

ORIGINAL ARTICLE

Assessment of clinical efficacy of CYT003-QbG10 in patients with allergic rhinoconjunctivitis: a phase IIb study

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Summary

Background Allergic symptoms are generally caused by exposure to substances to which people have become sensitized. Associated with this is an 'unbalanced' Th1/Th2 immune response with T cell responses skewed towards the production of Th2 cytokines, IL-4, 5, and 13 and high levels of IgE antibodies. Current immune modulating therapies require the use of allergens, carrying the risk to induce potentially severe allergic reactions.

Objective Goal of the present study was to assess the safety and efficacy of an allergen-free immune modulator in patients suffering from perennial allergy.

Methods In order to be protected from immediate degradation upon injection, a toll-like receptor 9 (TLR9) agonist was packaged into virus-like particles. These nanoparticles loaded with TLR9 ligands (CYT003-QbG10) were injected six times, at weekly intervals, into patients with house dust mite allergy in an attempt to ameliorate allergic symptoms by modifying the immune response towards allergens. Two different doses were compared against placebo in this double-blind, randomized phase IIb study ($n = 299$). Public trial registry: <http://clinicaltrials.gov> (NCT00800332).

Results The treatment was safe and generally well tolerated. Rhinoconjunctivitis symptoms were significantly lower in patients treated with high dose of CYT003-QbG10 as compared with placebo (scores 0.31 vs. 0.52, $P = 0.04$) based on a standardized average combined symptom and medication score. Furthermore, patients in the high dose group reported a significantly better quality of life score post-treatment than patients on placebo (scores 0.71 vs. 1.21, $P = 0.02$). The conjunctival provocation test revealed a median 10-fold increase in allergen tolerance in the high dose group while in the placebo group it remained unchanged. **Conclusion and Clinical Relevance** Treatment with high-dose CYT003-QbG10 improved disease symptoms and reduced medication use in allergic individuals thus providing first evidence for a new potential immunotherapeutic.

Keywords clinical trial, house dust mite allergy, immune modulator, toll-like receptor 9, virus-like particle

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Introduction

Specific immunotherapy (SIT) is a disease-modifying treatment for allergic rhinoconjunctivitis and allergic asthma and usually consists of a series of injections of an allergen extract. The SIT reduces both symptoms of allergic disease and the use of anti-symptomatic medication [1, 2]. Furthermore, SIT has a documented long-term effect [3, 4] and prevents the development of new sensitizations and asthma [5, 6]. So far, a broad range of allergen

extracts has been used for SIT. But despite good experiences with these preparations, they bear some disadvantages. For example, systemic allergic reactions can occur as adverse events (AEs) against the injected material [7]. To overcome this limitation, intensive research to develop faster, safer and more convenient SIT regimens is ongoing [8].

In general, allergic individuals exhibit an 'unbalanced' immune response to the allergen which is skewed towards type 2 T-helper cells (Th2). An alternative approach to

treat allergies may therefore be based on re-establishing a 'balanced' response against the allergen with cells of a more Th1-like phenotype. Indeed, it is thought that the underlying reason for the rapidly increasing incidence of allergic individuals is a vastly reduced exposure to environmental pathogens [9], an idea summarized under the umbrella of the hygiene hypothesis. Hence, an alternative way to treat allergies may be to mimic infections with natural pathogens.

Toll-like receptors (TLRs) are a key set of sensors for pathogens, recognizing so-called pathogen-associated molecular patterns. CpGs (DNA sequences containing non-methylated CG motifs) are over-represented in bacterial and viral genomes and recognized by TLR9. While A-type CpGs usually contain motifs with the ability to form palindromes, B-type CpGs are chemically stabilized by thioester bonds and do not form palindromes. These different structural properties result in distinct biological responses. The A-type CpGs typically induce type I IFNs while B-type CpGs induce mainly IL-12. The B-type CpGs have been successfully used as adjuvants to enhance antibody responses with hepatitis B surface antigen vaccine and influenza vaccine [10, 11]. They were also used as monotherapy or as adjuvants to enhance T cell-responses for treatment of a variety of tumours including lymphomas [12], melanomas [13], basal cell carcinomas [14], and glioblastomas [15]. Furthermore, data from an early-stage clinical trial of CpG oligonucleotides as an anti-allergic agent (CpG-oligonucleotides-ragweed allergen conjugate) were encouraging [16].

In contrast, experience with A-type CpGs is scarce, mostly as they consist of regular DNA, which is quickly degraded before a pharmacodynamic response can be induced *in vivo*. To overcome this limitation, the A-type CpG G10 has been encapsulated into virus-like particles (VLPs) to generate the CYT003-QbG10. These nanoparticles protect the CpGs from degradation by DNases. In addition, these particles efficiently drain to regional lymph nodes and are taken up by resident antigen presenting cells (APCs), including plasmacytoid dendritic cells (pDCs), which express high levels of TLR9 [17]. Within these APCs, the VLPs are shuttled to endosomes, where the protein shell is degraded and the packaged DNA exposed. Because TLR9 is expressed within endosomes, these biodegradable nanoparticles are ideally designed to deliver the CpGs to the right subcellular location [18]. Such CpG-loaded nanoparticles have been shown to efficiently induce tumour-specific cytotoxic T cells [19]. Furthermore, evidence of clinical efficacy in allergy has been seen in an initial clinical trial, where immunotherapy has been combined with specific allergen extracts [20]. This experience prompted us to investigate the effectiveness of the CpG-loaded nanoparticle without co-administration of allergen.

