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DOI:
[10.1016/j.jaci.2017.06.027](https://doi.org/10.1016/j.jaci.2017.06.027)

Document Version
Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

Silkoff, P. E., Flavin, S., Gordon, R., Loza, M. J., Sterk, P. J., Lutter, R., Diamant, Z., Turner, R. B., Lipworth, B. J., Proud, D., Singh, D., Eich, A., Backer, V., Gern, J. E., Herzmann, C., Halperin, S. A., Mensinga, T. T., Del Vecchio, A. M., Branigan, P., ... Johnston, S. L. (2017). TLR3 Blockade in Rhinovirus-Induced Experimental Asthma Exacerbations: A Randomized Controlled Study. *Journal of Allergy and Clinical Immunology*.
<https://doi.org/10.1016/j.jaci.2017.06.027>

Published in:
Journal of Allergy and Clinical Immunology

Citing this paper

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TLR3 Blockade in Rhinovirus-Induced Experimental Asthma Exacerbations: A Randomized Controlled Study

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Abstract

Background

Human rhinoviruses (HRV) commonly precipitate asthma exacerbations. TLR3, an innate pattern-recognition receptor, is triggered by HRV driving inflammation that may worsen asthma.

Objective

To evaluate an inhibitory monoclonal antibody to TLR3, CNTO3157, on experimental HRV-16 inoculation in healthy and asthmatic subjects.

Methods

In this double-blind multicenter randomized parallel-group study in North America and Europe, healthy and mild-moderate stable asthmatic subjects received single or multiple doses of CNTO 3157 or placebo, respectively, and were then inoculated with HRV-16 within 72 hours. All subjects were monitored for respiratory symptoms, lung function, and nasal viral load. The primary endpoint was maximal decline in forced expired volume in 1 second (FEV₁) during 10-days post-inoculation.

Results

In asthmatic subjects (N=63), CNTO3157 provided no protection against FEV₁ decline (LS mean [SE]: CNTO3157 (n=30) = -7.08 [8.15] % and placebo (n=25) = -5.98 [8.56] %), or symptoms post-inoculation. In healthy subjects (N=12), CNTO3157 versus placebo significantly attenuated upper (p=0.03) and lower (p=0.02) airway symptom scores, with area-under-the-curve increases of 9.1(15.1) vs 34.9 (17.6) and 13.0 (18.4) vs 50.4 (25.9) for the CNTO3157 group (n=8) and placebo group (n=4), respectively, after inoculation. All of the severe and three of the four non-serious asthma exacerbations occurred on CNTO3157.

Conclusion

In summary, CNTO3157 was ineffective in attenuating the impact of HRV-16 challenge on lung function, asthma control, and symptoms in asthma, but suppressed cold symptoms in healthy subjects. Other approaches, including blockade of multiple pathways, or antiviral agents, need to be sought for this high unmet medical need.

Key Messages

- TR3 signaling is triggered by common respiratory viruses and could play a role in the worsening airway inflammation in asthma exacerbations of viral origin.
- Blockade of TLR3 was ineffective in attenuating the respiratory manifestations of experimental rhinovirus challenge in mild-moderate persistent asthmatic subjects but did suppress cold symptoms in healthy volunteers.

Capsule summary

Blockade of TLR3, a major viral-sensing receptor, was ineffective in reducing the impact of rhinovirus infection, a common precipitant of asthma exacerbations, on asthma symptoms and lung function.

Key words: asthma, viral infection, inflammation, TLR3

Public registry numbers: The study was registered on the clinicaltrials.gov website (US registration number= NCT01704040) and the EU registration site (EudraCT), (registration number= 2011-005369-19).

The study was sponsored by Janssen R&D, Spring House, PA, USA

Abbreviations	
ACQ7	Asthma Control Questionnaire 7
ADA	antidrug antibodies
AE	adverse event
AM	morning
AUC	area under the curve
BD	bronchodilator
CCSS	Cold and Chest Symptom Scale
CCL	C-C motif chemokine ligand
CSAS	Cold Symptom Assessment Score
CPE	Cytopathic effect
CRP	C-reactive protein

CST	cystatin
CXCL	C-X-C motif ligand
dsRNA	Double stranded ribonucleic acid
ECG	electrocardiogram
FENO	fractional concentration of exhaled nitric oxide
FEV ₁	forced expired volume in 1 second
HC	Healthy controls
HRV	human rhinovirus
HSV-1	herpes simplex virus 1
ICS	inhaled corticosteroid
IFN	interferon
IL	interleukin
IP10	interferon gamma-induced protein 10
LS	least squares
MCP	macrophage chemoattractant protein
MDA-5	melanoma differentiation associated gene-5
mITT	modified intention to treat
MRC	Medical Research Council
PD	pharmacodynamics
PEFR	peak expiratory flow rate
PI	post-inoculation
PK	pharmacokinetics
poly-I:C	polyinosinic:polycytidylic acid

PRR	pattern recognition receptors
RANTES	Regulated on Activation, Normal T Cell Expressed and Secreted
RIG-I	retinoic acid inducible gene- I
Pre-BD	pre-bronchodilator
RNA	ribonucleic acid
sIL-33R	Soluble IL-33 receptor
TCID	tissue culture infective dose
TEAE	treatment-emergent adverse event
TLR3	Toll like receptor 3
TNOSS	Total Nasal and Ocular Symptom Score

Introduction

Acute asthma exacerbations are episodes of worsening symptoms that may lead to augmentation of asthma therapy, hospitalization, and on occasions death. Asthma exacerbations are most often associated with respiratory viruses, with an estimated 65-85% of all viral exacerbations in children and 50% in adults being caused by human rhinovirus (HRV).

