrates the therapeutic potential of QbG10 on top of medium dosage of steroids. It has important implications for the design of future studies, because it may allow improvement of more severe, uncontrolled asthmatic patients treated with ICSs with add-on QbG10 treatment, comparable to the role of anti-IgE therapy in current guidelines.<sup>36</sup> Interestingly, Roussel et al<sup>37</sup> recently reported suppressed activity of transcription factor 3 expression, a negative regulator of inflammation, in biopsies from severe, refractory asthma patients. This could reflect an unwanted effect of steroid treatment and was reversed by a TLR-9 agonist *in vitro*. Moreover, activation of the IgE receptor on human pDCs by circulating IgE leads to impaired TLR-9 immune responses in allergic subjects inversely correlated to high-affinity IgE receptor expression.<sup>38</sup> Thus, therapeutic effects of TLR-9 agonism by QbG10 may be complementary to and potentially synergistic with established treatments for chronic, severe allergic asthma.

Although the exact mechanism of action of QbG10 has not been elucidated in this study, it is likely that QbG10 exhibited anti-inflammatory properties because (1) Qb nanoparticles are efficiently taken up by pDCs *in vivo*,<sup>17,39</sup> (2) TLR-9-mediated activation of pDCs results in the induction of regulatory T cells<sup>19,40</sup> as well as production of T<sub>H</sub>2 downmodulating IFNs,<sup>18</sup> (3) pDCs may improve symptoms of asthma in preclinical mouse models,<sup>41</sup> and (4) QbG10 reduces blood eosinophils in this study, while FENO, despite lower mean values on active treatment, was not significantly reduced. The difficulties to better define the mechanism of action in humans is in line with findings from other studies, possibly owing to a lack of reliable and more specific surrogate or biomarkers that can be applied in clinical trials in this patient population.<sup>5</sup>

QbG10 has shown a good safety profile. No related serious AE occurred, and study discontinuation due to an AE occurred in 3 patients only. In fact, with respect to harms, most prominent were local AEs of mild to moderate intensity, which did not constitute a safety issue. No relevant changes in antinuclear antibodies could be observed. This confirms earlier results of a phase IIb study where QbG10 was tested in 299 individuals suffering from perennial allergic rhinoconjunctivitis (0.5 mg, 1 mg, and placebo).<sup>21</sup> Patients were followed up for a year, and no late AEs (such as development of antinuclear antibodies) occurred (unpublished data). Because the G10 DNA is packaged inside virus-like nanoparticles and therefore is “hidden” for B cells, it was also not expected that antinuclear antibodies would be induced. However, because QbG10 acts as an immune modulator, it will be important to exclude undue immunological reactions in larger patient cohorts.

Several questions remain to be addressed. Most important, no dose response has been established (the actually used dose was chosen following the results of the above-mentioned rhinoconjunctivitis study<sup>21</sup>). In addition, the duration of treatment effect has not been investigated. In the present study, no follow-up period was involved. Instead, patients were returned to their pre-study asthma medication immediately at the end of the last visit to ensure safety of research subjects in this steroid-reduction setting, regardless of the patients' actual level of asthma control and study treatment. Therefore, any leveling-off of the stabilizing effect of QbG10 could not be investigated. While the proposed mechanism

of action is compatible with a relatively long duration, additional studies need to address these points.

Finally, efficacy results of QbG10 were derived from allergic asthma patients only. Because the Global Initiative of Asthma 2011 report acknowledges that while a positive skin prick test result does not necessarily mean that the disease is “allergic” in nature, it is emphasized that a relevant exposure and its relation to symptoms must be confirmed by patient history. The criteria used in our protocol to define asthma as “allergic” were in line with Global Initiative of Asthma recommendations, although exceptional patients with sole seasonal allergy were at least partially studied outside their respective allergen season. Thus, it may be debatable whether these subjects should be labeled as suffering from “allergic asthma,” whereas the term “atopic” asthma may be more accurate.<sup>42</sup> The main reason for the inclusion of allergic or atopic subjects was to keep the atopy status of the study population consistent with prior studies with QbG10.<sup>21</sup> However, because of evidence indicating similar inflammatory patterns of atopic and nonatopic asthma,<sup>43</sup> and the importance of immune pathways driven by T<sub>H</sub>2-type cells for the pathobiology of severe asthma independent of atopy,<sup>44</sup> it is possible that QbG10 treatment may also be effective in a nonatopic asthma population.

