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Safety and pharmacodynamics of intranasal GSK2245035, a TLR7 agonist for allergic rhinitis: a randomized trial

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Running head: Intranasal TLR7 agonist for allergic rhinitis

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Abbreviations

AE, adverse event; AIT, allergen immunotherapy; AR, allergic rhinitis; AUC, area under the curve; BMI, body mass index; CI, confidence interval; CrI, credible interval; CytoRS, cytokine release syndrome; DV, dosing visit; ECP, eosinophil cationic protein; ELISA, enzyme-linked immunosorbent assay; FEV₁, forced expiratory volume over 1 second; FUV, follow-up visit; GCP, Good Clinical Practice; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSK, GlaxoSmithKline; ICH, International Conference on Harmonisation; IFN, interferon; IL, interleukin; i.n., intranasal; IP, interferon-inducible protein; LLQ, lower limit of quantification; MDC, macrophage-derived chemokine; NAC, nasal allergen challenge; PD, pharmacodynamic; pDC, plasmacytoid dendritic cell; PNIF, peak nasal inspiratory flow; PP, posterior probability; SD, standard deviation; TARC, thymus and activation-regulated chemokine; TLR, toll-like receptor; TNF, tumor necrosis factor; TNSS, total nasal symptoms score; TSLP, thymic stromal lymphopoietin; VAS, visual analog scale; WM, weighted mean.

ABSTRACT

Background Toll-like receptor 7 (TLR7) stimulation in the airways may reduce responses to aeroallergens by induction of type 1 interferons (IFNs). GSK2245035 is a novel selective TLR7 agonist in pharmaceutical development.

Objective Assessment of safety, pharmacodynamics and nasal allergic reactivity following repeated weekly intranasal (i.n.) GSK2245035.

Methods This randomized, double-blind, placebo-controlled study (TL7116958) was conducted over two pollen seasons (2013–2014) and follow-up study (204509) conducted one year later.

Participants with allergic rhinitis (n=42) were randomized to receive eight weekly doses of i.n. GSK2245035 (20 ng [2014 Cohort; n=14] or 80 ng [2013 Cohort; n=14]) or placebo (n=14). Adverse events (AEs), including cytokine release syndrome-AEs (CytoRS-AEs) and nasal symptoms were assessed. Nasal and serum IFN-inducible protein 10 (IP-10) were measured after doses 1 and 8, then 1 (follow-up visit [FUV] 1) and 3 (FUV2) weeks after final dose. Nasal allergen challenges (NAC) and allergic biomarkers assessment (nasal, serum) were conducted at baseline, FUV1, FUV2 and at a follow-up visit 1-year post final dose (FUV3; 2014 Cohort only). A Bayesian framework enabled probability statements for mean effect sizes.

Results GSK2245035 induced CytoRS-AEs (most commonly headache, median duration <1 day) in 93% of participants at 80 ng, while AE incidence at 20 ng was similar to placebo. There was no evidence of nasal inflammation. Dose-related increases in nasal and serum IP-10 were observed 24h after doses 1 and 8 (>95% certainty). Both doses showed a trend in reducing Total Nasal Symptom Score 15 minutes post NAC at FUV1 and FUV2 but there was no reduction evident at FUV3. Nasal levels of selected allergic biomarkers demonstrated trends for reductions at FUV1, FUV2 and FUV3.

Conclusions and clinical relevance Weekly i.n. GSK2245035 20 ng was well-tolerated and reduced allergic reactivity to nasal challenge for 3 weeks post treatment.

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INTRODUCTION

The immunologic environment of the airways plays a critical role in host responses to aeroallergens.

Induction of aberrant Th2 responsiveness drives respiratory allergy and asthma in susceptible individuals,^{1,2} whereas protection from allergy is associated with Th1/T regulatory responses.^{3,4}

Therapeutic modulation to rebalance Th2-dominated responses may offer clinical benefit for respiratory allergy and potential for disease remission.⁵

Toll-like receptors (TLRs) are considered the gatekeepers of the immune system. They recognize potential “danger signals” for the host and trigger immune cascades that elicit the most appropriate type of response to sustain homeostasis and health.⁶ Because of this critical role, TLRs have become attractive targets for therapeutic intervention in diseases characterized by dysregulated immunity.^{7,8}

In the context of allergy, there is experimental evidence that stimulation of several of the receptors from the TLR family with synthetic ligands produces Th1-type cytokines that attenuate allergen-specific immunoglobulin E production and inflammation in target organs, including the airways.^{7,9,10}

Clinical evidence exists for TLR4 or TLR9 agonists as adjuvants of allergen-specific immunotherapy,^{11,12} and for TLR7, TLR8 and TLR9 agonists as standalone allergy drug candidates.¹³⁻¹⁵

TLR7 (natural ligand viral ssRNA,¹⁶ is of particular relevance for respiratory allergies.¹⁷ TLR7 polymorphism has been strongly linked with susceptibility to asthma and other atopic disorders.¹⁸ TLR7 agonists effectively prevent Th2-mediated airway disease in animal models,^{9,10,19} while they also exhibit a lower pro-inflammatory profile in comparison with other classes of TLR ligands.²⁰ This is in part due to the relative restriction of TLR7 expression to plasmacytoid dendritic cells (pDC) resulting in predominantly type 1 interferon (IFN) responses, rather than proinflammatory cytokines such as tumor necrosis factor (TNF) α and interleukin (IL)-12, which are produced by monocytes and myeloid dendritic cells (MDC) in response to TLR8 agonists.²¹ Furthermore, TLR7-triggered type1 IFN elicits a protective anti-viral immune response.²²

GlaxoSmithKline (GSK) has developed GSK2245035, a highly potent and selective TLR7 agonist.²³ The immunomodulatory potential of GSK2245035 was demonstrated *in vitro* where it reduced IL-5 and IL-13 and enhanced secretion of IL-10 and IFN- γ , in cultures of peripheral blood mononuclear cells from donors with allergic rhinitis (AR).²³ Pharmacological assessment in healthy volunteers and participants with AR, indicated that intranasal (i.n.) administration of GSK2245035 results in a dose-dependent activation of TLR7-mediated signaling in the upper airways.²⁴ Repeat weekly i.n. administration of 40 and 80 ng for 4 weeks was well tolerated and did not result in amplification or tolerization of the pharmacological response.²⁴ A key challenge with this compound is finding a therapeutic window that allows the induction of the desired local immunomodulatory effects without associated adverse cytokine-mediated events.²⁵ In this study we explore the safety and tolerability and pharmacodynamics (PD) of repeat i.n. weekly dosing with GSK2245035 for 8 weeks, specifically the duration of pharmacological changes. Moreover, we examined the effect of treatment on allergic reactivity triggered by nasal allergen challenge (NAC) to generate hypotheses on the immunomodulatory potential of GSK2245035.

METHODS

Study design

Study TL7116958 (NCT01788813) was a randomized, double-blind, placebo-controlled, parallel-group Phase IIa study in participants with AR, with or without mild asthma, carried out at Kingston General Hospital, Kingston, Ontario, Canada. The study was initially designed to evaluate the safety and PD of treatment with GSK2245035 80 ng versus placebo during the 2013 ragweed pollen season (Cohort 1; GSK2245035 [n=14]; placebo [n=7]). However, blind review of the safety data from all participants revealed a high incidence of influenza-like symptoms, possibly related to TLR7-mediated cytokine induction (cytokine release syndrome-adverse events [CytoRS-AEs]), prompting the

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evaluation of a lower GSK2245035 dose. Therefore, the protocol was amended in January 2014 and a second cohort of new participants was included to evaluate the effect of GSK2245035 20 ng versus placebo during the 2014 tree/grass pollen season (Cohort 2; GSK2245035 [n=14]; placebo [n=7]). In both cohorts the participants had seasonal AR driven by pollen sensitization; for each participant a screening NAC was performed to confirm key pollen sensitivity (based on participant allergic history); subsequent NACs were performed with the same pollen allergen, at the threshold concentration that resulted in a positive response at the screening challenge (ie, a total nasal symptoms score [TNSS] ≥ 5 , peak nasal inspiratory flow [PNIF] reduction of $> 30\%$ from baseline). As it was hypothesized that the immunomodulatory effect of GSK2245035 on allergic responsiveness would be magnified with natural allergen exposure in parallel to treatment, the study was conducted during the relevant key pollen season.

Both cohorts underwent a screening period (scheduled during a 90-day window prior to and/or during the season), a treatment period during which participants were randomized 2:1 to receive eight weekly doses of either GSK2245035 or placebo i.n. (dosing visits [DV] 1–8) and an extended follow-up period comprising follow-up visit (FUV) 1 (1 week post final dose) and FUV2 (3 weeks post final dose) where NAC was performed. Participants in Cohort 2 were also eligible to participate in a further FUV (FUV3) approximately 1 year post final dose, which constituted the follow-up study, 204509 (NCT02446613) (Figure 1). This follow-up study was performed at the original study site with the same investigator, who remained blinded to the findings and treatment assignment from study TL7116958 (although the sponsor was unblinded to those data).

The randomization schedule was generated by the GSK Clinical Statistics department (Stevenage, Herts, UK) using validated internal software, RandAll NG. Institutional review board clearance was granted by the Queen's Health Sciences and Affiliated Hospitals Research Ethics Board (ROMEO/TRAQ numbers: 6007721 [study TL7116958]; 6015437 [study 204509]) in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of

Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP). The study was conducted in accordance with ICH GCP and ethical principles as outlined in the Declaration of Helsinki, 2008.²⁶ Written informed consent was provided by each participant prior to study commencement.

Participants

Males or females, aged 18–65 years, in good general health with the following key inclusion criteria were eligible: history of symptomatic seasonal AR, with or without mild asthma (asthma diagnosis was confirmed, per protocol, via positive methacholine challenge), for >2 years; positive skin prick test (wheal ≥ 3 mm over negative control) for pollen allergens, within a year from the study start; positive screening NAC with the key symptom-driving pollen allergen, defined by TNSS response of ≥ 5 (on a 12-point scale) and decrease in PNIF of >30%. Owing to low participant recruitment, and given that the primary endpoint related to safety, a protocol amendment in January 2014 removed the requirement for mild asthma; a methacholine provocation test (described in Supplementary materials) was then conducted only if the participant had a history of asthma. Exclusion criteria are listed in the Supplementary materials.

Treatments

GSK2245035 was formulated as a nasal spray solution in normal saline; placebo comprised normal saline spray. Further details are provided in Supplementary materials. In the 2013 cohort, participants received 80 ng GSK2245035 formulated at either 10 ng/actuation or 20 ng/actuation. In the 2014 cohort, participants received GSK2245035 at a concentration of 10 ng/actuation (total dose of 20 ng).

Endpoints and assessments

Primary endpoints

Primary endpoints comprised general safety endpoints, nasal tolerability endpoints and TLR7-induced serum and nasal PD biomarkers.

Safety endpoints

The primary safety endpoints included AEs and CytoRS-AEs. CytoRS-AEs (headache, fever, chills/rigor, nausea, arthralgia, myalgia, vomiting, diarrhea, hypotension) occurring within 24 hours post dose were graded 0–4 according to severity, based on the National Cancer Institute-Common Toxicity Criteria guidelines.²⁷ Also assessed were vital signs, clinical laboratory parameters, peak expiratory flow.

Nasal tolerability endpoints

Nasal tolerability was assessed via nasal examination and participants' scoring on a 10 cm visual analog scale (VAS) at 10 hours and 24 hours post dose to assess itching, discomfort, post-nasal drip, rhinorrhea and obstruction (ranging from 'No symptoms' to 'Worst symptoms ever experienced').

TLR7-induced serum and nasal PD biomarkers

The primary PD endpoints included levels of the TLR7-induced biomarkers used in the previously reported study²⁴ to confirm target engagement in all participants (IFN-inducible protein 10 [IP-10; serum and nasal]) and also to assess selectivity of GSK2245035 (TNF- α [serum and nasal], IFN- α [serum] and IL-6 [nasal]), determined as described in Supplementary materials. Serum and nasal PD biomarker sample collection took place pre-dose and 24 hours post 1st and 8th dose, and prior to NAC at FUV 1 and 2 to determine the reproducibility of the response after eight doses and the duration of the response post last dose.