While symptoms due to HRV are usually restricted to the upper airway in non-asthmatic subjects, in asthma, lower respiratory symptoms including cough, dyspnea, and wheezing are common. The mechanisms may include direct infection of the lower airway^{1,2}, with worsening of inflammation due to host defense mechanisms. The pathogenesis of HRV in the upper and lower airway has been extensively reviewed³. HRV infects a subset of cells in the respiratory epithelium, and viral replication initiates antiviral and pro-inflammatory responses through several molecular pathways.

Pattern recognition receptors (PRR) that respond to HRV include several toll like receptors (TLR)s including TLR3, as well as the ribonucleic acid (RNA) helicases, melanoma differentiation associated gene-5 (MDA-5) and retinoic acid inducible gene- I (RIG-I)⁴. Inflammatory mediators released due to PRR signaling pathways include Type I interferons (IFN- α / β), and Type III interferons (IFN- λ 1-4), interleukin (IL)-6, IL-12, and IL-15^{4,5}. Additionally, chemokines including C-X-C motif ligand (CXCL)10/IFN gamma-induced protein (IP-10)⁶ drive the recruitment of inflammatory cells, e.g. natural killer cells and Type 1 lymphocytes.

Preclinical studies demonstrated that an anti-TLR3 monoclonal antibody (mAb) can block polyinosinic:polycytidylic acid (poly(I:C))-induced inflammation in-vivo and in vitro⁷, and can down-regulate poly(I:C)-induced production of inflammatory cytokines/chemokines (IL-6, IL-8/CXCL8, CCL2/MCP-1, CCL5/ Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES), and CXCL10 /IP-10 in human lung epithelial cells)⁸. Antagonism of TLR3 also reduced mortality in an in-house mouse influenza model (unpublished data), consistent with published studies using TLR3-deficient mice⁹⁻¹¹. We hypothesized that blockade of TLR3 signaling would attenuate the impact of HRV infection in asthma.

CNTO3157 is a fully human IgG4 kappa mAb that binds cell-surface TLR3, prevents association of dsRNA with TLR3, and thereby inhibits TLR3 signaling-dependent generation of cytokines and other inflammatory mediators. Extensive published human in vitro and murine in vivo experiments supported the hypothesis that CNTO 3157 would be an effective agent to suppress the inflammatory effects induced by rhinovirus infection (see online repository Section E1)

Herein, we present the impact of CNTO3157 compared to placebo on the respiratory manifestations of inoculation with HRV-16 in healthy subjects and in subjects with mild-moderate persistent asthma.

Methods

Study Design

This was a 2-part, randomized, multicenter, double-blind, parallel-design, placebo-controlled study to evaluate the efficacy and safety of CNTO3157 preceding inoculation with HRV-16. The study was approved by regional health authorities and ethics committees relevant for each investigational site. All participants demonstrated understanding of the study procedures and provided written consent before any study procedures. The full study protocol can be found at the following link (TBD).

Subjects

Healthy non-smoking control subjects (HC) and subjects with mild to moderate persistent asthma aged 18-65 years were recruited. HC were required to have no clinically significant abnormalities as determined by medical history, physical examination, blood laboratory parameters, and electrocardiography.

Asthma subjects had a physician diagnosis of mild to moderate asthma for at least 6 months prior to screening, recently stable asthma based on physician assessment, an Asthma Control Score 7 (ACQ7)¹² symptom score of <1.5 (amended later in the study to <2.5) and a pre-bronchodilator (BD) FEV₁ ≥65 % predicted. Low to medium dose inhaled corticosteroids (ICS) (based on the National Heart, Lung, and Blood Institute Guidelines¹³ were permitted with additional controllers excluding oral corticosteroids and biologic therapies. Subjects with prior life-threatening asthma were excluded.

Subjects had a titer of serum neutralizing antibodies to HRV-16 ≤2-fold dilution, and previous serological confirmation of prior infection with herpes simplex 1 (HSV-1) due to associations of null TLR3 polymorphisms and childhood HSV-1 encephalitis¹⁴.

Randomization

Based on a computer-generated randomization schedule prepared before the study by an interactive voice or web response system provider, healthy subjects in Part 1 were randomly assigned to 1 of 2 treatment groups in a 2:1 ratio (CNTO 3157 versus placebo) and asthma subjects in Part 2 were randomly assigned to 1 of 2 treatment groups in a 1:1 ratio (CNTO 3157 versus placebo). The placebo used in the study consisted of the identical diluent used for CNO3157 with no discernible visual differences prepared on the day of administration by an independent person e.g. a pharmacist, who was not part of the study team.

Dose selection

The dosing regimen selected for CNTO3157 had been previously evaluated for safety, PK and PD effects in the first in human study in healthy volunteers, who received ascending single doses up to 10mg/kg, and in asthmatic subjects, who received 4 doses up to 10mg/kg of CNTO3157 at weekly intervals (NCT01195207). More details can be found in the online repository in **Section E1**.

Part 1: Healthy Subjects

The principal objective of Part 1 was to evaluate the safety of CNTO3157 followed by nasal inoculation of HRV-16 in approximately 12 healthy adult subjects.