Materials and methods

Study design overview

CYT003-QbG10 was tested in a randomized, double-blind, placebo-controlled, multi-center phase IIb study that evaluated the safety, tolerability, and efficacy of the immunotherapeutic. In total, 35 study sites, located in Europe [Estonia (five); Latvia (three); Lithuania (four); Germany (nine); Romania (ten); Greece (four)] included 299 patients suffering from perennial allergic rhinoconjunctivitis due to house dust mite (HDM) allergy. There was a competitive recruitment between the sites with a randomization block size of three, resulting in a homogeneous distribution of the three treatment arms over all sites and countries. Main inclusion criteria were a patient-reported HDM allergy history of at least 2 years, a positive skin prick test (SPT) for HDM and a conjunctival provocation test (CPT) score of ≥ 8 at 10 HEP/mL and ≥ 2 at 1 HEP/mL (HEP, histamine equivalent prick; i.e. biological activity of HDM; 100 HEP/mL are equivalent to an allergen concentration of 5 mg/mL; tests performed according to the manufacturer's instructions; LETI Laboratories, Madrid, Spain). All three criteria had to be fulfilled for inclusion. The HDM allergen comprised equal amounts of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* extract. Main exclusion criteria were, any clinically manifested allergies other than HDM allergy that could interfere with the study procedure and concomitant medications that potentially influence outcome assessments. In other words, patients with potentially interfering additional allergies (e.g. pollen) were not eligible, nor patients with asthma that required constant medication with inhaled corticosteroids. In contrast, patients who gave a positive signal in SPT to other allergens e.g. mould or pets, but who confirmed that they do not experience any symptoms when they have contact with mould or pets, could be included. In fact, according to the allergy anamnesis, the vast majority of patients (93%) included in the trial were mono-sensitive to HDM. Adult patients between 18 and 65 years of age were included. For a detailed list of inclusion and exclusion criteria see supporting information Appendix S1. All patients gave written informed consent before inclusion into the clinical trial. The patients received six weekly injections of either 0.5 mg CYT003-QbG10 ($N=99$), 1 mg CYT003-QbG10 ($N=103$), or placebo ($N=97$), subcutaneously. The study duration was 9 weeks for each patient. Primary efficacy was assessed by recording ocular and nasal allergy symptoms and relief medication use in a diary filled in by the patients over 2 weeks pre- and post-treatment. Secondary efficacy parameters were a validated mini rhinoconjunctivitis quality of life questionnaire (MiniRQLQ) [21], and a CPT with dilution series of HDM allergen. The safety and tolerability of the therapeutic was assessed by recording

adverse reactions and by conducting routine laboratory blood tests. The study obtained approval from all concerned independent ethic committees and the national regulatory authorities in the six countries involved. The study is registered in a publicly available database: <http://clinicaltrials.gov> (NCT00800332).

Study drug

CYT003-QbG10 is a VLP filled with an oligonucleotide, called 'G10' [22]. The repetitive and ordered characteristic of the virus shell is a reason for its strong immunogenicity in humans [23]. The VLPs were produced by Cytos Biotechnology AG (Schlieren, Switzerland) following ICH/GMP regulations. The described VLP spontaneously assembles from coat proteins of the bacteriophage Qb during recombinant expression [24]. The oligonucleotide G10 bears unmethylated CpG motives that are known to be recognized by the human TLR9 [25]. G10 is added during reassembly of the VLP leading to its encapsulation inside the particle. These CYT003-QbG10 VLPs consist of 180 Qb monomers with the same symmetry as the native Qb capsid and a size of about 30 nm. The oligonucleotide G10 content of the reassembled particle is approximately 20% of the total mass, corresponding to approximately 60 oligonucleotide molecules per CYT003-QbG10 VLP. Unlike whole viruses or bacteriophages, VLPs and in particular CYT003-QbG10, do not carry any replicative genetic information, are non-infectious, and therefore do not integrate into the host cell genome. They are typically non-toxic but very immunogenic and induce strong humoral and cellular immune responses [23]. In this context, G10 is thought to be acting as an immunostimulatory substance by promoting the secretion of cytokines and chemokines and the up-regulation of surface molecules on cells of the immune system.

Allergy symptom and medication diary

The average combined symptom and medication score (ACS) based on World Allergy Organization recommendations [26] was the primary end-point to assess the patient's clinical status pre- and post-treatment [27]. In order to record the ACS, patients completed an allergy diary before and after the treatment phase for a period of 14 days. The investigators trained the patients how to fill in the diary and monitored the diaries after return for soundness. Seven symptoms of rhinoconjunctivitis (nasal blockage, running nose, sneezing, nasal itching, ocular itching, eye reddening, eye secretion) were each scored as absent/none (= 0 points), mild (= 1 point), moderate (= 2 points), or severe (= 3 points). The single scores were averaged to result in a daily symptom score (range 0–3 points). In addition, patients documented the usage of three types (steps) of predefined relief medication that was provided

to them. No relief medication (= 0 points); Step 1 drugs: local nasal or ocular H1-antihistamines (= 1 point); Step 2 drug: oral antihistamine (= 2 points); Step 3 drug: nasal corticosteroid (= 3 points). In case patients used different types of relief medication on the same day, only the medication with the highest points was considered for that day. The average daily medication score (range 0–3) was calculated. The ACS was calculated for each day as the arithmetic mean of the respective average symptom score and the medication score [27]. The ACS ranges from 0 to 3 and values reported pre- and post-treatment represent the mean of daily ACS values over the 2 weeks of recording.

Quality of life questionnaire:

A validated MiniRQLQ [21] was used. The MiniRQLQ consists of a 14-item questionnaire and a 7-point-rating format (range 0–6 points). The questionnaires were filled out by the patients at the respective visits and covered the preceding 7 days.