Exploratory endpoints

Exploratory endpoints included efficacy as determined by reduction of nasal symptoms following allergen challenge and allergic reactivity biomarkers.

NAC was performed at screening, to confirm eligibility, prior to randomization to provide baseline data, and at FUV1, FUV2 (all participants) and FUV3 (Cohort 2 only). In the initial screening NAC, a series of nasal provocations took place approximately every 12 minutes. Four-fold increasing allergen concentrations (ragweed [starting concentration 0.1 Ragweed Antigen E units/mL], birch [starting concentration 19 Protein Nitrogen Unit/mL], or grass pollen [starting concentration 49 Bioequivalent Allergy Units/mL]; according to participants' key pollen sensitivity) were used to determine the concentration eliciting a TNSS ≥ 5 and decrease in PNIF of $>30\%$. The cumulative allergen concentration determined here was employed in subsequent NACs.

Nasal symptoms

Nasal symptoms (congestion, rhinorrhea, sneezing, itch) elicited by NAC at screening, FUV1, FUV2 and FUV3 were assessed individually and as a TNSS. TNSS and PNIF were assessed prior to the allergen challenge and at 15 (primary NAC comparison time-point) and 30 minutes and 1, 2, 3, 4, 5 and 6 hours after the end of the challenge. TNSS was derived from symptom scores graded as: 0=none: no symptom whatsoever, absent; 1=mild: symptom is present, noticeable but not bothersome; 2=moderate: symptom is bothersome, but tolerable; 3=severe: symptom is bothersome, hard to tolerate, requiring relief/treatment with a maximum score of 12. PNIF was determined using the In-Check™ PNIF meter (Alliance Tech Medical Inc. TX, USA).

Allergic reactivity biomarkers

Allergic biomarker samples were collected as nasal lavage samples or nasal lining fluid adsorbed onto synthetic absorptive matrix filters (Leuksorb®, Pall Corporation, Port Washington, NY, USA), pre- and 6 hours post NAC, at screening, FUV1, FUV2 and FUV3, and serum samples 6 hours post

NAC at screening, FUV1 and FUV2. A wide range of allergic biomarkers were measured reflecting potential TLR7-mediated effects on relevant target and effector cells. Further details are given in Supplementary materials.

Statistical analysis

No formal statistical hypotheses were planned to be tested and no formal sample size estimated. Following the protocol amendment, the anticipated sample size of 14 participants receiving active treatment was considered sufficient for the safety and PD profile of GSK2245035 supporting larger clinical trials. NAC and biomarker analyses were performed on the per-protocol population, which was defined post hoc and excluded three participants receiving incorrect levels of allergen at a FUV. Year-matched placebo data were used for comparisons of TNSS, body temperature, serum and nasal lavage biomarker levels (excluding analyses exploring TLR-7 target engagement during the dosing period; post hoc); placebo cohorts were pooled as planned for all other endpoints. Point estimates and corresponding 95% credible intervals [CrI] were obtained for selected assessments to determine the induction of TLR7-associated serum and nasal PD biomarkers. A Bayesian approach was employed to investigate mean effect sizes of exploratory endpoint findings. For reference a significant p-value from a two-sided test at the 5% level is equivalent to the posterior probability (PP) exceeding 0.975. PP >0.9 is generally defined as high certainty of the observed value representing a true treatment effect. Further details on the statistical analyses are presented in Supplementary materials.

RESULTS

Participant disposition and clinical characteristics

There were 42 participants (March 2013–August 2014), all receiving at least one dose of GSK2245035 or placebo and included in the safety analyses (Figure 2). Demographics and baseline characteristics are summarized in Table 1.

Safety and tolerability

AEs

Overall, 93% (13/14) of participants reported any on-treatment AE in the placebo group, 64% (9/14) in the 20 ng group and 100% (14/14) in the 80 ng group; headache was the most common, reported by 57% (8/14), 50% (7/14) and 86% (12/14) of participants in the placebo, 20 ng and 80 ng groups, respectively. Treatment-related AEs were reported by 71% (10/14) of participants in the placebo group, 43% (6/14) in the 20 ng group and 100% (14/14) in the 80 ng group (Table S1). No serious AEs or deaths were reported.

Only two AEs were reported during the follow-up study; both occurred in the same participant in the placebo group and were not considered related to study treatment.

CytoRS-AEs

CytoRS-AEs within 24 hours of dosing were observed in 93% (13/14) of participants in the 80 ng group and less frequently in the 20 ng (36%; 5/14) and placebo (29%; 4/14) groups (Table 2).

Headache was the most frequent CytoRS-AE in all treatments followed by fever, observed only in the 80 ng group. Apart from one participant in the placebo group who experienced severe headache, severe grade CytoRS-AEs were only observed in the 80 ng group (36%), where one participant was withdrawn owing to severe myalgia.

In the 80 ng group, headache and fever were reproducible CytoRS-AEs; two participants reported a CytoRS-related headache with each dose, one of whom also had CytoRS-related fever with seven doses (Figure 3). In contrast, of the five participants in the 20 ng group who reported a CytoRS-AE, three reported it at only one visit. The median duration of CytoRS-AEs induced by both doses was <24 hours, with the exception of chills/rigors in the 20 ng group (n=2; mean 26 hours).

Vital signs, clinical chemistry and hematology

In the majority of participants in all groups, vital signs were normal for the duration of the study. Further details are provided in the Supplementary materials. Decreases in peripheral blood lymphocyte numbers were observed at 24 hours post DV1 and DV8 in the 20 ng group, and at 8 hours and 24 hours post DV1 and post DV8 in the 80 ng group, with greater than 95% certainty (Figure S1).

Body temperature

Increases in mean body temperature within the normal range (36–37.5°C), were noted in the 20 ng and 80 ng groups compared with placebo, with the 80 ng group exhibiting a consistently greater increase than 20 ng (Figure 4). The mean body temperature increase after each dosing in both dose groups was less than 1°C, except in the 80 ng group after DV2.

Nasal tolerability

Nasal AEs were reported by one participant in the placebo group (moderate rhinorrhea), two participants in the 20 ng group (mild epistaxis and mild nasal congestion; moderate nasal dryness and moderate rhinorrhea) and two participants in the 80 ng group (mild rhinorrhea; mild epistaxis, mild nasal congestion, moderate nasal dryness and moderate nasal discomfort). One report of rhinorrhea in the placebo group, one report of nasal congestion in the 20 ng group and nasal discomfort reported by one participant at DV 2–6 inclusive in the 80 ng group were considered related to the study drug. Mean nasal VAS scores were generally higher for the 80 ng group, including baseline, compared with the 20 ng and placebo groups, at both DV1 and DV8. Mean changes in nasal VAS scores were small for all treatment groups at both DV1 and DV8 (Figure S2; nasal VAS scores were obtained at each dosing visit, data for DV1 and DV8 only shown in Figure S2).

Additional safety assessments including peak expiratory flow measurements, nasal examinations (Table S2) and nasal symptoms as recorded on daily diary cards did not reveal adverse effects with repeat dosing.

TLR7-induced serum and nasal PD biomarkers

Serum IP-10

Median fold changes in serum IP-10 levels increased with certainty (PP >0.98) at 24 hours post DV1 and DV8 for both doses versus placebo (Table 3). The fold changes compared with placebo [95% CrI] were greatest in the 80 ng group at both DV1 and DV8. In the 80 ng group, an increase in serum IP-10 level was observed at FUV1 (PP >0.99; Table 3).

Nasal IP-10

Median fold changes in nasal lavage IP-10 levels increased with certainty (PP=1.0) at 24 hours post DV1 and DV8 for both doses, versus placebo (Table 3). For the 20 ng group, the magnitude of fold change versus placebo (95% CrI) at 24 hours post DV8 was higher than post DV1. Median fold changes in the 80 ng group remained similar at 24 hours post DV1 and DV8. In the 80 ng group, an increase in nasal IP-10 levels was observed at FUV1 and at FUV2 (PP >0.96; Table 3).

TNF α , IL-6 and IFN α

Serum levels of TNF α were not detectable in any group (lower limit of quantification [LLQ] 9.38 pg/mL). Serum levels of IFN α were detectable (LLQ 12.5 pg/mL) at one or more study visits in two participants in the placebo group, eight in the 20 ng group and two in the 80 ng group.

Median fold changes of TNF α and IL-6 in nasal lavage samples increased for both doses (TNF α approximately 2.5- and 8-fold at 20 ng and 80 ng, respectively; IL-6 approximately 2.6- and 4.6-fold

at 20 ng and 80 ng, respectively; both post DV8). The absolute values were very low pre- and post DV1 and DV8 (TNF α <1 pg/mL; IL-6 <4 pg/mL) (data not shown).

Allergen challenge

TNSS

Median change from baseline in TNSS at 15 minutes post NAC was reduced in both GSK2245035 groups, compared with placebo, at FUV1 (PP=0.92 and 0.89 for 20 ng and 80 ng, respectively) and FUV2 (PP=0.84 for both doses) (Figure 5). The median treatment effect was similar for 20 ng and 80 ng at both FUV1 (-1.9, -1.8, respectively) and FUV2 (-1.3 and -1.4, respectively). In the 20 ng group, TNSS reduction was sustained up to 6 hours post NAC at FUV1 and up to 1 hour post NAC at FUV2; in the 80 ng group, the effects were sustained up to 6 hours post NAC at both FUV1 and FUV2 (Table S3). The median change from baseline in TNSS showed no reduction with GSK2245035 20 ng compared to placebo at FUV3 (Figure 5 and Table S3).

PNIF

No consistent changes were noted in PNIF post NAC at FUV1, FUV2 or FUV3 for any treatment group (data not shown).

Allergic reactivity biomarkers

Nasal lining fluid levels of Th2-associated cytokines/chemokines (IL-5, IL-16, IL-33, Eotaxin, thymus and activation-regulated chemokine [TARC], macrophage-derived chemokine [MDC]) and the immunoregulatory cytokine IL-10, and nasal lavage fluid levels of allergen specific IgA and effector mediator eosinophil cationic protein (ECP), were detectable in a majority of the samples and were analyzed. For the 20 ng group, a decrease in ECP was measured at FUV1 (PP >0.85), FUV2 (PP >0.97), and FUV3 (PP>0.7). Decreases were also noted for IL-5 at FUV 2 (PP>0.66) and FUV3 (PP>0.80) and for IL-16 at FUV1 (PP>0.78) and FUV2 (PP>0.50) (Table S4). Increases in allergen-specific IgA were

measured at FUV3 (PP>0.71). For the 80 ng group decreases were measured at both FUV1 and FUV2 in IL-5 (FUV1 PP >0.71; FUV2 PP >0.75), eotaxin (FUV1 PP >0.79; FUV2 PP >0.88), MDC (FUV1 PP >0.89; FUV2 PP >0.92), TARC (FUV1 PP >0.88; FUV2 PP >0.79), and an increase in allergen-specific IgA (FUV1 PP >0.94; FUV2 PP >0.88). No change in IL-10 was detected with either dose (Table S4).

Biomarkers assayed but with levels at or below LLQ included IFN γ , IL-12p70, IL-4, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-25, Thymic stromal lymphopoietin (TSLP), allergen specific IgE and allergen specific IgG4. Comparisons made with post NAC allergic biomarker levels in serum at FUV1 and FUV2 (adjusting for the screening value) showed no evidence for consistent treatment effects (data not shown).