Healthy subjects attended 2 screening visits to confirm eligibility and were then randomized on Day 1 using a 2: 1 ratio to receive a single IV dose of CNTO3157, 10mg/kg, or matching placebo, followed by inoculation of HRV-16 within 24 to 72 hours. Subjects attended the study unit daily for 5 days, at Day 7, and at Day 10 post-inoculation for study assessments and follow-up of adverse events (AEs). Additional follow-up visits occurred at approximately 4 and 8 weeks post-randomization. **Figure 1** presents a schematic of the study design for Part 1 (upper panel).

Part 1: Outcome Measures

Outcome measures included safety, pharmacokinetics (PK), and immunogenicity. The severity of the HRV-16-induced upper respiratory tract infection was assessed once daily using a cold symptoms assessment scale (CSAS) based on a modified Jackson scale ¹⁵, and a combined cold and chest symptom score (CCSS) that was based on Jackson et al ¹⁶ (questionnaire provided by SLJ). Additional assessments included exhaled nitric oxide (FENO), spirometry, blood biomarkers, and nasal lavage for viral assessments.

Part 2: Subjects with mild-moderate persistent asthma

The principal objective was to evaluate the efficacy of CNTO3157 for attenuating upper and lower respiratory manifestations following HRV-16 inoculation. We hypothesized that TLR3 blockade would attenuate the respiratory manifestations of HRV-16 in asthma.

Subjects attended up to 3 screening visits and were then randomly assigned using a 1:1 allocation to intravenous (I/V) CNTO3157 10mg/kg or placebo on Day 1, followed by 3 additional weekly doses of CNTO3157 3mg/kg I/V or placebo followed by inoculation of HRV-16 within 24-72 hours of the last dose. Subjects attended the study unit daily for 5 days, and then at Day 7 and Day 10 post-inoculation for study assessments including spirometry. Additional follow-up visits occurred at approximately 7 and 11 weeks post-randomization. **Figure 1 (lower panel)** presents a schematic of the study design for Part 2 .

Part 2 outcome measures

The primary endpoint was the maximum % decrease relative to pre-inoculation in all pre-BD FEV₁ measurements assessed at each visit from Day 1 to Day 10 post-inoculation (PI). Major secondary endpoints included the CCSS, the CSAS, area under the curve (AUC) AM peak flow rate (PEFR) and AUC pre-BD FEV₁, both over the 10 days post-inoculation, and the change from baseline in ACQ7 at day 10 post-inoculation.

Other endpoints include FENO, a total nasal and ocular symptom score (TNOSS), which was assessed during the pre-inoculation treatment phase to assess any anti-allergic benefit, nocturnal awakenings, and rescue medication use. Other assessments included the incidence of AEs, PK, and immunogenicity.

Biomarker assessments

Whole blood, serum, and nasal lavage samples and nasal brushing (Part 2 only) for biomarker analyses were collected and analyzed for the presence/absence of HRV-16, HRV-16 titers, and other biomarkers. Biomarkers were assessed in nasal lavage, blood, and sputum for proteomics (Somalogics platform) and transcriptomics. A subset of subjects participated in sputum induction during screening, before, and after inoculation with HRV-16. Detailed biomarker analyses can be found in **Section E2** of the online repository.

HRV challenge virus

The strain of HRV-16 used was isolated via nasal lavage from a subject in a clinical study at the University of Virginia. Details about the origin of the virus appear in Section E3 of the online repository. A confirmed infection with HRV-16 was defined as a positive culture from nasal lavage at any time in the 5 days post-inoculation, and/or a 4-fold serological conversion to HRV-16 assessed at the week 8 or 11 visits.

Safety

Safety was evaluated by assessment of adverse events, clinical laboratory tests (hematology, serum chemistry, and urinalysis), vital signs, physical examinations and electrocardiograms (ECG). Safety data obtained during the study were unblinded and reviewed on a routine basis by an independent data monitoring committee.

The safety analysis set included all subjects who received at least one dose of CNTO3157. If an event was judged by the investigator to be related to study agent, investigators had the option of attributing AEs to active drug, placebo, or the HRV-16 inoculum.

Asthma exacerbations were defined *a priori* as “severe” or “moderate”. Severe exacerbations were defined as worsening of asthma requiring use of systemic corticosteroids and/or hospitalization. Moderate exacerbations were defined as a deterioration in lung function ($\geq 30\%$

decrease in the mean AM PEFr from baseline) lasting for 2 days or more, and/or increased rescue bronchodilator use (≥ 3 additional puffs of rescue medication in 24 hours over the mean rescue medication use defined as the mean number of puffs taken during the 7 days prior to randomization).

Statistical analysis

The sample size calculation was based on the primary endpoint, the maximum percent decrease relative to baseline in the pre-BD FEV₁ measurements assessed at each visit through 10 days following inoculation with HRV-16. Based on Message et al¹⁷, for 80% power to detect a relative reduction in FEV₁ of 50% (from 13% decline for placebo to 6.5%) with a standard deviation (SD) of 10% using a 2-sided t-test at a 0.1 level of significance, 60 subjects (30/arm) were required. A 0.1 level of significance was selected because this was an early development proof of concept study.

Demographic and baseline disease characteristic data were summarized by treatment group. Descriptive statistics were used to summarize continuous variables. Counts and percentages were used to summarize categorical variables. Categorical data were analyzed using appropriate tests (chi-square tests, CMH chi-square tests, or logistic regression). Continuous responses were analyzed using the same statistical method as in the primary efficacy analysis. Nonparametric methods were adopted when the normality assumption was violated. For efficacy analysis, data was analyzed according to the assigned treatment group. No corrections were made for multiple comparisons.