Conjunctival provocation test

The CPT measures susceptibility of the conjunctiva towards the HDM allergen. Starting with diluent (no allergen), the CPTs were performed with up to six concentrations (0, 0.01, 0.1, 1, 10, and 100 HEP/mL, LETI Laboratories, Madrid, Spain) of a HDM allergen mixture (50% *D. pteronyssinus*: 50% *D. farinae*). Conjunctival and nasal symptoms were assessed 10 min after provocation and scored on a 4-point scale each with none (= 0 points), mild (= 1 point), moderate (= 2 points), severe (= 3 points). The five conjunctival symptoms (hyperaemia, secretion, itching, burning, and swelling of eyelids) resulted in a conjunctival sum score (CSS; range 0–15 points) and the three nasal symptoms (secretion, itching, sneezing), in a nasal sum score (range 0–9 points). The sequence of provocations was stopped as soon as the CSS had become ≥ 12 points or the highest concentration of the allergen was reached. The allergen concentrations used in the CPT were transformed into log CPT values for efficacy analyses. The CPT started with the negative control and continued with the lowest allergen concentration, $\log\text{CPT} = 1$. Then, the next higher concentration, $\log\text{CPT} = 2$ was used and so forth. The concentration = 0 was not considered for the calculation of $\log\text{CPT}$.

Statistical methods

Statistical analysis was performed for measurements at baseline (i.e. pre-treatment) and post-treatment using the two-sided Mann-Whitney test at a type I error probability of $\alpha = 0.05$. For calculation of variables resulting from the CPT, the log of the allergen concentration ($\log\text{CPT}$) at

which CSS reached or exceeded a threshold_{CSS} of 2 for the first time was determined. LogCPT could range from 1 to 5 according to the five concentrations of HDM extract from 0.01 to 100 HEP/mL. In case of CSS < threshold_{CSS} for the highest performed dose, a numerical value of 6 was used for logCPT. To assess efficacy and to show changes in allergen tolerance, the shift in logCPT (at threshold_{CSS} = 2) from baseline to post-treatment was calculated.

Results

Between November 2008 and April 2009 a total of 299 patients with HDM allergy were included into the clinical trial. The patients were randomized to three treatment groups (supporting information Appendix S1). Baseline

Table 1. Baseline characteristics of study patients

	Placebo (N = 97)	0.5 mg QbG 10 (N = 99)	1 mg QbG 10 (N = 103)
Gender (N)			
Male	35	49	50
Female	62	50	53
Age (years)			
Mean	31.6	30.0	32.5
Median	30	28	31
Range	18–59	18–56	18–64
Weight (kg)			
Mean	70.7	74.5	74.4
Median	68.6	70.0	70.0
Range	46–126	50–123	42–128
BMI (kg/m ²)			
Mean	24.2	25.2	25.0
Median	23.0	23.9	24.7
Range	16.7–38.9	18.7–45.2	17.0–38.9
Temperature (°C)			
Mean	36.6	36.5	36.6
Median	36.6	36.6	36.6
Range	35.0–37.3	35.3–37.1	34.5–37.4
Blood pressure systolic (mmHg)			
Mean	117	117	120
Median	115	119	120
Range	90–150	90–160	90–150
Blood pressure diastolic (mmHg)			
Mean	73	74	74
Median	72	74	75
Range	50–95	55–90	50–100
Heart rate (b/min)			
Mean	71	72	70
Median	71	70	70
Range	50–100	52–100	50–101
HDM allergy duration (years)			
Mean	8.8	8.4	10.2
Median	5	6	7
Range	2–44	2–38	2–44

BMI, body mass index; HDM, house dust mite.

characteristics of the three treatment groups were similar as shown in Table 1. Altogether, 16 patients discontinued the study prematurely, eight due to AEs, of which six were of suspected relationship to the study drug. Three patients experienced urticaria during the study that lasted for 2–4 days and resolved completely afterwards. Two patients developed local reactions with pain and flu-like symptoms. One patient showed worsening of his pre-existing asthma and one patient developed white spots on the skin of his forehead reported as 'vitiligo' that prompted the patient to discontinue the study. Finally, one patient experienced study drug unrelated influenza and decided to stop the trial because she was not able to keep the visit schedule. The latter two AEs were not thought to be related to the study drug, while the first six events were suspected AEs (supporting information Appendix S1). Overall, the most frequent AEs were mild to moderate injection site reactions in up to 18% of patients of the low dose group and in 14% of the high dose group (Fig. 1). Furthermore, erythema, lymphadenopathy, influenza-like illness, pyrexia, fatigue, and headache reached incidences of > 5% of patients in any treatment group and were more frequent in the active treatment groups. Changes of the mean and/or median values of laboratory parameters (haematology, clinical chemistry, urine) over time within patients of the different treatment arms were very small (within normal ranges). The minimal and maximal values reported, as well as the distribution of values outside the normal range, were similar at baseline and post-treatment. Overall there was no indication of a difference between treatments with respect to blood cell counts and liver enzymes (supporting information Appendix S1). No drug related serious adverse event (SAE) occurred. The only SAE during the reporting period was a hospitalization of a placebo-treated patient with fracture of the right elbow.