In conclusion, the current study provided preliminary evidence that 7 injections of QbG10 may contribute to continued asthma control. QbG10 injections resulted in an elevated incidence of local injection site reactions compared with placebo. It is suggested that the patient-reported and clinical/physiological improvements are associated with effects on inflammation. Further studies in larger patient populations are required to confirm and extend these findings.

**Clinical implications: The current study demonstrates in allergic asthma patients that QbG10 had a significant impact on asthma control, patient-reported disease symptoms, use of relief medication, and lung function despite inhaled steroid withdrawal.**

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**Table E1: Inclusion and Exclusion Criteria**

| Inclusion Criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"><li>• Able and willing to provide written informed consent</li><li>• Able and willing to complete all study protocol requirements</li><li>• 18 to 65 years of age</li><li>• History of persistent asthma according to GINA 2007 guidelines whose symptoms requiring long-term treatment with ICS</li><li>• Positive skin prick test to at least one aero-allergen and anamnesticly confirmed to cause asthma symptoms. In case of doubt the allergic nature of the asthma must be confirmed by nasal provocation with the suspected allergen.</li><li>• FEV<sub>1</sub> &gt;60% of predicted value</li><li>• FEV<sub>1</sub> improvement ≥12% after inhaled beta2-agonist (400 µg salbutamol) at screening</li><li>• Airway responsiveness to methacholine (PC20 &lt;8 mg/mL) at screening</li><li>• On stable therapy with ICS equivalent to a dose of ≥500 µg BDP daily (short-acting beta2-agonists (salbutamol) as needed may be used throughout the whole study)</li><li>• Clinically stable asthma or sufficiently controlled asthma at screening and prior to treatment phase as indicated by &lt;1.5 mean points in the Asthma Control Questionnaire (ACQ)</li><li>• Female participants must meet one of the following criteria:<ul style="list-style-type: none"><li>-No reproductive potential due to menopause (one year without menses, in case of doubts serum FSH will be determined and must be &gt;30 U/L and estradiol must be &lt;54.7pg/mL), hysterectomy, bilateral oophorectomy, or tubal ligation</li><li>-Patient agrees to consistently practice an effective and accepted method of contraception throughout the duration of the study and for 1 additional month after the last immunization (hormone-based, or intrauterine device, or double barrier contraception, i.e. condom + diaphragm, condom or diaphragm + spermicidal gel or foam)</li></ul></li></ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Exclusion Criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <ul style="list-style-type: none"><li>• Use of <u>oral</u> corticosteroids within past 3 months</li><li>• Hospitalization for asthma exacerbation within past 6 months</li><li>• Uncontrolled asthma defined by ACQ ≥1.5 points at screening or prior to treatment phase</li><li>• Intermittent or seasonal asthma not requiring ICS treatment</li><li>• Regular use of long-acting beta2-agonists (LABA). All LABAs should be discontinued after informed consent and at least 48h prior to the screening procedure to ensure appropriate washout.</li><li>• Smokers or quitters with a smoking history of &gt;10 packyears (one package per day for 10 years)</li><li>• Major surgery within 4 weeks prior to enrollment</li><li>• Presence or history of relevant cardiovascular, renal, pulmonary, endocrine, autoimmune, dermatological (in particular psoriasis), neurological and psychiatric (in particular depression) and ocular disease (in particular keratitis) as judged by the investigator. For psoriasis, family history should also be clean.</li><li>• Current diagnosis or history of malignancy; presence of suspicious lymphadenopathy or splenomegaly on physical examination</li><li>• Confirmed or suspected current infection with HIV, HBV, or HCV</li><li>• Presence of active infectious disease as judged by the investigator</li><li>• History of recurrent invasive streptococcal or staphylococcal infections, or known interleukin-1 receptor-associated kinase 4 (IRAK-4) deficiency</li><li>• Active autoimmune diseases</li><li>• Pregnancy (based on positive urine test at screening visit) or lactation</li><li>• Female planning to become pregnant during the study period</li><li>• Blood donation or loss of &gt; 400 mL within 8 weeks prior to inclusion</li><li>• Vaccination planned during study treatment period</li><li>• History of abuse of alcohol or other recreational drugs</li><li>• Lactose intolerance</li><li>• Any specific immunotherapy (SIT) planned during the whole study period or any former SIT within the last three years.</li><li>• Treatment with anti-IgE antibodies within past 6 months</li><li>• Use of any investigational drug within 30 days before enrolment, or planned use during the whole study period</li><li>• Previous participation in a clinical trial with a Qb-based vaccine</li><li>• Possible dependency of the patient on sponsor and/or investigator</li></ul> |