Primary efficacy analyses in Part 2 was based on a modified intention to treat (mITT) HRV set including randomized subjects who received at least 1 (partial or complete) dose of CNTO3157 or placebo, had at least 1 efficacy measurement prior to HRV-16 inoculation, were inoculated with HRV-16, and had at least 1 post-inoculation efficacy measurement during HRV-16 infection phase. The mITT set was defined as subjects who received at least 1 (partial or complete) dose of CNTO3157 or placebo, had at least 1 efficacy measurement prior to study agent infusion, and had at least 1 post treatment efficacy measurement during the treatment phase.

Safety, PK, and pharmacodynamic (PD) analyses in Part 1 and Part 2 included all subjects treated with study agent and were summarized based on the actual treatment received. Some safety, PK, and PD analyses were performed on the population inoculated with HRV-16.

The sponsor of the study, Janssen R&D Inc., wrote the protocol, and performed the analysis of the study. This manuscript was written by Janssen and reviewed by all authors.

Results

Disposition

Part 1 was conducted at a single center in Belgium, while **Part 2** was conducted at multiple sites in Canada, Denmark, Germany, Great Britain, and the Netherlands, from Sep 24th 2012 until Nov 17 2014. The screen failure rate was high (~93%) (771 subjects screened to randomize 63 subjects), driven primarily by serological entry requirements (HRV-16 negative and HSV-1 positive). **Figure 2** displays the disposition of subjects in the study, which had a high completion rate.

Part 1: healthy subjects

Thirteen healthy subjects were randomized into Part 1. Nine subjects received CNTO3157 and 4 placebo; 1 subject on active therapy was not inoculated due to an AE of vomiting, attributed to viral gastroenteritis. Baseline demographics were similar across the two treatment groups. All subjects were white with a mean age of 53.6 years (ranging from 34 to 65 years) and primarily (61.5%) male. All inoculated subjects on CNTO3157 and placebo had confirmed infection with HRV-16 as defined in the methods section. No deaths, serious AEs, or other significant AEs occurred. The vast majority of AEs were mild in severity and self-limiting, and none were reported as related to CNTO3157. Seven subjects on CNTO3157 experienced at least 1 AE (77.8%) compared with 4 subjects (100%) on placebo.

There was significant inhibition ($p=0.03$) of the CSAS in subjects on CNTO3157 compared with placebo, with a mean (SD) AUC of the change from pre-inoculation through 10 days post-inoculation of 9.1(15.1) for the CNTO3157 group vs 34.9 (17.6) for the placebo group. Similarly, there was significant inhibition ($p=0.02$) of the CCSS in subjects on CNTO3157 compared with placebo, with a mean (SD) AUC of the change from pre-inoculation through 10 days post-inoculation of 13.0 (18.4) for the CNTO3157 group vs 50.4 (25.9) for the placebo group, as depicted in **Figure 3**.

Part 2: asthmatic subjects

Demographical characteristics

The mean age of the asthmatic subjects was 38.9 years (range 18 to 65 years), and subjects were primarily (65.1%) male. Demographics and disease characteristics were generally similar between treatment groups. Sixty (95.2%) of the subjects were white. The asthma characteristics of randomized subjects are summarized in **Table 1**. Overall, disease characteristics were similar across treatment groups, with no significant between-group

differences. In general, the asthma of the subjects was mild in severity and well-controlled on enrolment (mean ACQ7 scores <1.0). A higher proportion of subjects in the placebo group (70%) vs 58% of subjects on CNTO3157 reported ICS use at enrolment.

Disposition

Sixty-three asthmatic subjects were randomized of whom 61 subjects received at least 1 dose of the study medication with 53 subjects completing all 4 doses. Thirty CNTO 3157 subjects and 25 placebo subjects were inoculated with HRV-16. All 61 randomized subjects completed all scheduled visits. The proportion of confirmed infected subjects in the CNTO3157 group (24/30 80.0%) was significantly lower than the placebo group (22/25; 88.0%), $p=0.031$.

Primary endpoint

For the primary analysis set inoculated with HRV (modified intention to treat [mITT] HRV), no significant difference ($p=0.60$) was found between treatment groups for percentage change post inoculation from pre-inoculation baseline in pre-bronchodilator (pre-BD) FEV₁ (LS mean [SE]: CNTO3157 group (n=30) = -7.08 [8.15] % and placebo group (n=25) = -5.98 [8.56] %).

Two pre-specified sensitivity analyses were performed for the primary efficacy endpoint. Sensitivity analysis 1 included only those subjects who had all scheduled pre-BD FEV₁ measurements for 10 consecutive days following inoculation with HRV-16 (24/25 on placebo and 27/30 on CNTO3157). Sensitivity analysis 2 directly compared the treatment effect among those subjects in the mITT HRV analysis set (n=55) who were infected after inoculation with HRV-16 (22 on CNTO3157 and 24 on placebo). No significant differences were found between subgroups for sensitivity analyses 1 and 2 of the primary efficacy endpoint.

The following pre-specified subgroups were analyzed using baseline disease characteristics and concomitant asthma therapy (use of ICS):

- Pre-BD FEV₁ (< Median, ≥ Median).
- Exhaled nitric oxide (FENO); <Median ≥ Median)
- Blood eosinophils (< Median, ≥ Median)
- ACQ7 symptom score (> 1.5, ≤ 1.5)
- ICS use (Yes, No)

There were no significant differences observed in any of the subgroup analyses that were conducted in regard to the primary endpoint.