DISCUSSION

Novel treatments to reduce aberrant immune responsiveness to allergen are needed for patients with respiratory allergies requiring daily medication. This i.n. TLR7 agonist, GSK2245035, was developed as a potential immunomodulatory treatment to maximize co-localization with both its target, pDCs, and aeroallergen while minimizing systemic activation. Here we report findings from a Phase IIa clinical trial of i.n. GSK2245035, which demonstrated trends for sustained reductions in responsiveness to NAC in participants with AR treated during pollen seasons. Our findings confirm target engagement with dose-related changes in PD markers and also reveal dose-related increases in the frequency and severity of CytoRS-AEs with repeated i.n. TLR7 agonism.

Previous clinical trials of i.n. GSK2245035 20–80 ng report a varying frequency of mild CytoRS-AEs.²⁴ Here, 80 ng was poorly tolerated, whereas the tolerability of 20 ng was similar to placebo with infrequent CytoRS-AEs and no fever. Regardless of severity, the duration of CytoRS-AEs was generally ≤ 1 day. The poor tolerability of 80 ng compared with the previous study may reflect

variability in individual responses, apparent with more individuals treated (n=14 vs n=4),²⁴ as GSK2245035 80 ng retained selective potency for type I IFNs over pro-inflammatory cytokines in this 8-week study. Systemic activation of TLR7 by GSK2245035 seems unlikely as plasma concentrations of GSK2245035 after i.n. administration have not previously been detected²⁴. Regarding nasal tolerability, the low frequency of nasal AEs, nasal examination findings and small increases in VAS scores, indicate that repeat administration of both doses were well tolerated locally.

Target engagement was demonstrated by dose-related increases in IP-10 levels and body temperature, and by decreases in peripheral blood lymphocytes, each consistent with production of type 1 IFNs.²⁸⁻³⁰ For GSK2245035 20 ng, previously only studied as a single dose, nasal lavage IP-10 levels increased with repeat dosing but not with the 80 ng dose. There was a 5-fold difference between 20 ng and 80 ng after one dose, decreasing to a 2-fold difference after eight doses. The difference in nasal lavage IP-10 may reflect increased recruitment of TLR7-responsive pDCs to the nasal mucosa following repeated exposure to GSK2245035 20 ng, as observed with a topical TLR7/8 agonist.³¹ In contrast, the apparent peak in nasal IP-10 after one 80 ng dose may indicate that a maximal response had already been reached. Notably, there were no systemic differences after 8 weeks' dosing at 20 ng and the nasal lavage IP-10 levels at 80 ng did not increase with repeated dosing. After repeat dosing of 80 ng, IP-10 levels remained elevated (approximately two-fold) compared with baseline at 1 (serum and nasal) and 3 (nasal) weeks post final dose suggesting ongoing activation of the IFN α pathway. The clinical consequences of this finding are uncertain as no AEs consistent with cytokine induction were noted during this period. Indeed, the prolonged activation of the IFN α pathway could be explored for a respiratory anti-viral effect as with inhaled IFN β for allergic asthma.³²

Both doses of GSK2245035 reduced clinical symptoms, although not reaching statistical significance, following a direct NAC. The effect sizes were clinically relevant (TNSS difference from placebo >1.0) and sustained (up to 3 weeks after treatment). The similar TNSS reductions observed with the 20 ng

and 80 ng doses indicate that efficacy is not likely related to ongoing pharmacologic effects that were only observed at the higher dose. No significant differences were noted between treatment and placebo groups with respect to PNIF measurements following allergen challenge. This was likely due to the variability noted in baseline PNIF readings and the use of a 30% reduction in PNIF to qualify for inclusion, which in hindsight appears less reliable than in protocols where a 50% reduction is required.³³

While no standardized minimum clinically important difference has been established for TNSS, these results are of a similar or greater magnitude to i.n. corticosteroid studies where the effects are present over a 24-hour dosing period.³⁴⁻³⁸ Here, the probability of a sustained treatment effect on TNSS for both doses was modest to high, about 90%, at 1 week and 84% at 3 weeks after treatment. Data on the long-term effects of GSK2245035 are limited to a subset of participants receiving GSK2245035 20 ng where a clinical effect was not present 1 year after treatment. Therefore, the reductions in nasal symptoms measured up to 3 weeks post treatment may have been due to an effect of locally induced IFN α on mast cell numbers or mediator release³⁹ rather than on adaptive immune responses.

Analyses of nasal allergic biomarkers revealed trends for a response to treatment. The observed reductions in selected Th2-associated cytokines, chemokines and ECP post NAC are consistent with the known *in vitro* effect of IFN α to suppress Th2 responses and eosinophilia^{23, 40-42}. In contrast to the effect on nasal symptoms, nasal biomarker data were not consistent for the two doses, likely reflecting the small sample size.

Exposure to allergen is thought to be important for TLR7-induced immunomodulatory changes. However, the conduct of these studies over multiple different pollen seasons, including grass, ragweed and tree, variable pollen exposure and differences in sensitization patterns among patients, represent important limitations, as indicated by differences in biomarker baseline values between the 2013 and 2014 cohorts. GSK2245035 treatment was therefore compared with placebo from the

same year rather than pooling as planned. A limitation regarding the detection of changes in nasal biomarkers is that, despite following standardized procedures, the TNSS induced by NAC were modest and may reflect baseline symptomatology from ongoing environmental exposure in polysensitized participants. As changes in TNSS were not measured over the 8-week treatment period, clinical efficacy is limited to the NAC model. Further limitations are the single time point for collecting allergic biomarker data meaning that the true effect on each biomarker may not have been established, a small sample size, and short initial follow-up period (3 weeks).

The 3-week duration of treatment effect has not previously been shown with similar treatments under investigation for AR and asthma, the TLR7 agonist AZD8848⁴³ and the TLR8 agonist VTX-1463.

¹⁴ The magnitude of treatment difference for TNSS (>1.0) provides strong evidence for the therapeutic potential of GSK2245035 despite the small sample size used in this study. The Bayesian statistical methods allow judgment on the strength of the data by providing a probability that the observed treatment effect is not chance. In this study, the probabilities of mean changes in IP-10 levels exceeded 0.975 and were equivalent to a p value <0.05. When applied to exploratory efficacy endpoints, Bayesian methods show the probability of each GSK2245035 dose inducing a reduction in nasal symptoms to be high at FUV1 and FUV2, not at the level generally associated with “proof” for definitive efficacy studies, rather as an informative way of assessing data in translational medicine studies such as this. Similarly, the probabilities of reducing individual Th2 cytokines or chemokines were moderate to high. These findings were demonstrated in a relatively small number of participants and would need to be confirmed in larger studies.

In summary, GSK2245035 is a potent, selective TLR7 agonist with clinical effects evident at 20 and 80 ng doses up to 3 weeks after treatment. Despite local administration, systemic influenza-like symptoms, in particular fever and myalgia, consistent with stimulation of the IFN α pathway were reported. Key questions arising from this study form objectives for future studies. The optimal dosing regimen for maximum clinical effect remains unknown. It is unclear if the difference in TNSS

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at each FUV represents a waning effect, and if so, the rate of decline has not been defined. Further, this study, and others with GSK2245035, have all been conducted during pollen seasons. It is important to understand if there is a threshold level of allergen exposure needed to coincide with GSK2245035 treatment as well as the relationship between allergen exposure and the magnitude and duration of clinical effect. Although the exact mechanism of action for GSK2245035 is yet to be determined, and Th1/regulatory responses were not detected, the i.n. reductions in IL-5, Th2 chemokines, and ECP provide evidence for a treatment effect on Th2-associated inflammation. Perhaps the greater challenge will be to link these changes in immune responses to the clinical benefits perceived by the patient.

By altering established immune responses to aeroallergens, TLR7 agonism has broad therapeutic utility. I.n. GSK2245035 is conveniently administered once weekly and the relevant therapeutic comparison will be to a daily i.n. corticosteroid. As an immunomodulatory treatment that works with natural allergen exposure, a key advantage might be the low risk of anaphylaxis, and its safe use also in season. Lastly, although the serum allergic biomarkers did not reveal systemic treatment-related effects, the PD data (ie, raised serum IP-10 and body temperature, and decreased blood lymphocyte counts), suggest that an effect of i.n. GSK2245035, via the induced type 1 IFN response, may not be restricted to the local nasal environment. Therefore, TLR7 remains a valid target with the prolonged local clinical effects and self-resolving AEs providing evidence for a therapeutic window.

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Conflict of interest

AKE has received honoraria for participation in an advisory board for Circassia Ltd, GSK, Merck and Novartis; been a speaker for Novartis, Merck, Pfizer, Takeda, Meda and AstraZeneca; received research grants from GSK, Circassia Ltd, SunPharma, Merck, Pfizer and Novartis. LAL, DQ and WP are employees of and shareholders in, GSK; DCT was an employee of and shareholder in GSK at the time this research was conducted.

Author contributions

AKE and DQ were involved in the conception and design of the study, acquisition of data, analysis and interpretation of data and drafting of the manuscript. LAL and DCT were involved in the conception and design of the study, analysis and interpretation of data and drafting of the

manuscript. WP was involved in the conception and design of the study and analysis and interpretation of data. All authors reviewed and critically revised the manuscript prior to approval.

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TABLES AND FIGURES

Table 1. Participant demographics and baseline characteristics

	Placebo N=14	GSK2245035 20 ng N=14 [Cohort 2, 2014]	GSK2245035 80 ng N=14 [Cohort 1, 2013]
Age (years), mean (SD)	39.4 (11.91)	35.9 (12.54)	38.9 (12.67)
Female, n (%)	10 (71)	11 (79)	9 (64)
BMI (kg/m ²), mean (SD)	30.8 (7.03)	30.8 (7.95)	29.1 (5.67)
Race, n (%)			
American Indian or Alaskan Native	2 (14)	0	1 (7)
Asian-Japanese/East or South-East Asian Heritage	1 (7)	0	0
White	11 (79)	14 (100)	12 (86)
American Indian or Alaskan Native and White	0	0	1 (7)
Allergen used in NAC, n (%)			
Birch	3 (21)	5 (36)	0
Ragweed	7 (50)	0	13 (93)
Timothy grass	4 (29)	9 (64)	1 (7)
Allergens with positive responses, n (%)			
Ragweed	12 (86)	13 (93)	14 (100)
Pigweed	2 (14)	5 (36)	3 (21)
Plantain	3 (21)	5 (36)	4 (29)
Cocklebur	5 (36)	8 (57)	8 (57)
Mugwort	4 (29)	8 (57)	6 (43)
Birch	9 (64)	9 (64)	12 (86)
Oak	3 (21)	7 (50)	7 (50)
Tree Mix (9)	4 (29)	3 (21)	9 (64)
Rye grass	13 (93)	12 (86)	12 (86)
Timothy grass	10 (71)	11 (79)	12 (86)
Lamb's quarters	3 (21)	5 (36)	5 (36)
Cat dander/hair	10 (71)	8 (57)	6 (43)
Dog dander/hair	2 (14)	4 (29)	3 (21)
<i>Dermatophagoides pteronyssinus</i>	8 (57)	9 (64)	9 (64)
<i>Dermatophagoides farinae</i>	8 (57)	9 (64)	10 (71)
<i>Alternaria</i>	2 (14)	0	2 (14)

BMI, body mass index; NAC, nasal allergen challenge; SD, standard deviation.

Table 2. Summary of CytoRS-AEs (occurring within 24 hours post dose)

CytoRS event n (%)	Severity	Placebo N=14	GSK2245035 20 ng N=14 [Cohort 2, 2014]	GSK2245035 80 ng N=14 [Cohort 1, 2013]
Any event	All	4 (29)	5 (36)	13 (93)
Headache	Mild	1 (7)	2 (14)	2 (14)
	Moderate	1 (7)	2 (14)	4 (29)
	Severe	1 (7)		5 (36)
Fever	Mild			4 (29)
	Moderate			4 (29)
	Severe			
Chills/rigors	Mild	1 (7)	1 (7)	3 (21)
	Moderate			3 (21)
	Severe			
Nausea	Mild			3 (21)
	Moderate	1 (7)		1 (7)
	Severe			
Arthralgia (joint pain)	Mild			1 (7)
	Moderate		1 (7)	3 (21)
	Severe			
Myalgia (muscle pain)	Mild			1 (7)
	Moderate		1 (7)	4 (29)
	Severe			1 (7)*

AE, adverse event; CytoRS, cytokine release syndrome.