**Table E2: Immunogenicity**

|          |         |    | Total IgE<br>(kU/L) |         | Total IgG<br>(mg/dL) |          | Anti-Qb IgG<br>(ELISA titer*) |                 |
|----------|---------|----|---------------------|---------|----------------------|----------|-------------------------------|-----------------|
| N        |         |    | Geom.<br>mean       | 95% CI  | Geom.<br>mean        | 95% CI   | Geom.<br>mean                 | 95% CI          |
| Baseline | Placebo | 30 | 183                 | 113-298 | 984                  | 923-1048 | 22                            | 15-33           |
|          | QbG10   | 33 | 153                 | 100-237 | 989                  | 938-1042 | 50                            | 33-75           |
| Week 6   | Placebo | 29 | 192                 | 117-314 | 954                  | 892-1020 | 26                            | 18-37           |
|          | QbG10   | 31 | 149                 | 95-234  | 990                  | 932-1051 | 162'767                       | 133'071-199'089 |
| Week 12  | Placebo | 28 | 186                 | 113-306 | 949                  | 885-1017 | 26                            | 17-40           |
|          | QbG10   | 31 | 143                 | 91-225  | 980                  | 924-1039 | 303'882                       | 247'968-372'403 |

kU= kilo Units

\*As no reference standard of Q $\beta$ -specific human IgG is available, a quasi-quantitative ELISA was developed and antibody levels are reported as ELISA titers.

**Table E3: Injection Site Reactions**

| Preferred term (PT)       | Placebo |          |        | QbG10 |          |        |
|---------------------------|---------|----------|--------|-------|----------|--------|
|                           | mild    | moderate | severe | mild  | moderate | severe |
| Fatigue                   | 0       | 0        | 0      | 0     | 1        | 0      |
| Influenza like illness    | 0       | 0        | 0      | 0     | 1        | 0      |
| Injection site erythema   | 0       | 0        | 0      | 3     | 4        | 1*     |
| Injection site induration | 0       | 0        | 0      | 2     | 0        | 0      |
| Injection site oedema     | 0       | 0        | 0      | 2     | 0        | 0      |
| Injection site pain       | 0       | 0        | 0      | 2     | 0        | 0      |
| Injeciton site pruritus   | 0       | 0        | 0      | 3     | 1        | 0      |
| Injection site swelling   | 0       | 0        | 0      | 3     | 1        | 0      |
| Pyrexia                   | 0       | 0        | 0      | 0     | 1        | 0      |

The table is showing all AEs coded under general disorders and administration site conditions as primary system organ class (SOC) using MedDRA version 13.0 including their grading.

\*The only erythema that was rated as severe occurred in a patient on the day of the last (7<sup>th</sup>) injection and had a maximal diameter of 3 cm. The reaction was treated with Cetrizin tablets and resolved completely within 4 days.

## **Specific Safety Measures described in the Clinical Trial Protocol adhered to during the Steroid Reduction Phase**

To ensure safety of subjects during the steroid reduction phase, additional visits may be performed if deemed necessary to monitor for symptoms, lung function, and beta2-agonist use. After ICS reduction to 0 at Visit 8 (week 8), visits are scheduled for safety reasons on a weekly basis. In all patients the 7 days preceding the start of reduction will be used as individual baseline. For this period, mean daily symptom scores, mean morning PEF, and mean use of rescue SABA will be calculated. Moreover, clinic FEV<sub>1</sub> and ACQ score prior to steroid reduction will be used as baseline. With these baseline criteria, the following guidance in which case omitting the ICS reduction to 0 is issued.

On any of the consecutive visits after the ICS reduction to 50% of starting dose, no further reduction in ICS dose should be performed if:

- FEV<sub>1</sub> is reduced by more than 20% versus FEV<sub>1</sub> at Visit 6 (week 4).
- Morning PEF is reduced by 20% on two of three consecutive days in the diary compared to mean baseline PEF prior to ICS reduction (mean of 7 days prior to Visit 6, week 4).
- Rescue use of SABA has increased by >100% versus mean baseline usage (mean of 7 days prior to Visit 6, week 4) and exceeds more than 10 puffs/day (in absolute values).
- Nighttime awakening due to asthma in at least two of any three consecutive nights throughout the week before clinic visits.
- Doubling of the mean ACQ score and mean ACQ score  $\geq 1.5$ .