Figure 4 shows the percentage change in pre-BD FEV₁ from pre-inoculation baseline to 21 days post-inoculation for the primary analysis set. The fall in FEV₁ was approximately 50% of expected based on powering assumptions as detailed in the methods section.

Secondary endpoints

Major secondary analyses are presented in **Table 2**. Both treatment groups showed worsening in all major secondary endpoints with no significant difference between treatment with CNTO3157 or placebo. Except for the AUC over 10 days post-inoculation for Pre-BD FEV₁, the changes were numerically higher in the CNTO3157 group but not to a clinically-meaningful degree. The changes in CSAS and CCSS scores from pre-inoculation baseline are shown in **Figure 5**. Following HRV-16 inoculation, both treatment groups showed acute elevations in mean scores for both symptom scales, which peaked around Day 3 post-inoculation but resolved more quickly in the placebo group. The scores for the CSAS and CCSS were numerically greater for those subjects on CNTO3157 compared with placebo.

There was a trend for improvement in TNOSS scores ($p=0.07$) for the CNTO3157 group compared to placebo at Week 4. The CCSS and CSAS scores were stable in both treatment groups during this phase.

Figure 6 shows the percentage change from baseline for pre-BD FEV₁ during the treatment phase before HRV-16 inoculation in Part 2 from the mITT analysis set, to evaluate the impact of CNTO3157 vs placebo on lung function after HRV-16 inoculation. There were no significant or clinically meaningful differences for pre-BD FEV₁ between CNTO 3157 and placebo.

The pharmacokinetic (PK) profiles of CNTO3157 in Part 1 and Part 2 were similar to the profiles seen in the first in human study (NCT01195207) for similar doses and dosing regimens (data not shown). Only 1 subjects in Part 2 had antidrug antibodies. **See Section E4** of the online repository for further details.

Safety

There were no serious adverse events in Part 1 or Part 2. In Part 2, five CNTO3157 treated subjects (17%) had asthma exacerbations post-inoculation. Two of the 5 subjects had protocol-defined severe exacerbations (use of systemic steroids). The 2 severe exacerbations occurred on Days 3, and 13 post-inoculation while the 3 moderate exacerbations occurred on Days 2, and at Weeks 7 and 11 post-inoculation. All of the post-inoculation asthma exacerbations occurred in subjects treated with CNTO3157. More detailed safety information is presented in Section E6 and Table E1 in the online repository.

Viral load and infectivity

Viral load was not significantly different between the treatment groups (data not shown).

Biomarkers

During the treatment phase, FENO was stable in both treatment groups with no significant difference after the treatment phase compared with the pre-treatment baseline ($p=0.91$). FENO showed slight increases in both treatment groups after inoculation compared with the pre-HRB16 inoculation baseline but returned to pre-inoculation levels during follow-up evaluations with no significant difference between treatment groups (data not shown).

Nasal lavage

In Part 2 acute phase proteins were significantly up-regulated selectively in the CNTO3157 group. C reactive protein (CRP) was significantly elevated on CNTO3157 compared to pre-inoculation on Days 3 and 4 post-inoculation, and on Day 4 post-inoculation in the placebo group, with significantly higher elevations in the CNTO3157 group compared to the placebo group. IL-6 was significantly elevated in both treatment groups on Days 3 and 4 post inoculation (See online supplement **Figure E1**).

IFN-induced chemokines CXCL10 and CXCL11 were up-regulated in both the placebo and CNTO3157 groups in Part 2. **Figure E2 (online supplement)** shows the AUC and maximal value for CXCL10 in Part 1 and Part 2 after inoculation with HRV-16. For Part 1, there was a non-significant suppression of AUC CXCL10 ($p=0.19$) and maximal CXCL10 ($p=0.58$) in the CNTO3157 group vs. the placebo group, whereas in Part 2, there was a trend for elevation of AUC CXCL10 ($p=0.08$), and significant elevation of maximal CXCL10 ($p=0.03$) in the CNTO3157 group vs. the placebo group. There was significant suppression of AUC and maximal CXCL10 levels ($p=0.01$; $p=0.01$, respectively) for the placebo group in Part 2 (asthma) compared to Part 1 (healthy subjects) suggesting an intrinsic suppression of CXCL10 responses to viral inoculation in asthma, not seen in the CNTO3157 group.

Of note, several Type 2-associated analytes were modestly increased by HRV-16 in CNTO3157 but not on placebo, including IgE, IL-5 and soluble IL-33 receptor (IL-1 R4) (See **Figure E3, online supplement**).

From exploratory analyses of nasal lavage analytes measured using the SomaLogic SOMAscan v3 platform, all analytes significantly increased ($FDR<0.05$) at least 2-fold (day 4/baseline) in asthma patients after RV16 infection in CNTO 3157 treatment group and where such change was at least 2-fold ($p<0.05$) that in the placebo group are reported in Table E2 in the Online Repository. These results further support the observations that inflammation induced with

RV16 infection, including up-regulation of acute phase proteins, leukocyte chemoattractant chemokines, and neutrophil- and cytotoxic T cell- associated proteins, was further increased with CNTO 3157 treatment relative to placebo.

No analytes were significantly modulated during the pre-inoculation treatment phase in either treatment group.

Discussion

This is the first study evaluating an inhibitory anti-TLR3 mAb in asthma, and to evaluate the effects of this mAb on experimental viral challenge. Despite preclinical support for the concept, antagonism of TLR3 signaling was ineffective in attenuating the effects of HRV-16 infection on lung function, or upper and lower airway symptoms in asthma.