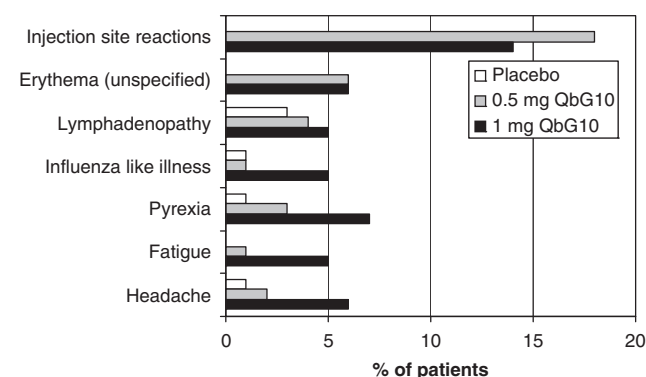


Fig. 1. Incidence of adverse events suspected AEs with an incidence of > 5% in any treatment arm are presented. 'Injection site reactions' comprise injection site-erythema/-induration/-pruritus/-swelling/-pain/-reaction, local -swelling/-reaction, and induration. Patients with more than one related term were counted once. The preferred term of 'erythema' is shown separately as it was not further specified regarding localization.

Consequences of allergy in daily life were assessed as a combined score of allergy symptoms and use of relief medication, where both aspects were rated equally (Fig. 2). At baseline, all three treatment groups revealed similar median scores between 0.60 and 0.63. Possible score-range was between 0 and 3. After the treatment phase with either 0.5 mg CYT003-QbG10, or 1 mg CYT003-QbG10, or placebo the median of the ACS were reduced compared with pre-treatment values. The ACS had fallen in the CYT003-QbG10 high dose treatment group from 0.60 points pre-treatment to 0.31 points post-treatment (–48%). The effect was less pronounced in the low dose group with 0.61 points pre-treatment and 0.45 points post-treatment (–26%). In the placebo group only a moderate decrease of median ACS from 0.63 points pre-treatment to 0.52 points post-treatment (–17%) was observed. The difference between placebo and the 1 mg-treatment group reached statistical significance at study

end ($P = 0.04$) but not pre- to post-treatment comparisons within the three treatment arms.

The quality of life scores derived from the MiniRQLQ (Table 2) were also comparable at baseline in all treatment groups. In the placebo group the median MiniRQLQ score decreased 26% from 1.64 points to 1.21 points post-treatment. In contrast, the decrease in both active treatment arms was more pronounced by –55% (from 1.57 to 0.71 points) in the 1 mg CYT003-QbG10 group and by –52% (from 1.66 to 0.79 points) in the 0.5 mg group. The disease scores were significantly different from placebo ($P = 0.0498$ for the 0.5 mg and $P = 0.02$ for the 1 mg CYT003-QbG10 treatment arm). This effect was not yet discernible at weeks 2 and 4 while the treatment was still ongoing.

The allergen tolerance as measured by CPT (Table 3) was improved after treatment with 1 mg of CYT003-QbG10 compared with placebo. The difference of the 1 mg CYT003-QbG10 group to placebo reached statistical

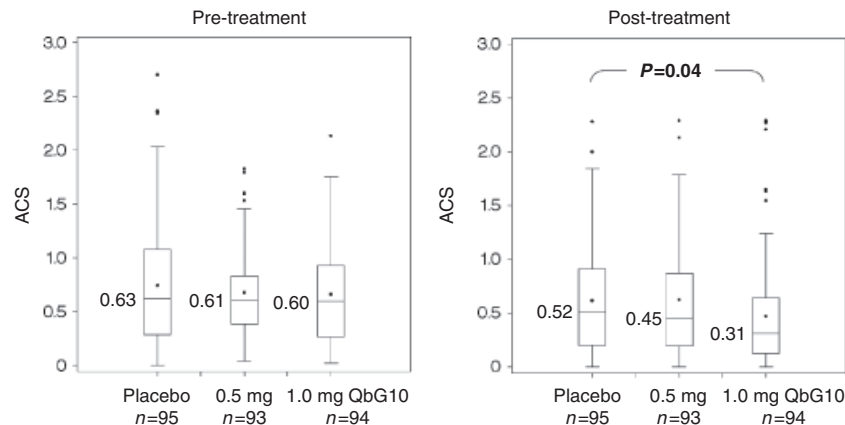


Fig. 2. Average combined symptom and medication score (ACS) The box-plot shows the median (line), the mean (dot), and the lower and upper 25-75 percentile. Statistical significance is given post-treatment for the difference 'placebo-group' vs. '1 mg QbG10-group'. When scores are baseline adjusted (i.e. corresponding baseline ACS was taken as covariate [35]), the P -value is 0.03.

Table 2. Mini rhinoconjunctivitis quality of life questionnaire (MiniRQLQ)

	Treatment group	N	MiniRQLQ Score (points)			P-value vs. Placebo
			Median	25–75%	Min–Max	
Pre-treatment	Placebo	95	1.64	1.00–2.29	0.07–4.93	
	0.5 mg QbG10	92	1.66	1.07–2.32	0.00–3.79	0.86
	1 mg QbG10	94	1.57	1.00–2.21	0.14–5.00	0.37
During treatment (day 14)	Placebo	94	1.32	0.57–1.93	0.00–4.64	
	0.5 mg QbG10	93	1.14	0.57–1.71	0.00–3.86	0.38
	1 mg QbG10	94	1.25	0.64–1.79	0.00–4.23	0.60
During treatment (day 28)	Placebo	95	0.93	0.43–1.86	0.00–4.86	
	0.5 mg QbG10	93	0.93	0.43–1.64	0.00–3.57	0.45
	1 mg QbG10	94	0.86	0.50–1.71	0.00–4.29	0.55
Post-treatment (day 49)	Placebo	95	1.21	0.57–1.71	0.00–4.64	
	0.5 mg QbG10	92	0.79	0.39–1.46	0.00–4.50	0.0498
	1 mg QbG10	94	0.71	0.43–1.50	0.00–4.29	0.02

P -values based on Mann–Whitney test; six weekly injections were applied (days 0, 7, 14, 21, 28, and 35).

