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

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Background: Allergen-specific T_H2 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_H1 response, and thereby suppresses the allergic T_H2 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_H1-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_1, 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_1 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;131:866-74.)

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,[1,2] 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.[3] 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_H2 cells found in the airways of asthmatic patients. Upregulation of T_H2-mediated immune responses provokes an increase in IgE levels and secretion of T_H2 cytokines, which inhibit the differentiation of counterbalancing T_H1 cells. Moreover, cytokines secreted by T_H2 cells can directly be linked to the underlying allergic inflammation (eg, IL-5 and eotaxin)[4] or to excessive mucus production in airways (IL-13). Allergen-specific immunotherapy (SIT) aims at therapeutically interfering with T_H2 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.[5,6] An immune-modifying treatment without administration of allergen-redirecting allergic T_H2 responses to a more balanced mixed T_H1/T_H2 response may therefore be an attractive addition to conventional asthma therapies. Such an immunological response may be achieved by mimicking infections with natural pathogens.[7] 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: Plasmocytoid 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])[8] and is a potent ligand for Toll-like receptor 9 (TLR-9).[9] In contrast to chemically stabilized B-type CpGs, which have previously been used in the clinic,[5,10-15] G10 is an A-type CpG and induces production of IFN-α by plasmocytoid dendritic cells (pDCs) rather than IL-12.[16] 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*.[17] Interestingly, in humans but not in mice, IFN-α is a potent inhibitor of existing T_H2 responses by inducing degradation of the transcription factor GATA-3.[18] 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_H2 responses.[19] Findings from trials in allergic diseases indicate that the immunotherapeutic QbG10 results in clinical benefits when used in combination with specific allergen extracts[20] or alone.[21]

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 μg 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.[21] 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, 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-μg 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).[22] 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).[23] 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_1. 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[24]). 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 α 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_1, 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.