CNTO3157 demonstrated slightly worse outcomes compared with placebo for both the primary as well as the major secondary outcomes in asthmatics exposed to multiple weekly doses over 3 weeks. CNTO3157 also failed to reduce nasal and serum CXCL10, a downstream marker for viral signaling. Finally, there were more moderate and severe asthma exacerbations reported in subjects receiving CNTO3157 compared to those receiving placebo after inoculation, which further suggests that CNTO3157 not only failed to attenuate the manifestations of HRV-16 infection but may have made them slightly worse. In contrast, in healthy subjects there was some evidence to suggest that a single dose of CNTO3157 attenuated cold and chest symptoms, albeit in small numbers of subjects.

Typically, viral challenge results in a clinical cold with upper airway symptoms that peak at around 3 days after inoculation. In healthy subjects, there are little to no chest symptoms, in contrast to asthma where chest symptoms (e.g. cough, wheeze, dyspnea, phlegm production) are more commonly seen. Much more variable is the decline in lung function post-inoculation which can be absent or only minimal in mild and moderate asthma¹⁸⁻²⁰ in some reports, but has been reported to be greater in uncontrolled asthma with a lower FEV₁²¹. Our assumptions for this study were that HRV-16 challenge would result in a decline of 13% in pre-BD FEV₁ and that CNTO3157 would attenuate this decline by 50% based on Message et al¹⁷.

To increase the probability of seeing a moderate FEV₁ decline, we allowed not only mild but also moderate persistent asthmatics on ICS therapy, and allowed subjects with a pre-BD FEV₁ as low as 65% of predicted. Despite these criteria, the enrolled population was well-controlled with preserved lung function, and this reduced the chance to demonstrate FEV₁ decline (the maximal decline for pre-BD FEV₁ on placebo was approximately 6%). Considering all efficacy endpoints, and the excess of asthma exacerbations in the CNTO3157 group, these results provide compelling evidence that CNTO3157 compared with placebo was ineffective in attenuating and may have augmented the respiratory manifestations of HRV-16 in asthma.

Our primary hypothesis for the unsuccessful outcome of this study is that HRV-16 also interacted with other receptors, e.g. RIG-I, and MDA5, that were upregulated by the repeated dosing regimen in Part 2 and drove the increases in CXCL10 and other acute phase responses to HRV-16^{22, 23}. Compatible with this notion is the significant elevation in Type 2 mediators (IgE, IL-5, sIL33R) seen in the CNTO3157 group but not in the placebo group in Part 2. Of note, recent evidence suggests that stimulation of RIG-I increases Type 2 inflammation through IL-33 production²⁴. Second, blockade of TLR3, by reducing interferon signaling, could conceivably have left viral replication unchecked resulting in worsening inflammation. This underpins a current theory attributing viral induced asthma exacerbations to an acquired deficiency in interferon responses^{25, 26}. However, we found no evidence for an increased nasal viral load in those who received CNTO3157. Finally, the dose of CNTO3157 was more than adequate for blockade of TLR3 based on a prior study, where in an ex-vivo assay on whole blood stimulated with poly I:C, CNTO3157 administered with the same regimen suppressed cytokine release as described in the methods section.

In keeping with our findings of increased Type-2 inflammation, recent observations from a human model of HRV infection in asthmatic subjects indicate a potential role of IL-33-dependent Type 2 inflammation. Nasal lavage levels of IL-33, IL-4, IL-5 and IL-13 and bronchial lavage levels of IL-5 and IL-13 were significantly increased by HRV infection in subjects with asthma and nasal and bronchial IL-33 correlated with clinical outcomes and viral load¹⁶. In another report, a subset of asthmatic subjects infected with HRV-16 (61%) had increased levels of secreted IL-25, a cytokine that can also augment Type-2 inflammation in the nasal mucosal fluid²⁷. We observed a similar Type-2-associated response to infection with HRV-16 in asthmatic subjects, with the additional novel finding that antagonism of TLR3 appears to enhance the Type-2 response, including levels of sIL-33R relative to the placebo group. Our findings are consistent with previous reports indicating higher levels of soluble IL-33R in response to respiratory syncytial virus infection in infants²⁸, and may represent a protective host response mechanism similar to that described in models of allergic asthma²⁹ and lipopolysaccharide-induced acute lung injury in mice³⁰.

The HRV challenge model has been utilized for a number of years to study asthma pathogenesis, and has been helpful in elucidating the mechanisms underlying viral-induced asthma exacerbations as summarized in a recent review³¹. This challenge model has also been used extensively for common cold research. Based on our literature review, this study is one of the first to study an intervention with a mAb against TLR3 in asthma. Significant disadvantages of this model include the need for subjects to have low titers against HRV leading to a screen failure rate of ~50% for this reason alone, the need for parallel group designs, the meager FEV₁ response in well-controlled asthma, and the limited number of investigators available who are well-versed in the conduct of this approach. The additional requirement for this study for

subjects to be HSV-1 seropositive ¹⁴ contributed significantly to the screen failure rate which was in excess of 90%. Despite these challenges, this study, in a modest number of subjects, provided a clear no-go for efficacy of CNTO3157 as an intervention to reduce asthma exacerbations.

Current approaches to reduction of asthma exacerbations include inhaled steroids, and emerging anti-inflammatories including anti-IgE, anti-IL-4R, anti-IL-13 and anti-IL-5 mAbs. Despite these interventions, there is still significant unmet need with regards to the prevention of exacerbations including particularly in those who do not meet the Type 2 inflammatory phenotype suitable for these mAb therapies e.g. anti-IL-13 or anti-IL-5 mAbs. A diametrically opposite approach to blockade of TLR3 which inhibits the interferon axis, is the administration of nebulized IFN which aims to boost antiviral host defense. This approach showed efficacy for ACQ, PEF, and moderate asthma exacerbations in a post hoc analysis ³². In this regard ACQ has been shown to be a strong predictor of future risk of exacerbations ³³.