CytoRS-AEs were analyzed per protocol, whether considered drug related or not. Note that a participant can experience >1 event. Values represent distinct participant(s) experiencing at least one event, and displayed at highest level of severity for those experiencing >1 grade of an event.

*Participant met protocol defined stopping criteria and was withdrawn after two doses.

Table 3. Median fold changes* in serum and nasal lavage IP-10 (95% credible interval†)

	Treatment Comparison Active vs Placebo	DV1	DV8	FUV1	FUV2
Serum	GSK2245035 20 ng	1.66 (1.05, 2.61) PP=0.98	2.19 (1.15, 4.20) PP=0.99	1.00 (0.70, 1.41) PP=0.50	0.85 (0.65,1.11) PP=0.12
	GSK2245035 80 ng	6.06 (3.80, 9.63) PP=1.0	6.43 (3.26, 12.68) PP=1.0	1.66 (1.15, 2.38) PP=1.0	1.00 (0.76,1.33) PP=0.51
Nasal lavage	GSK2245035 20 ng	4.34 (2.65, 7.13) PP=1.0	10.30 (5.12, 20.93) PP=1.0	0.92 (0.49, 1.73) PP=0.39	0.92 (0.47, 1.76) PP=0.39
	GSK2245035 80 ng	21.03 (12.52, 35.11) PP=1.0	19.05 (9.28, 38.93) PP=1.0	2.47 (1.29, 4.74) PP=1.0	1.86 (0.93, 3.72) PP=0.96

DV, dosing visit; FUV, follow-up visit; IP-10, interferon-inducible-protein-10; PP, posterior

probability. ≥ 1.0 . *Ratio of GSK2245035:placebo; †range within which the true value lies with 95%

probability (certainty). Median absolute nasal IP-10 levels (pg/mL) 24 hours post dose: placebo 256

(DV1), 204 (DV8); 20 ng 1111 (DV1), 2104 (DV8); 80 ng 5380 (DV1), 3891 (DV8).

Figure 1. Study design

DV, dosing visit; FUV, follow-up visit

Figure 2. Participant disposition

AE, adverse event; NAC, nasal allergen challenge.

*n=7 in Cohort 1; n=7 in Cohort 2.

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**n=7 in Cohort 1; †Cohort 2 (2014); ‡Cohort 1 (2013); ^data from the placebo and 20 ng arms of TL7116958 study were re-used, even if the participants did not participate in Study 204509. Therefore, there are more participants in the per-protocol population than there are participants who had a NAC in the follow-on study. See note in statistical analysis section within Supplementary materials.

Figure 3. Frequency of CytoRS-AEs over eight dosing visits, per participant

AE, adverse event; CytoRS, cytokine release syndrome; DV, dosing visit; i.n., intranasal.

*Includes one participant who withdrew after receiving four doses (owing to a protocol violation [non-compliance at DV5, meaning last dose received at DV4]).

†Includes one participant who withdrew after receiving two doses (owing to severe myalgia).

Figure 4: Change in body temperature at each DV

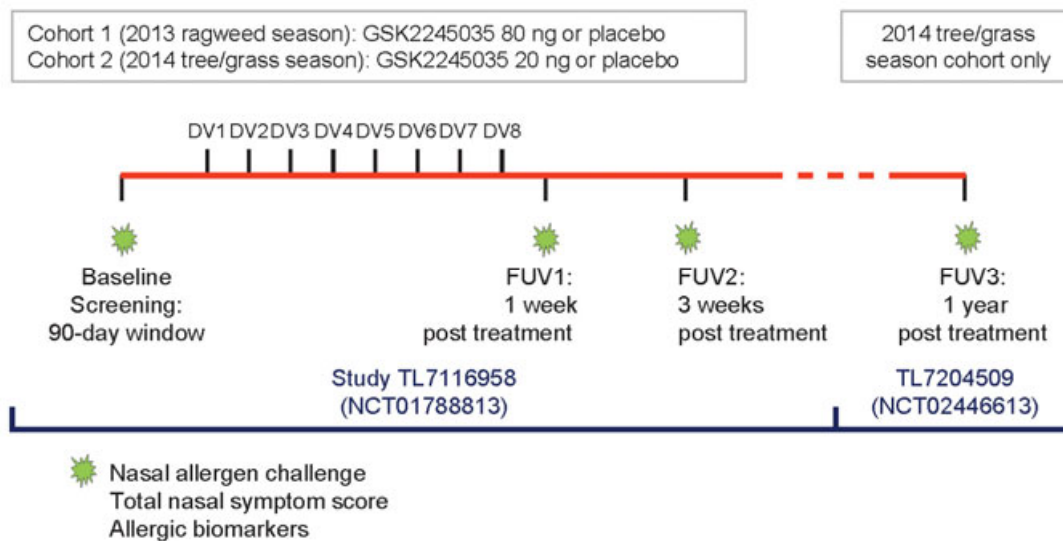
DV, dosing visit; i.n., intranasal.

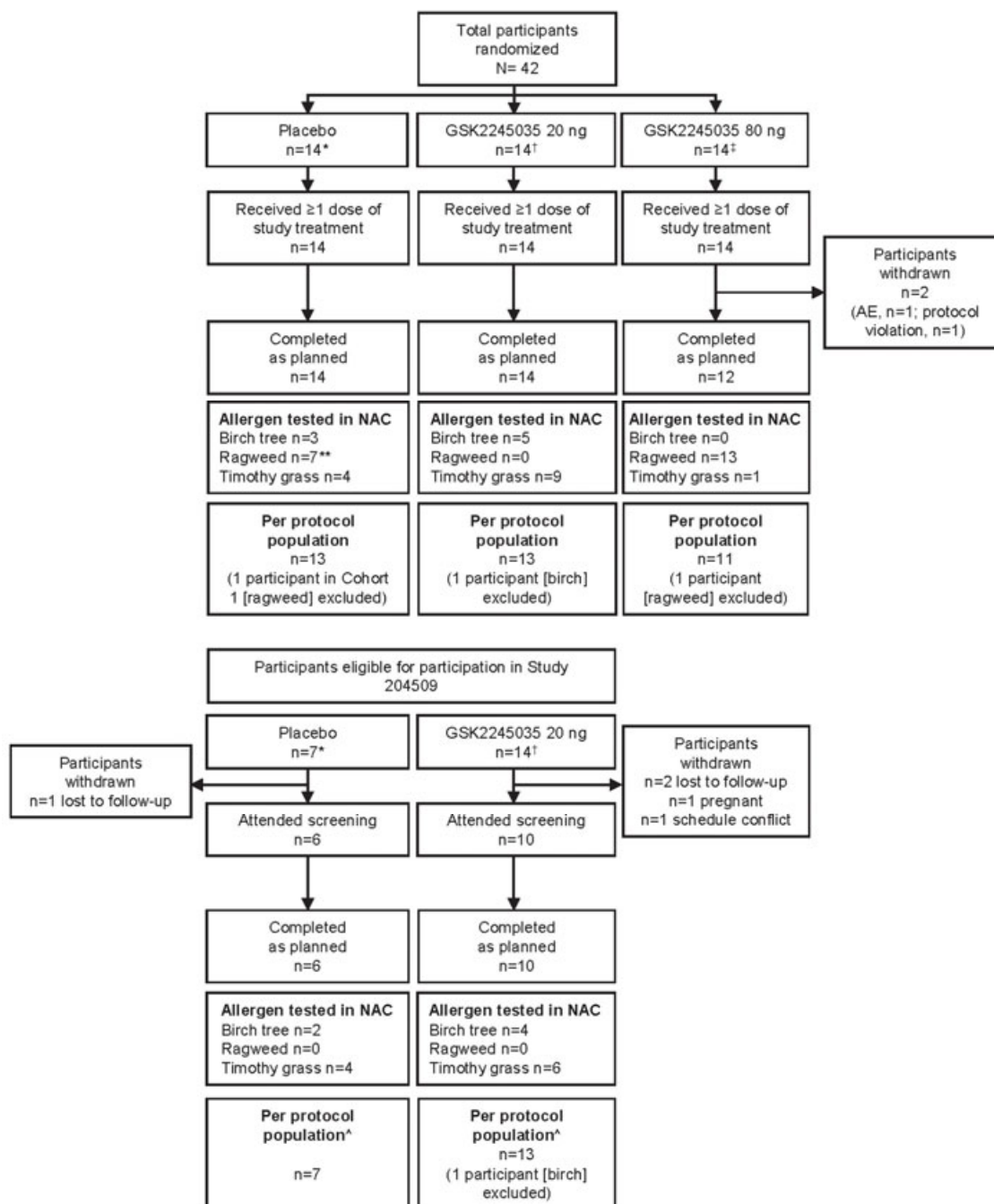
The increases in body temperature were within the normal reference range (36–37.5°C).

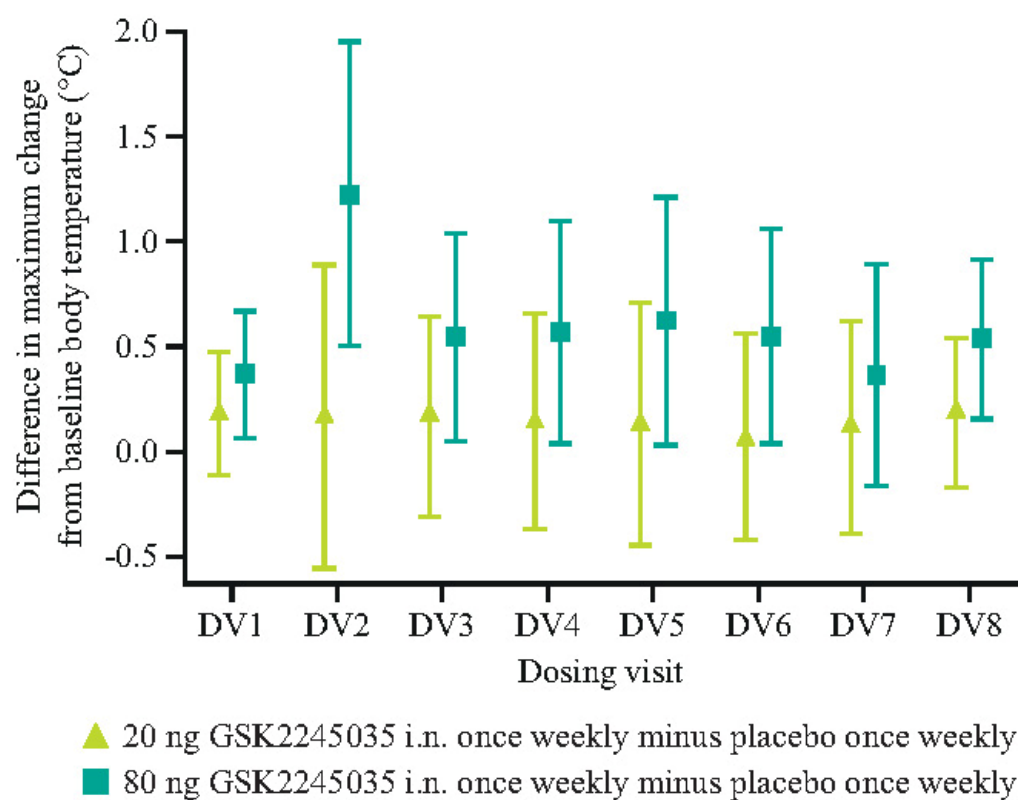
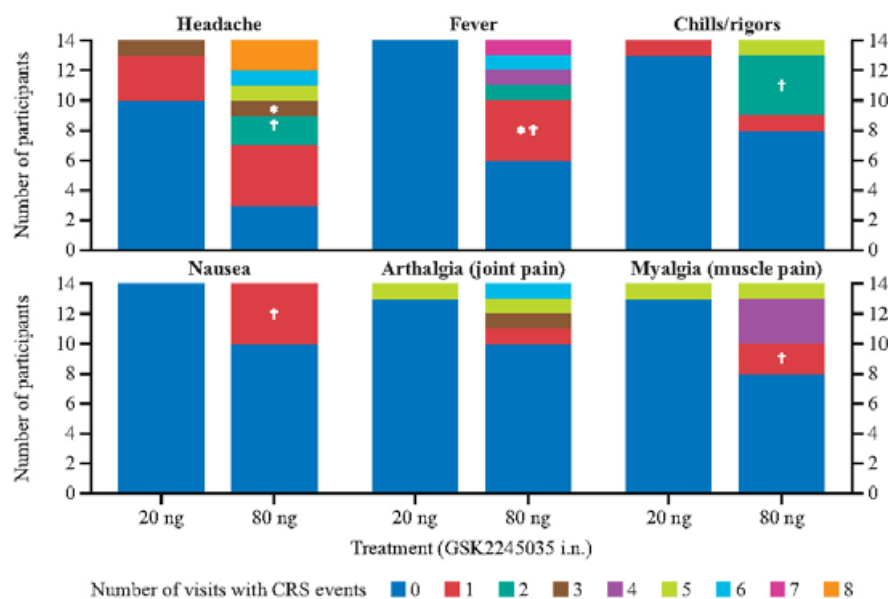
Figure 5. Change from baseline in TNSS at 15 minutes post NAC at FUV1, FUV2 and FUV3