Table 3. Conjunctival provocation test (CPT)

	Treatment group	N	logCPT		P-value vs. Placebo
			Mean	SD	
Pre-treatment	Placebo	95	2.27	0.75	
	0.5 mg QbG10	93	2.24	2.00	0.84
	1 mg QbG10	94	2.26	0.84	0.95
Post-treatment	Placebo	95	2.66	1.19	
	0.5 mg QbG10	93	2.68	1.17	0.95
	1 mg QbG10	94	3.02	1.12	0.032

The table shows the allergen concentration (expressed as logCPT) necessary to induce an ocular irritation score of 2 or more. *P*-values based on Mann-Whitney test.

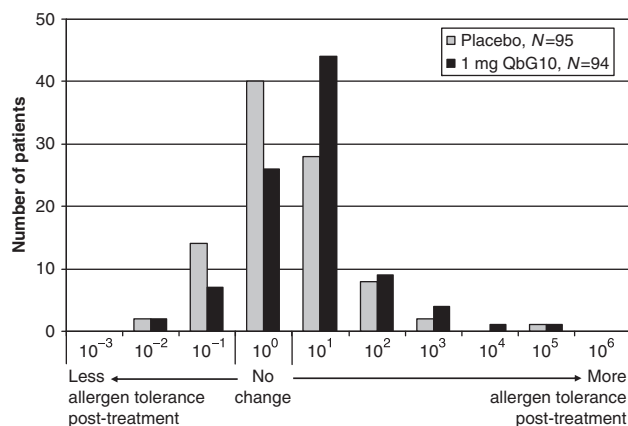


Fig. 3. Conjunctival provocation test shift individual allergen tolerance shifts from baseline to post-treatment evaluation for a conjunctival sum score (CSS) with threshold of 2. A shift of one log step to the right indicates that the patients tolerated post-treatment 10-fold higher concentrated allergen compared with baseline, and consequently a shift to the left indicates that the patient experienced symptoms above threshold already at a 10-fold lower allergen concentrations.

significance ($P = 0.032$). The CPT was also analysed within the groups (pre- vs. post-treatment). After placebo and 0.5 mg CYT003-QbG10 there was no change in allergen tolerance from pre- to post-treatment (median shift of logCPT = 0). After 1 mg CYT003-QbG10 the concentration to induce a threshold reaction of 2 was increased by a factor of 10 (median shift of logCPT = 1), which was statistically significant compared with placebo ($P = 0.007$). Figure 3 presents the distribution of individual logCPT values from pre- to post-treatment for the placebo and the high dose group. The majority of patients in the placebo group did not experience any change of allergen tolerance pre- to post-treatment (change in logCPT = 0), whereas in the high dose group the majority of patients tolerated 10 times higher allergen concentrations after the treatment (change in logCPT = 1).

Discussion

At present, allergic rhinoconjunctivitis due to HDMs is mostly treated with symptom-relieving medication (anti-

histamines and local corticosteroids). Allergen-specific immunotherapy is able to change the disease-causing allergic state of the immune system. However, this treatment-modality takes long and carries the potential risk of inducing systemic allergic reactions [28]. An immune-modulating treatment with more rapid onset of action without the risk of causing anaphylaxis would therefore be an attractive alternative. The immunomodulator CYT003-QbG10 described here offers an allergen-independent alternative treatment, with a favourable safety profile and a therapeutic benefit within 2 months.

The immunomodulator CYT003-QbG10 was tested in a double-blind, randomized, placebo-controlled clinical trial in patients suffering from HDM allergy. With six weekly doses of 1 mg CYT003-QbG10 subjective and objective rhinoconjunctivitis-related study end-points (ACS, MiniRQLQ, and CPT) were improved to a statistically significant and clinically relevant degree. The lower 0.5 mg dose showed reduced efficacy compared with the 1 mg dose.

ACS is a combined score for symptoms and medication intake. The ACS was improved in patients treated with 1 mg of CYT003-QbG10 and this improvement was mainly due to improved rhinoconjunctivitis symptoms ($P = 0.02$ vs. placebo), while medication use was hardly affected. This was likely caused by the fact that medication use was overall low. Interestingly, the nasal symptoms were more strongly reduced than the conjunctival symptoms. This may indicate that CYT003-QbG10 is mostly active in the respiratory tract (supporting information Appendix S1). Further experiments and clinical studies are needed to explore this hypothesis and to learn more about the duration of the treatment effect. However, first observations after 6 months still revealed a slight difference between the active treatment groups and placebo, while after 12 months the effect was not visible anymore.

Qb-based vaccines have so far been safe and generally well tolerated in several clinical trials and indications by subjects who received the active treatment (healthy volunteers [29], smokers [30], allergic/atopic patients [20], melanoma patients [19], and patients with hypertension [31]). No adverse systemic effect on any specific organ system could be identified. Local reactions were frequent, mostly mild or moderate and spontaneously resolving within days. Vital sign and clinical laboratory parameters remained generally uninfluenced. Anti-nuclear or anti-DNA antibodies did not change under therapy.

The exact mechanism by which treatment with CYT003-QbG10 ameliorates allergic symptoms in patients with rhinoconjunctivitis is not completely understood. The TLR9 agonistic effect of G10 is likely to play a central role for the treatment. By binding TLR9 in endosomes of pDCs, a cascade of cytokines (in particular type I IFNs) is stimulated that directs the immune response into the Th1 direction and at the same time suppresses the allergic Th2 responses. Indeed, it has been shown that exposure to

CpGs results in blunted Th2 responses [32]. Additionally, recent investigations suggest a TLR cross-talk via B cells regulating cytokine production by multiple pathways [33]. Finally, it is also possible that the TLR9 agonist acts on mast cells as shown *in vivo* for murine mast cells [34].