Limitations of this study include the milder than expected severity and good asthma control of the asthmatic participants which may have reduced the chances of observing any benefit from TLR3 blockade. In addition, the impact of repeat dosing in the asthmatic cohort compared to a single dose as utilized in Part 1, the healthy control group, was not evaluated.

In summary, CNTO3157, a TLR3 antagonist mAb, was ineffective in attenuating the impact of a HRV-16 challenge on asthma control, asthma symptoms and lung function. Other approaches, including blockade of multiple pathways, and antiviral agents, need to be sought for this high unmet medical need.

Author Contributions

Study design: PES, ESB, SF, SLJ, PJS, DP, AMD, PB, LS, RBT, JG, FB

Investigational site acquiring data: RL, ZD, BJL, DS, AE, VB, CH, SAH, TTM,

Data analysis: RG

Manuscript preparation: PES, PB

Review and approval of the manuscript: All authors

Declaration of Interests

FB, MG, ESB, SF, MJL, RG, PB, LS, AMD, PES report that they were/are full-time employees and shareholders of Janssen R&D, LLC; **CH** reports grants from Janssen during the conduct of the study; personal fees from Genzyme, personal fees from Hexal, personal fees from AbbVie, outside the submitted work; **ZD** reports for HAL Allergy, AstraZeneca, and Gilead, outside the submitted work; **TTM** reports fees from Janssen Research & Development for the conduct of the study; **RL** has nothing to disclose, **PJS** reports grants from Johnson and Johnson, during the conduct of the study; **VB** has no conflicts to report; **AE** has nothing to disclose; **DS** reports grants from Johnson and Johnson during the conduct of the study; grants and personal fees from Almirall, grants and personal fees from AstraZeneca, grants and personal fees from Boehringer Ingelheim, grants and personal fees from Chiesi, grants and personal fees from GlaxoSmithKline, grants and personal fees from Glenmark, grants and personal fees from Merck, grants and personal fees from NAPP, grants and personal fees from Novartis, grants and personal fees from Pfizer, grants and personal fees from Takeda, grants and personal fees from Teva, grants and personal fees from Therevance, grants and personal fees from Verona, personal fees from Genentech, personal fees from Skyepharma, outside the submitted work; **SAH** has nothing to disclose; **SLJ** reports grants and personal fees from Centocor, grants and personal fees from Sanofi Pasteur, grants and personal fees from GSK, grants and personal fees from Chiesi, grants and personal fees from Boehringer Ingelheim, personal fees from Grünenthal, grants and personal fees from Novartis, grants, personal fees and Shareholding from Synairgen, outside the submitted work; In addition, Dr. Johnston has a patent Blair ED, Killington RA, Rowlands DJ, Clarke NJ, Johnston SL. Transgenic animal models of HRV with human ICAM-1 sequences. UK patent application No. 02 167 29.4, 18 July 2002 and International patent application No. PCT/EP2003/007939, 17 July 2003. licensed, a patent Wark PA, Johnston SL, Holgate ST, Davies DE. Anti-virus therapy for respiratory diseases. UK patent application No. GB 0405634.7, 12 March 2004. licensed, a patent Wark PA, Johnston SL, Holgate ST, Davies DE. Interferon-Beta for Anti-Virus Therapy for Respiratory Diseases. International Patent Application No. PCT/GB05/50031, 12 March 2004. licensed, a patent Wark

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Acknowledgements

Janssen study personnel: Rasa Vitonyte, MD, MBA and Jennifer Lane, MS, as well as all the investigational site personnel who conducted the study.

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Figure legends

Figure 1: Study design for Part 1 and Part 2. In Part 1, healthy volunteers received 10mg/kg of CNTO3157 or placebo IV, and were then inoculated with HRV-16 within 72 hours and monitored closely for 10 days post inoculation with safety follow-up visits at weeks 4 and 8. In Part 2, mild-moderate persistent asthmatics received 10mg/kg, 3mg/kg, 3mg/kg and 3mg/kg of CNTO3157 or placebo IV at weekly intervals and were then inoculated with HRV-16 within 72 hours and monitored closely for 10 days post inoculation with safety follow-up visits at weeks 7 and 11.

Figure 2: Disposition of participants for Part 1 (healthy subjects) and Part 2 (mild-to moderate persistent asthma). Where reasons for discontinuation are recorded as “other”, there is no documented reason in the database. AE= adverse events.

Figure 3: Change over the 10-day post-inoculation from pre-inoculation baseline in mean (\pm SD) CSAS and CCSS (symptom scales) for Part 1 (healthy subjects), where there was a significant attenuation of both symptom scales on CNTO3157 (n=8) vs placebo (n=4).

Figure 4: The primary endpoint, % change from pre-inoculation baseline in LS mean (SE) Pre-BD FEV1 (mITT HRV-16 analysis set) for CNTO3157 and placebo in Part 2 (persistent asthmatic subjects). There was no significant difference for maximal fall or AUC day 1-Day 10 post-inoculation between CNTO3157 and placebo.

Figure 5: Change in mean (SD) CSAS and CCSS post HRV inoculation from pre-inoculation baseline over the 10-day post-inoculation period for Part 2 (persistent asthmatic subjects) where both symptom scales were numerically higher on CNTO3157.