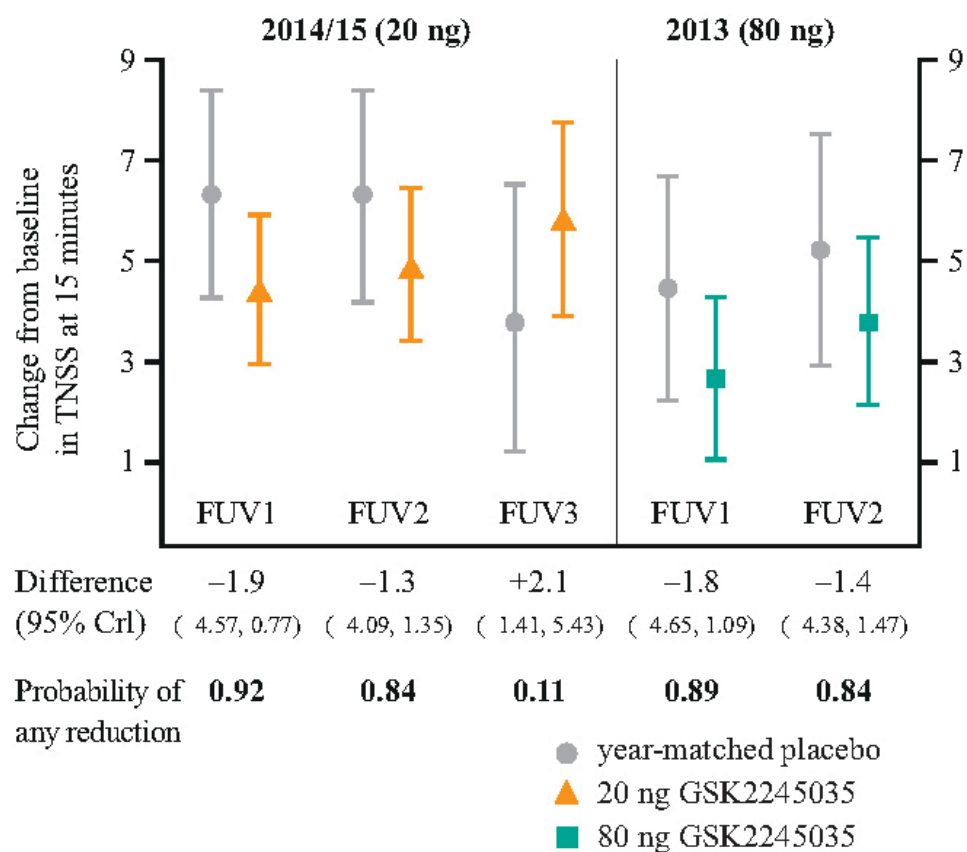
CrI, credible interval; FUV, follow-up visit; NAC, nasal allergen challenge; TNSS, total nasal symptom score (0–12).

Difference represents placebo minus GSK2245035.





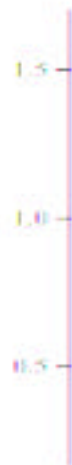




Ratio to Dosing Visit Baseline

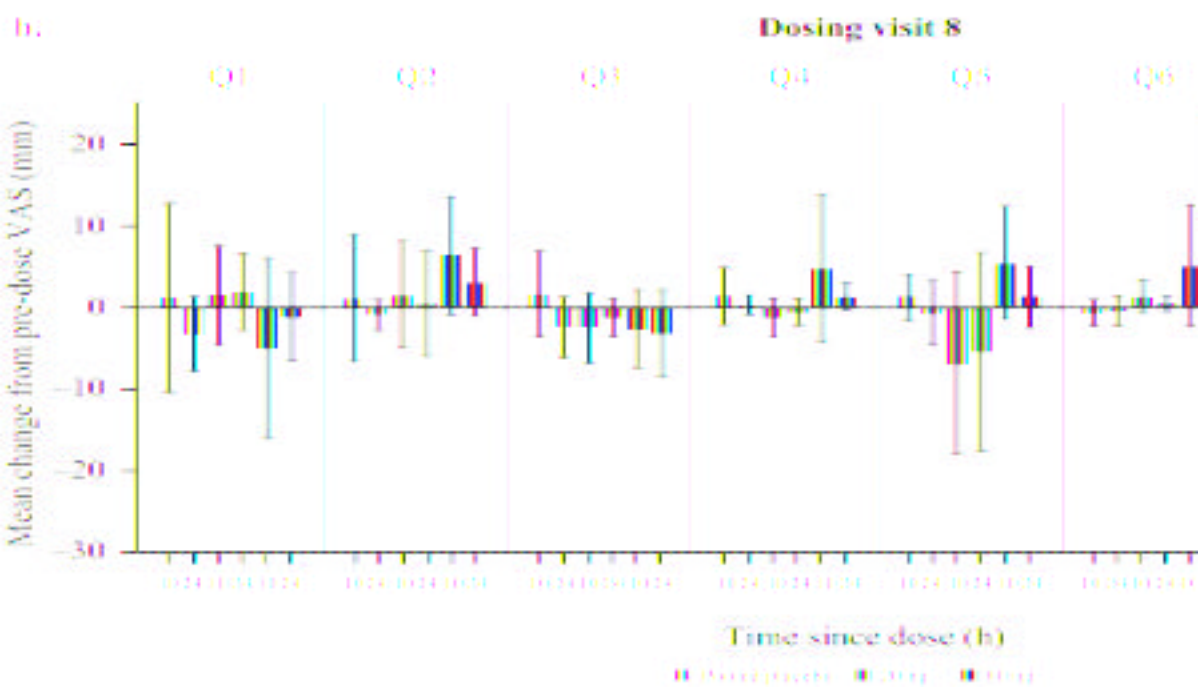
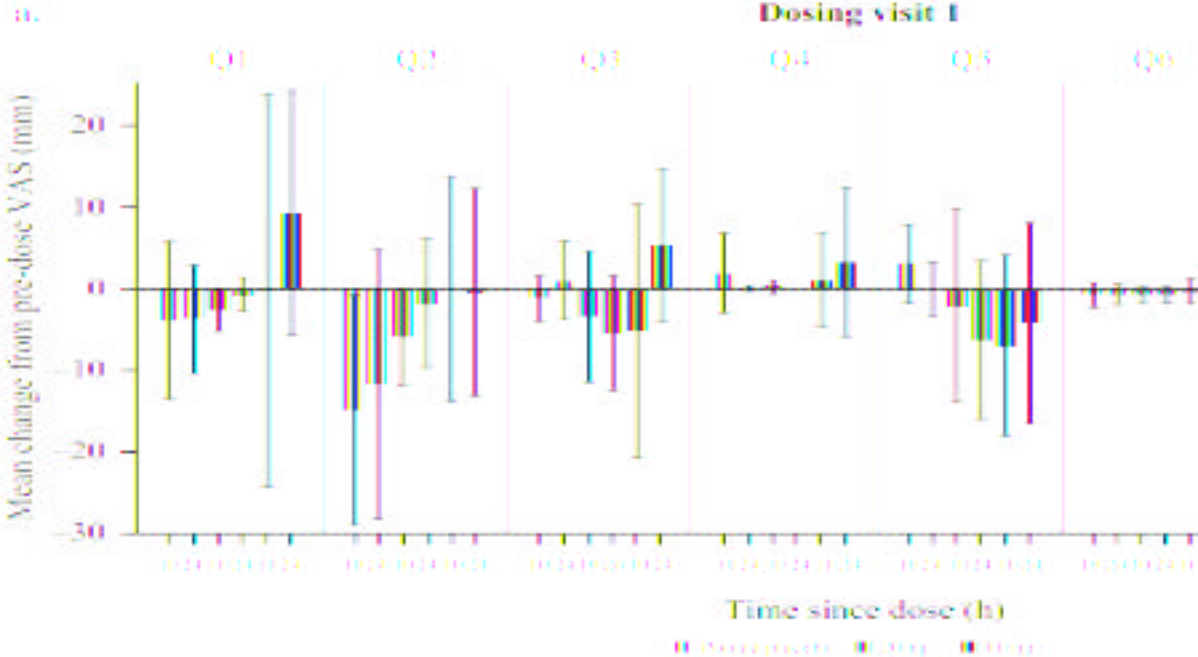
Placebo

10 mg



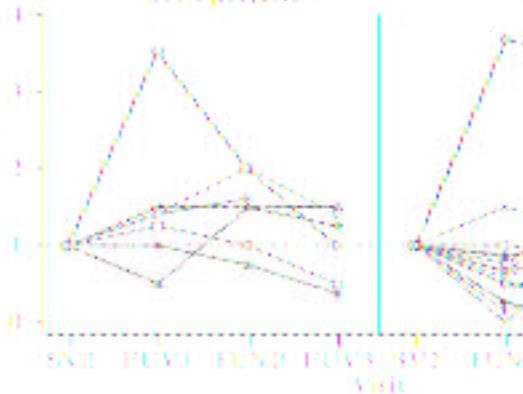
Planned Time

OV1 Pre-Dose
OV1 8 HRS
OV1 24 HRS
OV8 Pre-Dose
OV8 24 HRS
OV1 Pre-Dose
OV1 8 HRS
OV1 24 HRS
OV8 Pre-Dose
OV8 24 HRS
OV1 Pre-Dose

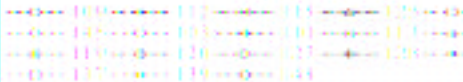


2014 placebo

Multiple Baseline Experimental Comparison
 Number of Discontinuation Events



Participant ID



Supplementary methods

Participants

Prior to the protocol amendment in January 2014, participants were required to have mild asthma, as determined by a positive screening inhaled methacholine provocation test, defined as a decrease of baseline forced expiratory volume over 1 second (FEV₁) by 20% (PC20) with ≤16 mg/mL methacholine. Individuals could participate only once in this study, even though the study was conducted in two separate allergy seasons.