If none of the above criteria apply, the subject may proceed with last ICS reduction step to 0. However, if only one criterion applies, the investigator may judge upon his own discretion whether to continue with the ICS reduction. In all cases where two or more criteria apply, the subject must not undergo any further ICS reduction. The investigator decides in favor for the patient whether or not the ICS dose has to be increased. The patient may stay in the study as regular.

SABA= short acting beta2-agonist, i.e. salbutamol

### **Figure Legend E1: Change of mean Beclomethasone dipropionate (BDP) inhaled**

The curves represent the mean dose of inhaled BDP  $\pm$  standard error of the mean of changes from baseline. Imputation for missing data with the last observed value carried forward was adopted for four patients who discontinued prematurely: two QbG10 patients from week one and four and two placebo patients from week two and six, respectively. Analysis of repeated measures and mean differences between placebo and QbG10 between weeks 4 and 12 showed no statistical significance.

### **Figure Legend E2: Change in mean peak expiratory flow (PEF) from baseline**

PEF measurement was made by patients at home as a control measure for patients and investigators to identify situations of imminent exacerbation of asthma. Analysis of repeated measures and mean differences between placebo and QbG10 between weeks 4 and 12 showed no statistical significance. Error bars represent standard error of the mean.

### **Figure Legend E3: Change in mean PC20 from baseline**

The measurement of airway hyperresponsiveness to methacholine followed the American Thoracic Society (ATS) guideline (Crapo RO et al, Am J Respir Crit Care Med; 1999; 161:309-29). The curves represent the mean  $\pm$  standard error of the mean of change to baseline methacholine concentration. Analysis of repeated measures from week 4 to week 12 reports a p-value of 0.477.

### **Figure Legend E4: Change in mean FeNO from baseline**

FeNO was measured according to the guidelines of the American Thoracic Society (2005). The arithmetic mean of three measurements per patient was documented. Analysis of repeated measures and mean differences between placebo and QbG10 between weeks 4 and 12 showed no statistical significance ( $p=0.156$ ). Error bars represent standard error of the mean.