In summary the success of this short-term study of an allergen-free immune modulator opens a door towards a safe and fast-acting alternative to conventional desensitization therapies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Assessment of Clinical Efficacy of CYT003-QbG10 in Patients with Allergic Rhinoconjunctivitis: A Phase IIb Study.

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Title:**Assessment of Clinical Efficacy of CYT003-QbG10 in Patients with Allergic Rhinoconjunctivitis: A Phase IIb Study.**

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Supplemental Data 1)

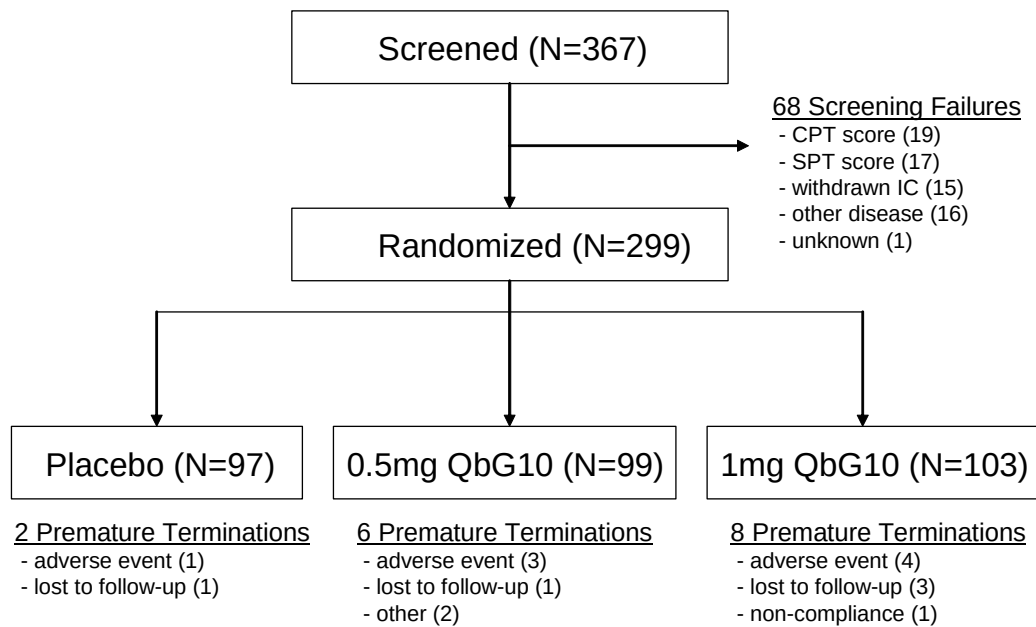
Inclusion criteria

- 18 to 65 years of age
- Perennial allergic rhinoconjunctivitis due to clinically relevant allergy towards house dust mite allergens (*Dermatophagoides pteronyssinus* and/or *D. farinae*) as evident from:
 - history of ≥ 2 years, and
 - positive skin prick test (SPT) with a mean wheal diameter ≥ 3 mm larger than negative control to house dust mite allergen solution (50:50 mixture of *D. pteronyssinus* and *D. farinae*) and
 - positive conjunctival provocation test (CPT) with a score ≥ 2 at 1 HEP/mL and a score ≥ 8 at 10 HEP/mL concentrations of a dilution series of house dust mite allergen (50:50 mixture of *D. pteronyssinus* and *D. farinae*).
- Patient did not perform a house dust mite sanitation to physically avoid house dust mite allergens within 2 months prior to his/her screening visit and patient does not plan to perform such an environmental intervention during study participation
- Female participants must meet one of the following criteria:
 - No reproductive potential due to menopause (one year without menses, in case of doubts serum FSH will be determined and must be >30 U/mL and Estradiol must be <54.7 pg/mL), hysterectomy, bilateral oophorectomy, or tubal ligation
 - 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)
- Patient gave written informed consent
- Patient is willing and able to comply with all trial requirements (such as diary completion, sticking to the study's visit schedule and also to the study rules regarding use of anti-allergic and other concomitant medication)

Exclusion criteria:

- Clinically manifested seasonal allergy/-ies which is/are expected to interfere with the patient's study treatment schedule and/or assessments (e.g. via usage of anti-allergic drugs or presence of allergy symptoms) as judged by the investigator (the patient should not suffer from clinically relevant seasonal allergy symptoms between January and May)
- Clinically relevant perennial allergy/-ies other than house dust mites allergy (e.g. cat, moulds)
- Contraindication for Conjunctival Provocation Test and/or Skin Prick Test
- Use of any concomitant medication that could affect the patient's study treatment response and/or allergic sensitivity or assessment results during the trial or that represents a contraindication for any study assessment as judged by the investigator (contraindicated drugs see Table 3.4.7-1).