Participants were excluded if they had existing nasal conditions (ie, nasal septal perforation, nasal polyps, other nasal malformations or history of frequent nosebleeds) that in the opinion of the investigator may have affected the outcome of the study, respiratory tract infection within 4 weeks prior to the first dose or a serious asthma exacerbation requiring a hospital visit and/or treatment with oral steroids or high doses of inhaled steroids within 6 weeks prior to screening. Other exclusion criteria included a history of immunological disorders or treatment with allergen-specific immunotherapy within 5 years. Individuals receiving steroid treatment for allergic rhinitis and/or asthma were able to participate in the study if they remained free of medication throughout the treatment and follow-up period starting from the following periods prior to screening: nasal steroids: 4 weeks; oral steroids: 12 weeks; inhaled steroids: 4 weeks. Those using other medications for allergic rhinitis and/or asthma on an as-needed basis were able to participate if they abstained from: xanthines (including theophylline, but not including caffeine), anticholinergics, cromoglycates, leukotriene antagonists, 5-lipoxygenase inhibitors and long-acting inhaled beta-agonists from 1 week prior to screening and throughout the treatment and follow-up period. Nasal and oral decongestants were not permitted for 24 hours prior to each visit, nasal antihistamines and short-acting inhaled beta-agonists for 48 hours prior to each visit, and oral antihistamines for 76 hours prior to each visit.

Treatments

For participants randomized up to and including July 1, 2013 (Cohort 1), the device delivered 10 ng GSK2245035 per actuation; those receiving 80 ng of study treatment received eight actuations in alternate nostrils (four per nostril) and participants randomized to the placebo group until this date also received eight actuations of placebo to match the treatment group. From July 2, 2013, participants randomized to the 80 ng treatment group received four actuations (two per nostril) of a preparation delivering 20 ng GSK2245035 per actuation; participants randomized to the placebo group between July 2, 2013 and December 31, 2013 also received four actuations to match the treatment group. From January 1, 2014, participants randomized to the 20 ng dose group (Cohort 2)

received two actuations (one in each nostril) of a preparation delivering 10 ng GSK2245035 per actuation; participants randomized to the placebo group after this date also received two actuations to match the treatment group.

Sentinel dosing in Cohort 1 was planned; five participants with ragweed sensitivity (two in the placebo group; three in the 80 ng treatment group) initially received study treatment, so that 24-hour safety data could be reviewed prior to the main dosing visit. As there was only one participant with grass sensitivity, sentinel dosing did not take place in that circumstance. Sentinel dosing was not required for the 2014 cohort due to the lower dose of the study treatment.

Assessments

Primary endpoints

Adverse events (AEs) were monitored at each visit; cytokine release syndrome (CRS)-AEs (headache, fever, chills/rigor, nausea, arthralgia, myalgia, vomiting, diarrhea, hypotension; occurring within 24 hours post dose) were graded 0–4 according to severity, based on the National Cancer Institute–Common Toxicity Criteria guidelines. [1] Physical examination was performed and vital signs monitored at 10 hours post 1st dose, and 24 hours post 1st and 8th dose. Body temperature was measured pre-dose and 10, 12 (for the 2014 cohort) and 24 hours after each dose; this was performed by the participant if they were not present at the study site. Safety laboratory test samples were taken at 8 hours post 1st dose (hematology) and at 24 hours post 1st and 8th dose (hematology, clinical chemistry, urinalysis). Nasal tolerability assessment was performed via participants' symptom scoring on a 10 cm visual analog scale (VAS) at 10 hours and 24 hours post each dose to assess itching, discomfort, post-nasal drip, rhinorrhea and obstruction (ranging from 'No symptoms' to 'Worst symptoms ever experienced'). Nasal examination to assess tolerability was performed pre-dose and 24 hours post 1st and 8th dose.

Pharmacodynamic (PD) biomarker assays

Serum interferon(IFN)-inducible protein (IP)-10, tumor necrosis factor (TNF)- α and IFN- α levels were analyzed by Quest Diagnostics Clinical Laboratories (Madison, NJ, USA), using Searchlight™ IP-10 (lowest limit of quantification [LLQ] 54.6 pg/mL) and TNF α (LLQ 9.38 pg/mL) assays and IFN α enzyme-linked immunosorbent assay (ELISA; PBL, Piscataway, NJ, USA; LLQ 12.5 pg/mL). Nasal lavage IP-10, IL-6 and TNF- α levels were measured at GSK using R&D Systems (Minneapolis, MN, USA) human IP-10 ELISA (LLQ 8–16 pg/mL) and MSD (Rockville, MD, USA) V-Plex IL-6 (LLQ 0.13 pg/mL) and TNF α (LLQ 0.11 pg/mL) assays.

Allergic biomarker assays

Serum and nasal filter eluate samples were assayed at GSK using MSD (Rockville, MD, USA) V-Plex Th1/Th2 panel assays for interleukin (IL)-4, IL-5, IL-10, IL-12p70, IL-13, IL-16, IFN gamma, granulocyte-macrophage colony-stimulating factor (GM-CSF), thymus and activation-regulated chemokine (TARC), Eotaxin and macrophage derived chemokine (MDC); MSD custom Plex assays were used to measure IL-25, IL-33 and thymic stromal lymphopoietin. Serum total and allergen specific immunoglobulin (Ig) E levels were measured by Quest Diagnostics Clinical Laboratories; allergen specific IgG4 levels were assayed at GSK by ImmunoCAP (Thermo Scientific; Uppsala,

Sweden). Nasal lavage samples were analyzed for allergen specific IgE, IgA, IgG4 and eosinophil cationic protein (ECP) by ImmunoCAP.

Statistical analyses

The Bayesian approach to investigate mean effect sizes of exploratory endpoint findings allowed quantitative statements to be constructed from the posterior distributions that are more flexible/aligned to early stage clinical development (since non-informative priors were used for all parameters, the set of statistical analyses in this study could be reproduced using frequentist methods). The structure of the analyses is very similar; namely repeated measures modeling (participant as the repeated effect, unstructured variance covariance matrix common to each treatment arm). In the repeated measures model a baseline by visit by year interaction term was fitted along with a treatment by time by year interaction term. The additional interaction with year was added post hoc after reviewing data from 2013 and 2014 for several PD endpoints and noting differences in the range of values between years (prohibiting the planned pooling of placebo data and necessitating the year interaction term akin to the active dose treatments being compared with their respective year's placebo). Posterior distributions for the pre-specified treatment comparisons were constructed using appropriate combinations of the fitted model terms. These posterior distributions for treatment effects have been summarized using their medians and 95% equi-tailed credible interval. Probability statements are derived from the areas under the curve (e.g. probability of any reduction = $\text{AUC} [-\infty, 0]$); they can be interpreted as the levels of certainty.

The modeling approach was extended to include the 1-year follow-up visit as an additional time point. However this was done as a separate modeling step and only data from the 2014 cohorts were used (i.e. 20 ng and matching placebo participants). Note: If a 2014 participant did not participate in the 1-year follow-up study their FUV1 and FUV2 data were still used in the model to obtain better estimates of residual variability. This is the reason why the per-protocol population in study 204509 is larger than the number of participants who participated in the 1-year nasal allergen challenges. Results presented in the manuscript for FUV3 have come from a separate modeling activity to those presented for FUV1 and FUV2. To check the consistency the resulting treatment estimates from the FUV1 and FUV2 visits were compared between the two modeling activities and found to be acceptably similar (data not presented).

Supplementary results

Vital signs, clinical chemistry and hematology

There were four reports of high systolic blood pressure of potential clinical importance, three of which were recorded at screening (placebo n=1, 80 ng n=2) and one event at Dosing Visit (DV)1 (20 ng).

Clinical chemistry abnormalities of potential clinical importance were reported in 21 participants (placebo, n=9; 20 ng, n=5; 80 ng, n=7) and included elevated C-reactive protein levels, elevated alanine aminotransferase (placebo, n=3) and aspartate aminotransferase (placebo, n=2) levels, low potassium levels (placebo, n=1; 20 ng, n=2; 80 ng, n=1) and one report of increased blood glucose level in the placebo group at DV4, which was recorded as an AE.

A post hoc analysis revealed decreases in lymphocyte numbers at 24 hours post DV1 and DV8 in the 20 ng group, and at 8 hours and 24 hours post DV1 and 24 hours post DV8 in the 80 ng group, with greater than 95% certainty (Figure S1).

TABLES AND FIGURES

Table S1. Summary of treatment-related adverse events

Preferred term	Number (%) of participants		
	Placebo (N=14)	GSK2245035 20 ng (N=14)	GSK2245035 80 ng (N=14)
Any events	10(71)	6(43)	14(100)
Ear discomfort	0	0	1(7)
Ear pain	0	0	1(7)
Eye pruritus	1(7)	0	0
Photophobia	0	0	1(7)
Nausea	0	0	4(29)
Abdominal discomfort	0	0	1(7)
Hypoesthesia oral	1(7)	0	0
Pyrexia	0	0	10(71)
Chills	1(7)	1(7)	6(43)
Fatigue	1(7)	1(7)	2(14)
Feeling hot	0	1(7)	3(21)
Pain	0	0	3(21)
Influenza like illness	0	0	2(14)
Chest discomfort	0	0	1(7)
Feeling cold	0	0	1(7)
Subcutaneous abscess	0	0	1(7)
Upper respiratory infection	1(7)	0	0
Body temperature decreased	0	0	2(14)
Body temperature increased	0	0	1(7)
Arthralgia	0	1(7)	4(29)
Myalgia	0	1(7)	4(29)
Back pain	0	0	2(14)
Musculoskeletal stiffness	0	0	1(7)
Neck pain	0	0	1 (7)
Pain in extremity	0	0	1(7)
Headache	5(36)	5(36)	11 (79)
Dizziness	0	1(7)	3(21)
Lethargy	0	0	4(29)
Sinus headache	0	0	2(14)
Allodynia	0	0	1(7)
Dysgeusia	1(7)	0	0
Parosmia	0	0	1(7)
Somnolence	1(7)	0	0
Abnormal dreams	0	1(7)	0
Oropharyngeal pain	1(7)	0	4(29)
Cough	1(7)	0	2(14)
Rhinorrhea	1(7)	0	1(7)
Dyspnea	0	0	1(7)
Nasal congestion	0	1(7)	0
Nasal discomfort	0	0	1(7)
Oropharyngeal discomfort	0	0	1(7)
Throat irritation	0	0	1(7)
Tonsillar hypertrophy	0	0	1(7)
Upper-airway cough syndrome	0	1(7)	0
Hyperhidrosis	0	0	5(36)
Cold sweat	0	0	1(7)
Hot flush	0	0	1(7)