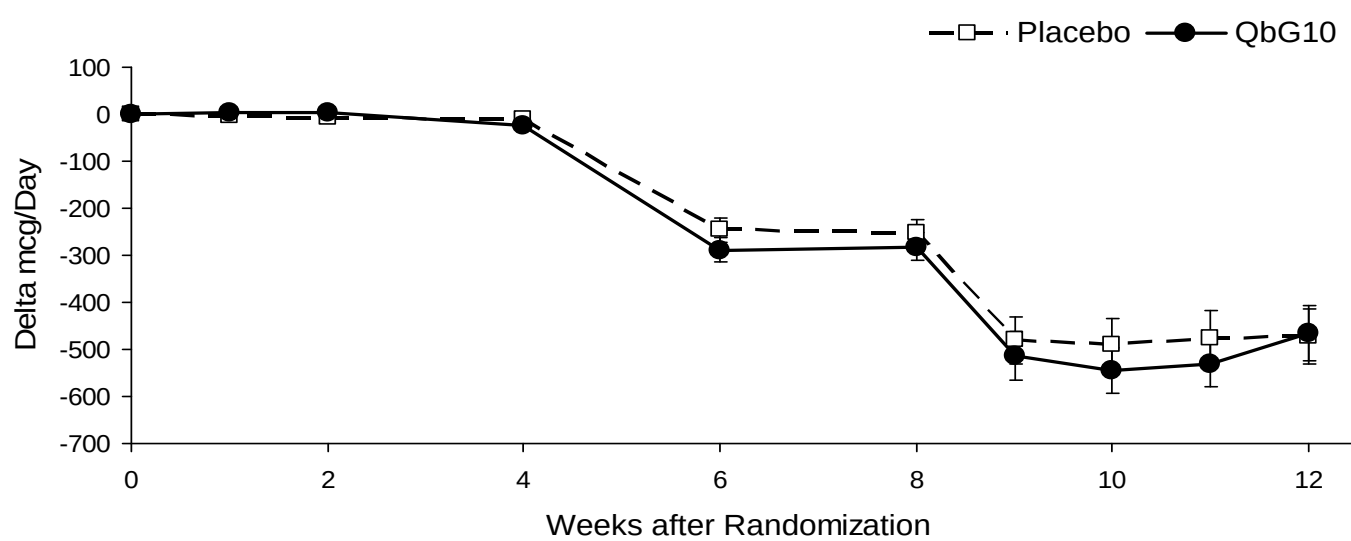


Figure E1

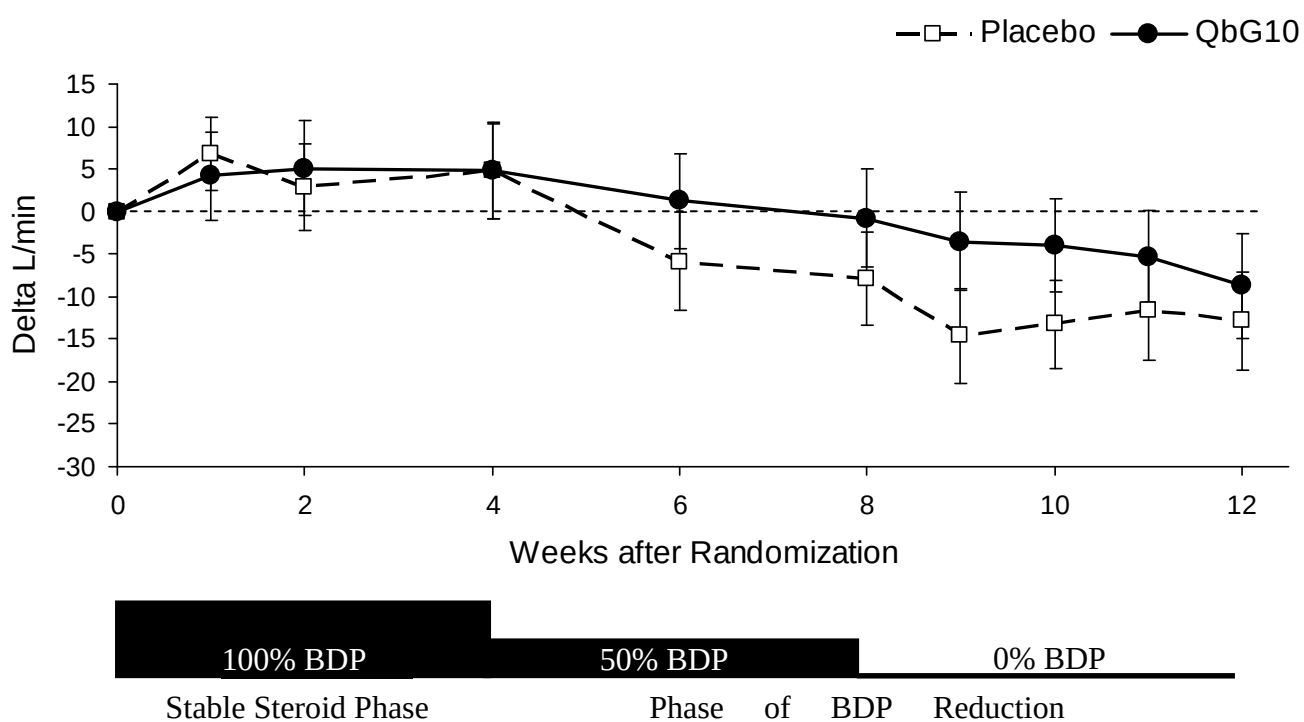


Figure E2