- Any other specific immunotherapy (SIT) planned during the whole study period or any former specific immunotherapy (SIT) within the last five years with house dust mite allergens (*Dermatophagoides pteronyssinus* and/or *D. farinae*)
- History of anaphylaxis
- Contraindication for adrenaline/epinephrine as allergic shock rescue medication (e.g. acute or chronic symptomatic coronary heart disease, severe arterial hypertension)
- Actual significant obstructive pulmonary disorder as judged by the investigator
- Current diagnosis of asthma that requires treatment with oral or daily inhaled corticosteroids; intermittent and mild persistent asthma according to the asthma severity definitions of the Global Initiative for Asthma (GINA 2006) are permitted.
- Any vaccination planned during study treatment period
- 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.
- Completed or ongoing treatment with tranquilizers or other psychoactive drugs for treatment of a psychiatric disease/condition
- Presence of active infectious disease as judged by the investigator
- History of recurrent invasive streptococcal or staphylococcal infections, or known interleukin-1 receptor-associated kinase 4 (IRAK-4) deficiency
- Confirmed or suspected current infection with HIV, HBV or HCV
- Current diagnosis or history of malignancy; presence of suspicious lymphadenopathy or splenomegaly on physical examination
- Pregnancy (based on positive urine test at screening visit) or lactation
- Female planning to become pregnant during the study period
- History of abuse of alcohol or other recreational drugs
- Use of an investigational drug within 30 days before enrolment, or planned use during the whole study period
- Previous participation in a clinical trial with a Qb-based vaccine
- Possible dependency of the patient on sponsor and/or investigator

Supplemental Data 2)**Patient Disposition (CONSORT)**

The first patient started in Dec-2008 and the last patient was included in Apr-2009. The last patient finished the study in Jun-2009. The study duration for each patient was 8 weeks. For analyses, all patients were included for whom a pre- and a post-treatment assessment of the respective efficacy variable was available.

Supplemental Data 3)

Premature Terminations

Patient number	Age at screening /gender	No of injections applied	Reason for premature study termination	Treatment group
01S008	24/M	2	Lost to follow-up	0.5mg QbG10
01S016	55/M	1	Non-compliance	1mg QbG10
03S014	57/M	4	AE (flu-like symptoms, local reactions)	1mg QbG10
04S002	20/M	3	Other (withdrawn IC)	0.5mg QbG10
21S021	26/M	2	AE (urticaria)	0.5mg QbG10
22S010	35/F	6	Lost to follow-up	1mg QbG10
24S006	23/M	4	AE (vitiligo)	1mg QbG10
26S013	31/F	6	AE (asthma exacerbation)	0.5mg QbG10
29S002	34/M	2	AE (local reactions incl. pain)	0.5mg QbG10
31S001	57/F	6	Lost to follow-up	1mg QbG10
31S003	46/F	6	Lost to follow-up	Placebo
31S005	43/F	4	Other (withdrawn IC, patient moved away)	0.5mg QbG10
31S008	30/F	6	Lost to follow-up	1mg QbG10
34S008	30/F	1	AE (influenza)	Placebo
41S002	29/M	1	AE (urticaria)	1mg QbG10
41S005	38/M	5	AE (urticaria)	1mg QbG10

M = male; F = female; IC = informed consent; AE = adverse event; Additional information about reasoning are given in brackets, if available.

In total, 16 patients discontinued the study prematurely, of those 8 patients due to adverse events.

Three patients (21S021, 41S002, 41S005) experienced urticaria during the study that lasted for 2 to 4 days and resolved completely afterwards. Two patients (03S014, 29S002) developed local reactions with pain and flu-like symptoms. One patient (26S013) showed worsening of his pre-existing asthma and one patient (24S006) developed white spots on the skin of his forehead reported as “vitiligo” that prompted the patient to discontinue the study. Finally, one patient (34S008) experienced study drug unrelated influenza and decided to stop the trial because she was not able to keep the visit schedule. The latter two AEs of patients 24S006 and 34S008 were of not suspected relation to the study drug, while the first six events were judged as suspected AEs.

Further recorded reasons for premature terminations were “lost to follow-up” in 5 cases, “other” in 2 cases and “non-compliance” in one case.

Patient 21S021 (0.5mg QbG10), a 26-year-old male, discontinued the study after the 2nd injection due to adverse events. About 2 days after the 2nd injection the patient developed urticaria of suspected relationship on his legs and shoulders that was treated successfully with cetirizine (Zyrtec[®]). Additionally, a transient angioedema of the lips was reported. The investigator recognized enlarged lymph nodes. Already after the 1st injection the patient reported itching and reddening of the injection site followed by a general weakness. However, no symptoms were recorded in the local reaction diaries by the patient.

Patient 41S002 (1mg QbG10), a 29-year-old male, experienced redness and induration at the injection site one day after vaccination. However, no symptoms were recorded in the local reaction diary. A generalized urticaria or hives occurred 2 days after injection and disappeared spontaneously 3 days later. The information was obtained over the telephone since the patient did not return to the study site. The patient was considered as drop-out at Visit 1 with reason “AE; urticaria” of suspected relationship.

Patient 41S005 (1mg QbG10), was a 38-year-old male. Four days after the 5th injection the symptoms of the patient were described as large papulas all over the body, and considered of suspected relationship to study treatment. One week later, the patient was withdrawn from the study with reason “AE, urticaria”. The entry in the physical examination section of the CRF at the termination visit was “urticaria rash on chest, upper and lower extremities, abdomen”. The symptoms were treated with prednisone and cetirizine and resolved completely 4 days after occurrence. The local reaction diary revealed no findings except transient erythema after the 4th and 5th injection.