Table S2. Nasal examination findings at Dosing Visits 1 and 8

Nasal symptom	Dosing Visit 1						Dosing Visit 8					
	Pre-dose			24 h post dose			Pre-dose			24 h post dose		
	Placebo	20 ng	80 ng	Placebo	20 ng	80 ng	Placebo	20 ng	80 ng	Placebo	20 ng	80 ng
L nostril mucosal colour												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Normal	12 (86)	12 (86)	14 (100)	12 (86)	12 (86)	11 (79)	13 (100)	14 (100)	12 (100)	13 (100)	11 (85)	10 (83)
Abnormal	2 (14)	2 (14)	0	2 (14)	2 (14)	3 (21)	0	0	0	0	2 (15)	2 (17)
Red	1 (50)	2 (100)	0	1 (50)	1 (50)	3 (100)	0	0	0	0	1 (50)	2 (100)
White	1 (50)	0	0	1 (50)	1 (50)	0	0	0	0	0	1 (50)	0
R nostril mucosal colour												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Normal	12 (86)	12 (86)	14 (100)	11 (79)	13 (93)	11 (79)	13 (100)	13 (93)	12 (100)	12 (92)	11 (85)	11 (92)
Abnormal	2 (14)	2 (14)	0	3 (21)	1 (7)	3 (21)	0	1 (7)	0	1 (8)	2 (15)	1 (8)
Red	1 (50)	1 (50)	0	2 (67)	1 (100)	3 (100)	0	0	0	1 (100)	2 (100)	1 (100)
White	1 (50)	1 (50)	0	1 (33)	0	0	0	1 (100)	0	0	0	0
L nostril concha media turbinate swelling												
n, %	2 (100)	6 (100)	2 (100)	5 (100)	9 (100)	2 (100)	4 (100)	10 (100)	0	9 (100)	7 (100)	7 (100)
Absent	2 (100)	3 (50)	2 (100)	4 (80)	7 (78)	1 (50)	2 (50)	7 (70)	0	5 (56)	5 (71)	5 (71)
Mild	0	3 (50)	0	1 (20)	2 (22)	1 (50)	2 (50)	3 (30)	0	4 (44)	2 (29)	2 (29)
R nostril concha media turbinate swelling												
n, %	2 (100)	6 (100)	1 (100)	4 (100)	9 (100)	3 (100)	4 (100)	10 (100)	0	9 (100)	8 (100)	6 (100)
Absent	2 (100)	5 (83)	1 (100)	3 (75)	7 (78)	2 (67)	2 (50)	6 (60)	0	6 (67)	7 (88)	5 (83)
Mild	0	1 (17)	0	1 (25)	2 (22)	1 (33)	2 (50)	4 (40)	0	3 (33)	1 (13)	1 (17)
L nostril concha inferior turbinate swelling												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Absent	4 (29)	7 (50)	9 (64)	10 (71)	8 (57)	5 (36)	11 (85)	7 (50)	8 (67)	11 (85)	8 (62)	9 (75)
Mild	10 (71)	7 (50)	5 (36)	4 (29)	5 (36)	7 (50)	2 (15)	7 (50)	4 (33)	2 (15)	5 (38)	3 (25)
Severe	0	0	0	0	1 (7)	2 (14)	0	0	0	0	0	0
R nostril concha inferior turbinate swelling												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Absent	5 (36)	11 (79)	6 (43)	7 (50)	8 (57)	7 (50)	8 (62)	11 (79)	9 (75)	9 (69)	9 (69)	7 (58)
Mild	8 (57)	3 (21)	8 (57)	6 (43)	6 (43)	5 (36)	5 (38)	3 (21)	3 (25)	4 (31)	3 (23)	5 (42)
Severe	1 (7)	0	0	1 (7)	0	2 (14)	0	0	0	0	1 (8)	0

Table S2 continued. Nasal examination findings at Dosing Visits 1 and 8

Nasal symptom	Dosing Visit 1						Dosing Visit 8					
	Pre-dose			24 h post dose			Pre-dose			24 h post dose		
	Placebo	20 ng	80 ng	Placebo	20 ng	80 ng	Placebo	20 ng	80 ng	Placebo	20 ng	80 ng
L nostril secretions												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Absent	9 (64)	7 (50)	7 (50)	13 (93)	12 (86)	13 (93)	6 (46)	8 (57)	9 (75)	8 (62)	9 (69)	8 (67)
Normal	5 (36)	5 (36)	6 (43)	1 (7)	2 (14)	1 (7)	7 (54)	6 (43)	3 (25)	5 (38)	4 (31)	1 (8)
Abnormal	0	2 (14)	1 (7)	0	0	0	0	0	0	0	0	3 (25)
Clear	0	1 (50)	1 (100)	0	0	0	0	0	0	0	0	3 (100)
Opaque	0	1 (50)	0	0	0	0	0	0	0	0	0	0
R nostril secretions												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Absent	11 (79)	8 (57)	8 (57)	12 (86)	11 (79)	13 (93)	5 (38)	8 (57)	9 (75)	8 (62)	9 (69)	9 (75)
Normal	3 (21)	6 (43)	6 (43)	2 (14)	2 (14)	1 (7)	8 (62)	6 (43)	3 (25)	5 (38)	4 (31)	2 (17)
Abnormal	0	0	0	0	1 (7)	0	0	0	0	0	0	1 (8)
Clear	0	0	0	0	0	0	0	0	0	0	0	1 (100)
Purulent	0	0	0	0	1 (100)	0	0	0	0	0	0	0
Nasal infection												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Absent	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	11 (92)
Viral infection	0	0	0	0	0	0	0	0	0	0	0	1 (8)
Nasal post nasal drip												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Absent	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	10 (83)
Present	0	0	0	0	0	0	0	0	0	0	0	2 (17)
Clear	0	0	0	0	0	0	0	0	0	0	0	1 (50)
Opaque	0	0	0	0	0	0	0	0	0	0	0	1 (50)
Nasal crusting												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
Absent	13 (93)	12 (86)	13 (93)	14 (100)	14 (100)	14 (100)	13 (100)	11 (79)	10 (83)	11 (85)	10 (77)	11 (92)
Mild	1 (7)	2 (14)	1 (7)	0	0	0	0	3 (21)	2 (17)	2 (15)	3 (23)	1 (8)
Nasal signs of bleeding												
n, %	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	12 (100)	13 (100)	13 (100)	12 (100)
No	14 (100)	13 (93)	14 (100)	14 (100)	14 (100)	14 (100)	13 (100)	14 (100)	11 (92)	13 (100)	12 (92)	12 (100)
Yes	0	1 (7)	0	0	0	0	0	0	1 (8)	0	1 (8)	0

Table S3. Change from baseline in TNSS at 15 minutes post NAC, WM 0–1 hour and WM 0–6 hour post NAC

Treatment groups	Visit	Endpoint	Median	SD	95% CrI	PP (active minus placebo) (PP<0)
GSK2245035 20 ng	FUV1	15 min	-1.9	1.352	-4.57, 0.77	0.922
		WM 0–1 h	-1.8	0.622	-3.00, -0.54	0.997
		WM 0–6 h	-1.6	0.529	-2.64, -0.55	0.998
	FUV2	15 min	-1.3	1.374	-4.09, 1.35	0.841
		WM 0–1 h	-0.9	0.909	-2.69, 0.90	0.842
		WM 0–6 h	-0.0	0.698	-1.37, 1.38	0.495
	FUV3	15 min	+2.1	1.716	-1.41, 5.43	0.107
		WM 0–1 h	+1.0	1.443	-1.98, 3.75	0.242
		WM 0–6 h	+0.4	0.741	-1.08, 1.87	0.288
GSK2245035 80 ng	FUV1	15 min	-1.8	1.457	-4.65, 1.09	0.892
		WM 0–1 h	-2.0	0.646	-3.30, -0.76	0.999
		WM 0–6 h	-1.6	0.554	-2.71, -0.53	0.998
	FUV2	15 min	-1.4	1.487	-4.38, 1.47	0.838
		WM 0–1 h	-1.0	0.943	-2.89, 0.83	0.867
		WM 0–6 h	-1.4	0.723	-2.87, -0.02	0.976

CrI, credible interval; FUV, follow-up visit; NAC, nasal allergen challenge; PP, posterior probability; SD, standard deviation; TNSS, total nasal symptom score; WM, weighted mean.

*If these probabilities exceed 0.975 it would be the equivalent of a statistically significant difference from a two-sided test at 5% type 1 error rate. If the active is identical to placebo the probability would be 0.5.

FUV3 visit only applicable to 20 ng treatment and results obtained from separate statistical modeling activity to FUV1 and FUV2. The inverted mean treatment effect at FUV3 may be due to the small sample size meaning that atypical response patterns from individuals had a high influence on the means (see Figure S3 for individual participant TNSS).

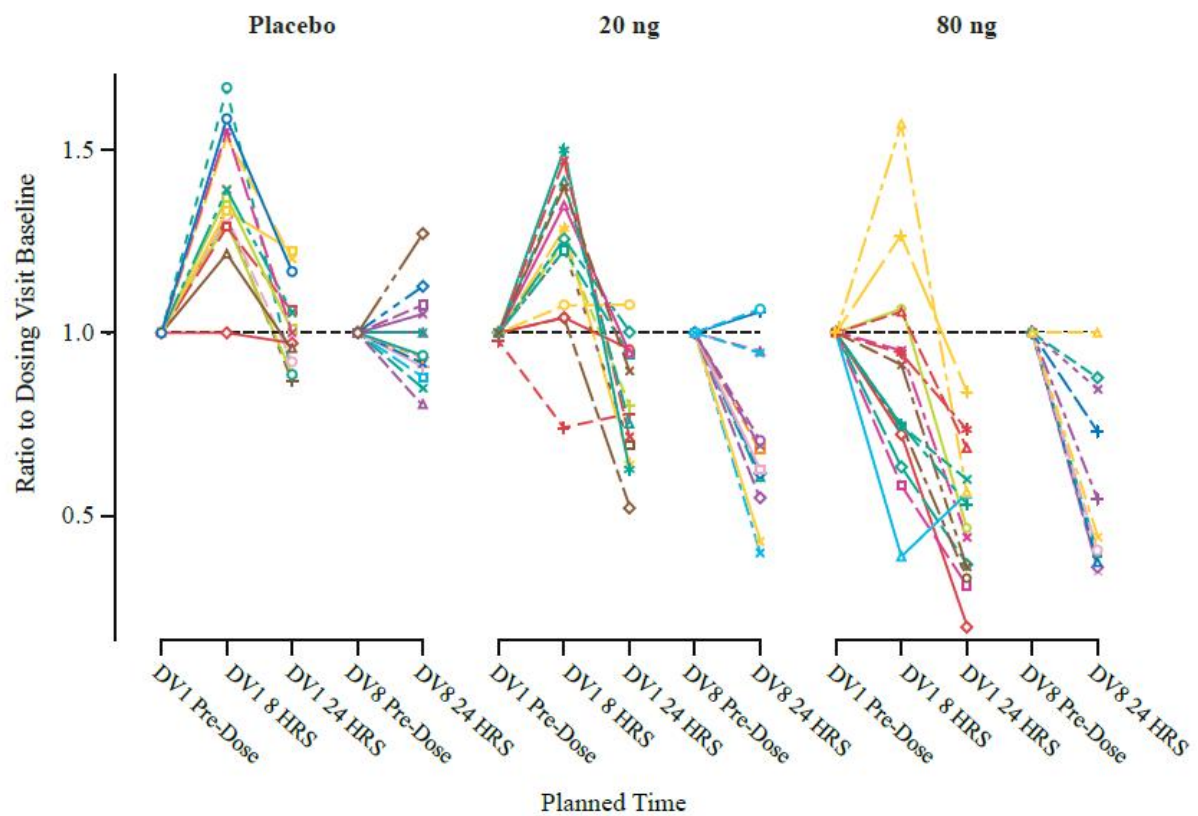
Table S4. Nasal allergic biomarkers (all participants)