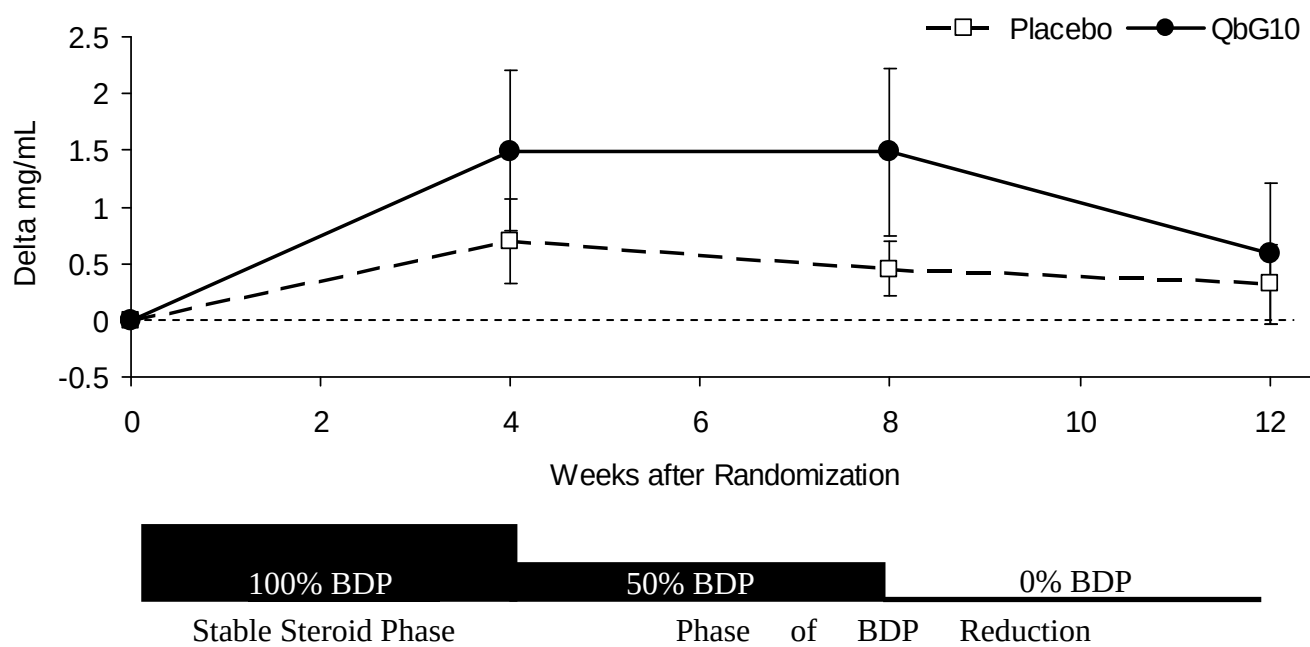


Figure E3

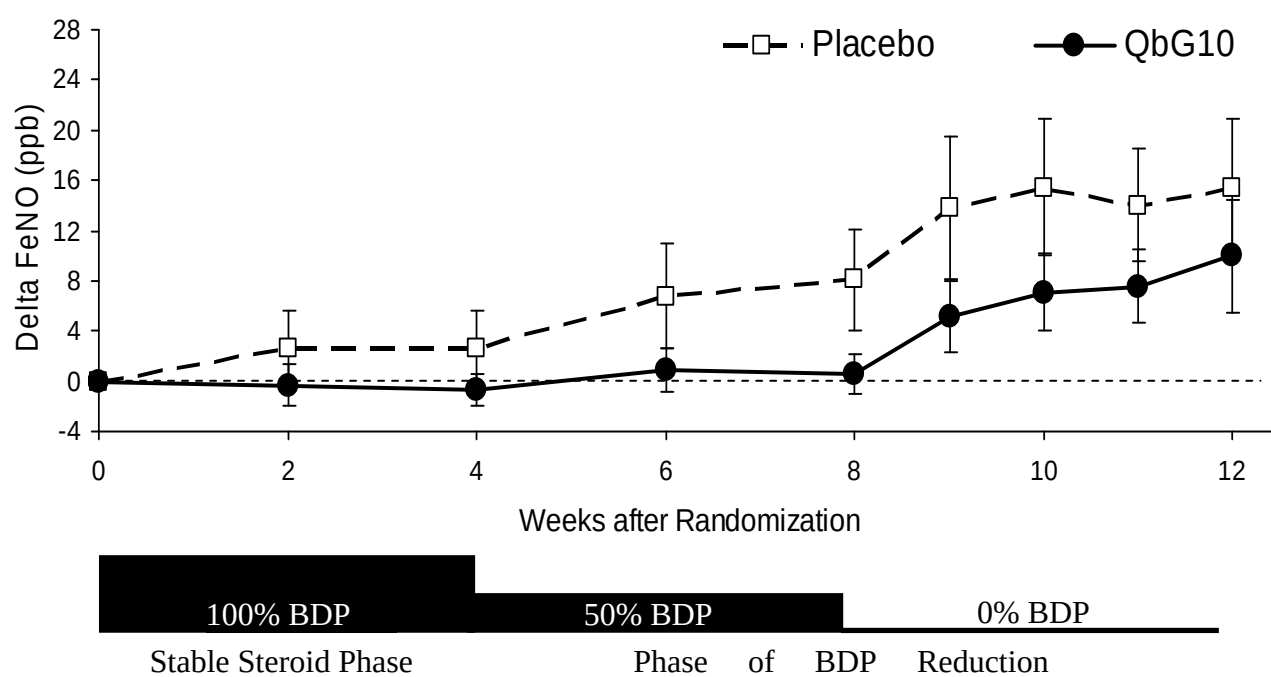


Figure E4