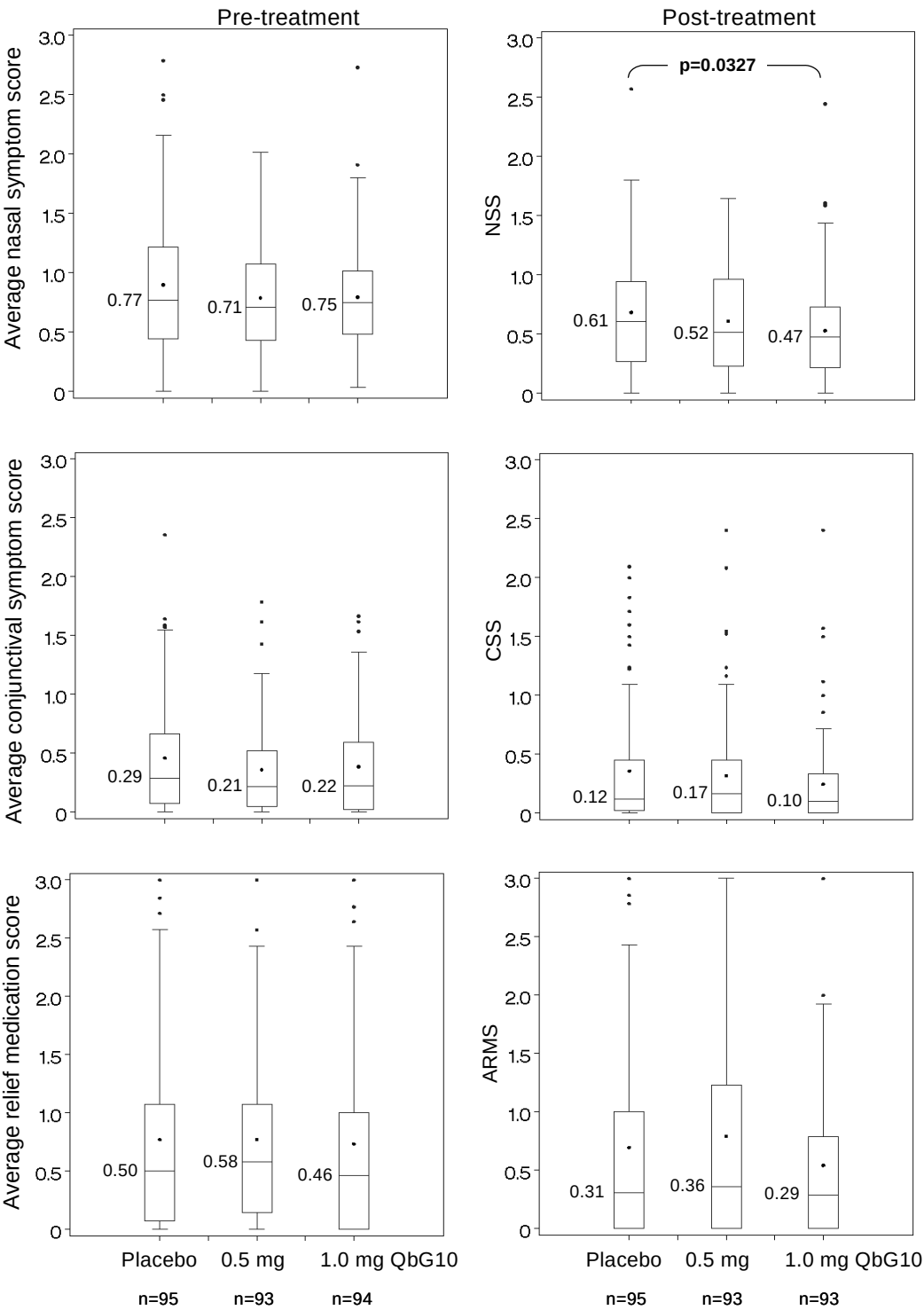
Patient 24S006 (1mg QbG10), was a 23-year-old male. Two days after the 4th injection “vitiligo” was recorded by the investigator as an adverse event with a duration of one week. The AE was not considered to be study drug related. In detail, up to 8 white spots with a diameter of 5-7 mm occurred on the forehead of the patient. The patient used Mometasoni-17-Furoate locally (Elocom[®] cream, a Group III corticosteroid). Nevertheless, the patient discontinued the study prematurely.

Supplemental Data 4)

Blood cell counts and liver enzymes given in mean (SD)

	Placebo		0.5mg QbG10		1mg QbG10	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Leucocytes [cells/nL]	6.0 (1.4)	5.8 (1.4)	6.4 (1.6)	6.0 (1.7)	6.4 (1.8)	5.9 (1.4)
Lymphocytes [cells/nL]	1.8 (0.5)	1.8 (0.5)	1.9 (0.6)	1.9 (0.6)	1.9 (0.5)	1.9 (0.5)
Neutrophils [cells/nL]	3.4 (1.2)	3.3 (1.1)	3.6 (1.2)	3.3 (1.3)	3.7 (1.5)	3.3 (1.2)
Thrombocytes [cells/nL]	258 (72)	257 (64)	251 (51)	242 (47)	259 (59)	251 (49)
AST (GOT) [U/L]	24.8 (9.8)	24.5 (7.0)	26.3 (10.3)	26.2 (11.1)	25.4 (6.8)	26.1 (17.2)
ALT (GPT) [U/L]	25.2 (14.9)	25.7 (13.0)	29.8 (19.2)	31.7 (36.4)	27.9 (15.2)	29.0 (24.3)
GGT [U/L]	19.2 (10.6)	19.0 (10.8)	23.1 (19.7)	22.2 (19.9)	23.6 (28.0)	22.2 (16.9)

Supplemental Data 5)



The 14 day diary recordings contain on one hand patient's score for their allergic symptoms and on the other hand the usage of standardized commercial relief medication. The average combined symptom and medication score (ACS) as presented in the paper takes both aspects into account.

In the box-plots above the ACS is separated into the sub-groups nasal symptoms, conjunctival symptoms, and medication use. The figures show the median (line) , the mean (dot), and the lower and upper 25-75 percentile. Statistically significance is given post-treatment for the difference "placebo-group" vs. "1mg QbG10-group".

A significant improvement is seen for the nasal symptoms that contributes most to the beneficial treatment effect of CYT003-QbG10 with respect to the ACS