Analyte	Treatment comparison*	Median ratio (95% CrI)	PP (median ratio active/placebo)	
Th1/T regulatory cytokine				
IL-10 (pg/mL)	20 ng FUV1	1.05 (0.133, 8.175)	PP≥1	0.5180
	80 ng FUV1	0.57 (0.065, 5.049)		0.3011
	20 ng FUV2	1.10 (0.226, 5.347)		0.5460
	80 ng FUV2	0.95 (0.173, 5.157)		0.4775
	20 ng FUV3	0.49 (0.026, 10.313)		0.3136
Th2 cytokines/chemokines				
IL-33 (pg/mL)	20 ng FUV1	0.54 (0.100, 2.891)	PP≤1	0.7671
	80 ng FUV1	0.87 (0.139, 5.361)		0.5640
	20 ng FUV2	6.75 (1.135, 39.747)		0.0179
	80 ng FUV2	1.25 (0.179, 8.504)		0.4088
	20 ng FUV3	1.31 (0.299, 6.251)		0.3534
IL-5 (pg/mL)	20 ng FUV1	1.58 (0.248, 10.069)	PP≤1	0.3117
	80 ng FUV1	0.56 (0.074, 4.236)		0.7138
	20 ng FUV2	0.69 (0.120, 3.964)		0.6636
	80 ng FUV2	0.53 (0.077, 3.479)		0.7504
	20 ng FUV3	0.28 (0.014, 5.610)		0.8068
IL-16 (pg/mL)	20 ng FUV1	0.59 (0.159, 2.197)	PP≤1	0.7891
	80 ng FUV1	1.00 (0.240, 4.172)		0.5031
	20 ng FUV2	0.99 (0.265, 3.768)		0.5067
	80 ng FUV2	1.10 (0.258, 4.565)		0.4480
	20 ng FUV3	0.22 (0.057, 0.841)		0.9853
Eotaxin (pg/mL)	20 ng FUV1	1.46 (0.245, 8.717)	PP≤1	0.3336
	80 ng FUV1	0.45 (0.066, 3.178)		0.7935
	20 ng FUV2	1.56 (0.376, 6.405)		0.2663
	80 ng FUV2	0.41 (0.089, 1.872)		0.8807
	20 ng FUV3	2.45 (0.596, 10.299)		0.1013
MDC (pg/mL)	20 ng FUV1	2.11 (0.507, 8.606)	PP≤1	0.1435
	80 ng FUV1	0.39 (0.085, 1.746)		0.8930
	20 ng FUV2	1.26 (0.307, 5.000)		0.3705
	80 ng FUV2	0.34 (0.076, 1.517)		0.9246
	20 ng FUV3	2.52 (0.944, 7.256)		0.0321
TARC (pg/mL)	20 ng FUV1	1.96 (0.302, 12.635)	PP≤1	0.2341
	80 ng FUV1	0.30 (0.040, 2.221)		0.8835
	20 ng FUV2	1.08 (0.167, 6.900)		0.4657
	80 ng FUV2	0.44 (0.060, 3.250)		0.7929
	20 ng FUV3	1.88 (0.417, 8.270)		0.1939
Effector mediators				
ECP (µg/L)	20 ng FUV1	0.44 (0.093, 2.071)	PP<1	0.8580
	80 ng FUV1	0.99 (0.202, 4.956)		0.5041
	20 ng FUV2	0.28 (0.080, 0.961)		0.9783
	80 ng FUV2	1.68 (0.458, 6.353)		0.2141
	20 ng FUV3	0.74 (0.227, 2.284)		0.7061
Allergen Specific IgA (µg/mL)	20 ng FUV1	0.64 (0.269, 1.543)	PP>1	0.1562
	80 ng FUV1	2.17 (0.820, 5.661)		0.9433
	20 ng FUV2	0.43 (0.188, 1.023)		0.0276
	80 ng FUV2	1.77 (0.689, 4.514)		0.8875
	20 ng FUV3	1.24 (0.564, 2.707)		0.7134

CrI, credible interval; ECP, eosinophil cationic protein; FUV, follow-up visit; IL, interleukin; MDC, macrophage-derived chemokine; NAC, nasal allergen challenge; PP, posterior probability; TARC, thymus and activation-regulated chemokine.

*([GSK2245035 post/pre-NAC at FUV] / [GSK2245035 post/pre-NAC at screening]) / ([placebo post/pre-NAC at FUV] / [placebo post/pre-NAC at screening]). FUV3 visit only applicable to 20 ng treatment and results obtained from separate statistical modeling activity to FUV1 and FUV2.

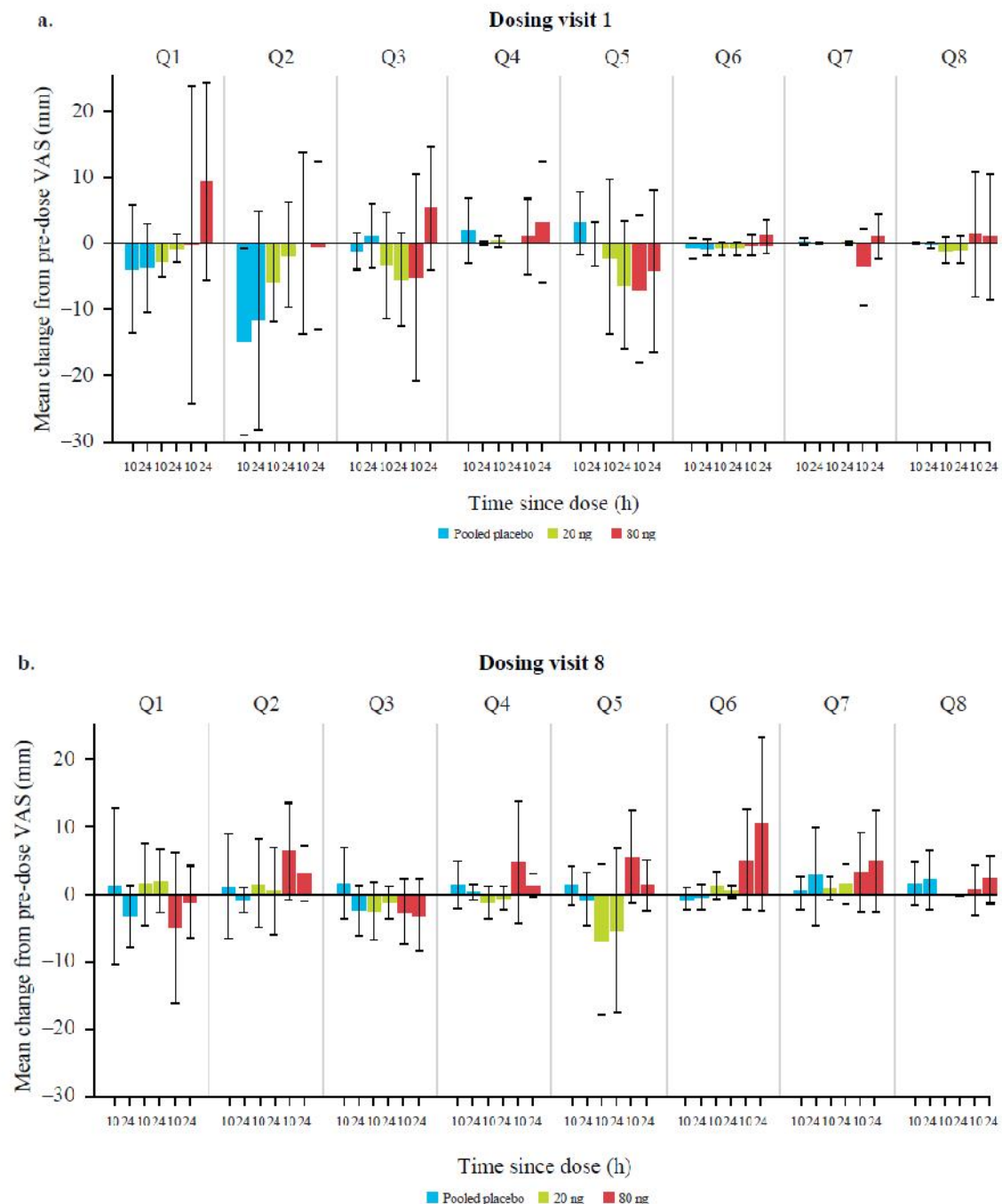
Figure S1. Change in lymphocyte numbers at DV1 and DV8 as a ratio of baseline



DV1, Dosing Visit 1; DV8, Dosing Visit 8.

Each line represents an individual participant.

Figure S2. Mean change from respective visits pre-dose nasal tolerability VAS scores and 95% CIs* at (a) DV1 and (b) DV 8

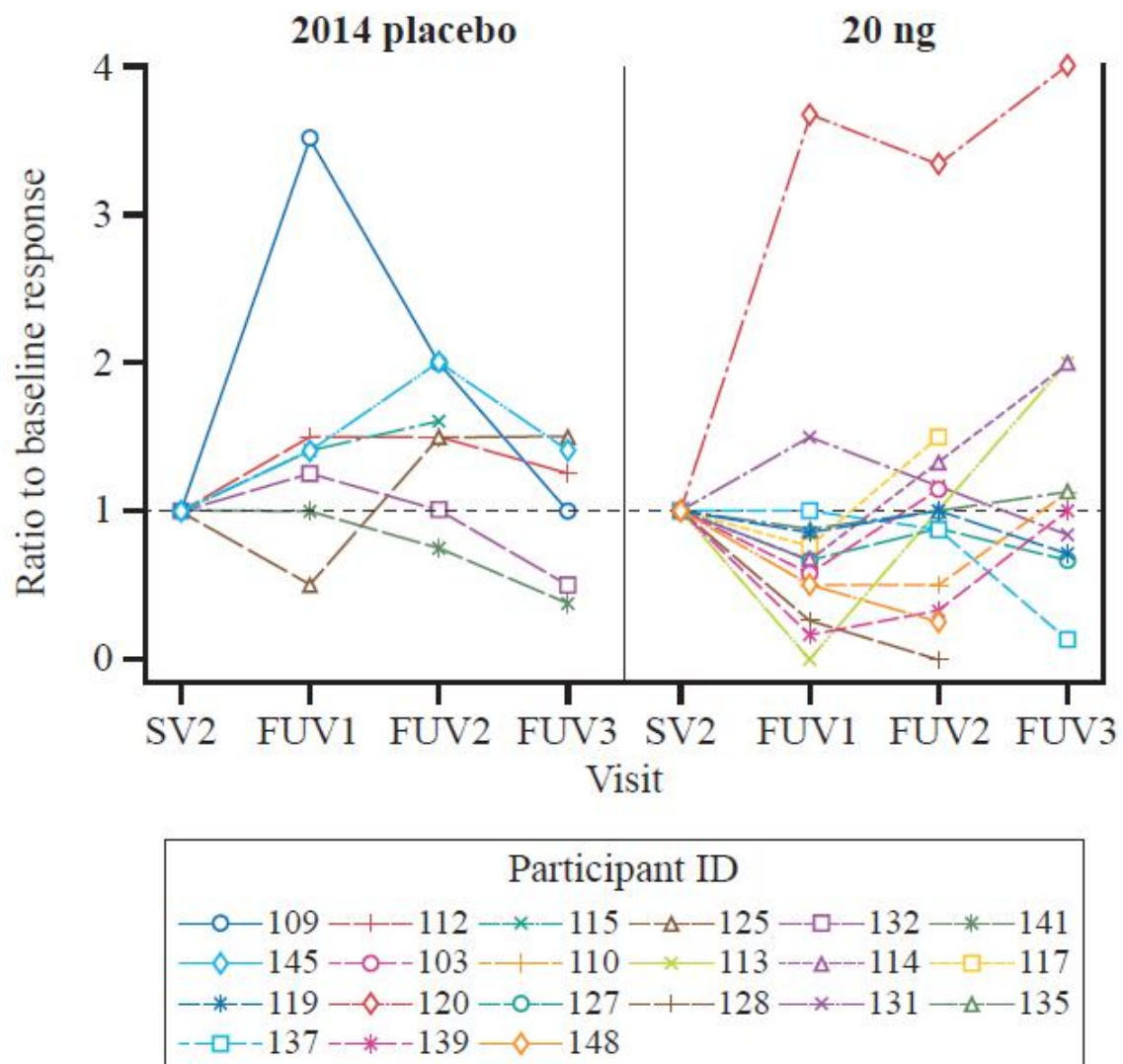


CI, confidence interval; DV, Dosing Visit; VAS, visual analog scale.

*Derived from the raw data points.

Q1,nose not/extremely runny; Q2,nose clear/completely blocked; Q3,nose not/extremely itchy; Q4,nose not sore/as sore as it has ever been; Q5,nose not dry/as dry as it has ever been; Q6,throat not sore/as sore as it has ever been; Q7,sense of taste normal/completely abnormal; Q8,sense of smell normal/completely abnormal; VAS: 0=no symptom, 100=worst symptom.

Figure S3: Change from baseline (ratio to screening visit) in TNSS at 15 minutes post NAC by individual participant



FUV, follow-up visit; NAC, nasal allergen challenge; TNSS, total nasal symptom score; SV, screening visit.

At FUV1 the consistent reduction in TNSS in the majority of participants was strong enough for this “signal” to override Participant 120’s profile. However, at FUV3 the reduction signal is not as strong, and Participant 120’s influence is more evident, especially as the placebo mean response is reduced at FUV3.

REFERENCE

[1] (CTEP) NCICTEP. Common Terminology Criteria for Adverse Events (CTCAE). 2009. Available from: <http://evs.nci.nih.gov/ftp1/CTCAE/About.html> [25 February 2015].