Figure 6: For the mITT analysis set, percentage change from screening baseline in LS mean (SE) Pre-BD FEV1 for CNTO3157 and placebo in Part 2 during the treatment phase only (before HRV-16 inoculation). This demonstrates the effect of blockade of TLR3 compared to placebo on lung function. While there was a numerical difference between CNTO 3167 and placebo, this was not significant.

Table 1: Demographic and Disease characteristics for Part 2

	Placebo	CNT03157
All Randomized Subjects (n)	31	32
Age (years) Mean (SD)	38.1 (12.15)	39.6 (14.28)
Female	10 (32.3%)	12 (37.5%)
Male	21 (67.7%)	20 (62.5%)
Race		
Asian	1 (3.2%)	0
Black / African American	1 (3.2%)	0
Other	0	1 (3.1%)
White	29 (93.5%)	31 (96.9%)
BMI (kg/m ²) Mean (SD); range	26.1 (3.5); 20.3-38.6	25.7 (3.8); 19.4-34.3
pre-BD FEV ₁ % predicted; mean (SD)	89.65 (12.44)	88.70 (10.83)
Log FENO [ppb]–mean (SD)	3.73(0.63)	3.50 (0.80)
Reported Allergies	51.6%	34.4%
ACQ ₇ [0–6]; mean (SD)	0.65 (0.43)	0.78 (0.54)
Blood Eosinophils (x 10 ⁹ /L); mean (SD)	0.197 (0.1009)	0.178 (0.1650)
ICS Use – Yes	22 (71.0%)	18 (56.3%) (p=0.23)

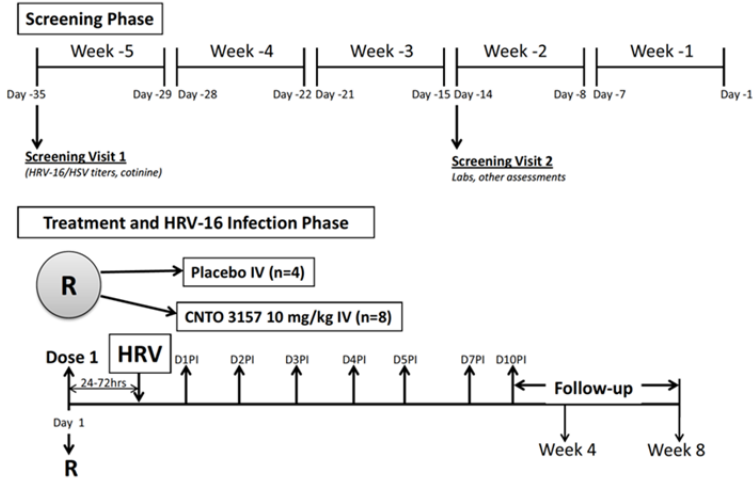
BMI: body mass index; SD: standard deviation; pre-BD: pre-bronchodilator; FEV₁: forced expired volume in 1 second; FENO: fractional concentration of exhaled nitric oxide; ACQ: asthma control questionnaire; ICS: inhaled corticosteroids. There were no significant between-group differences for demographic and disease characteristics.

Table 2: Major secondary endpoints assessed as change in the 10 day post-inoculation period in Part 2

Mean (SD)	LS mean CNTO3157/placebo	P value
AUC of the % change from pre-inoculation in pre-BD FEV ₁	-4.26 (11.06)/-13.04 (12.11)	0.60
AUC of the change from pre-inoculation in the CCSS	48.9 (9.87)/34.5 (10.63)	0.33
AUC of the change from pre-inoculation in the CSAS	32.2 (6.09)/25.0 (6.56)	0.43
AUC of the change from pre-inoculation in AM PEFr	-183.2 (72.67)/-8.5 (79.64)	0.11
Change from baseline in ACQ7 symptom scores	0.20 (0.68)/0.06 (0.62)	0.43

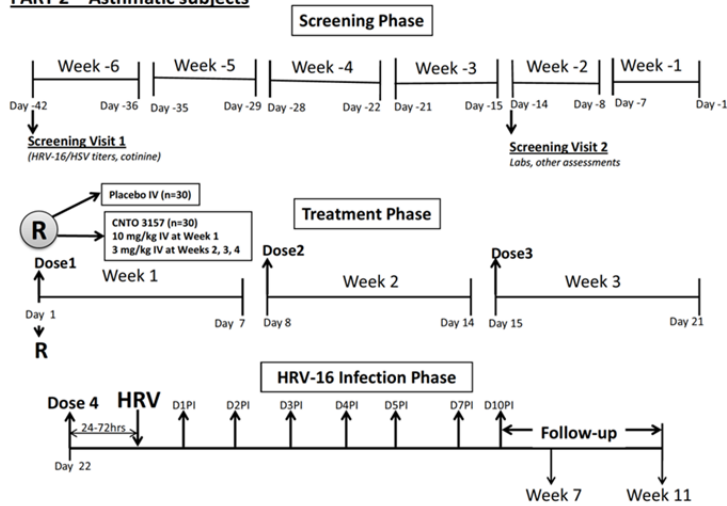
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PART 1 – Healthy subjects



Dose = Dose study agent; R = randomization; HRV = Inoculate with HRV-16; D#PI = Day # post-inoculation

PART 2 – Asthmatic subjects



Dose = Dose study agent; R = randomization; HRV = Inoculate with HRV-16; D#PI = Day # post-inoculation

Figure No.2

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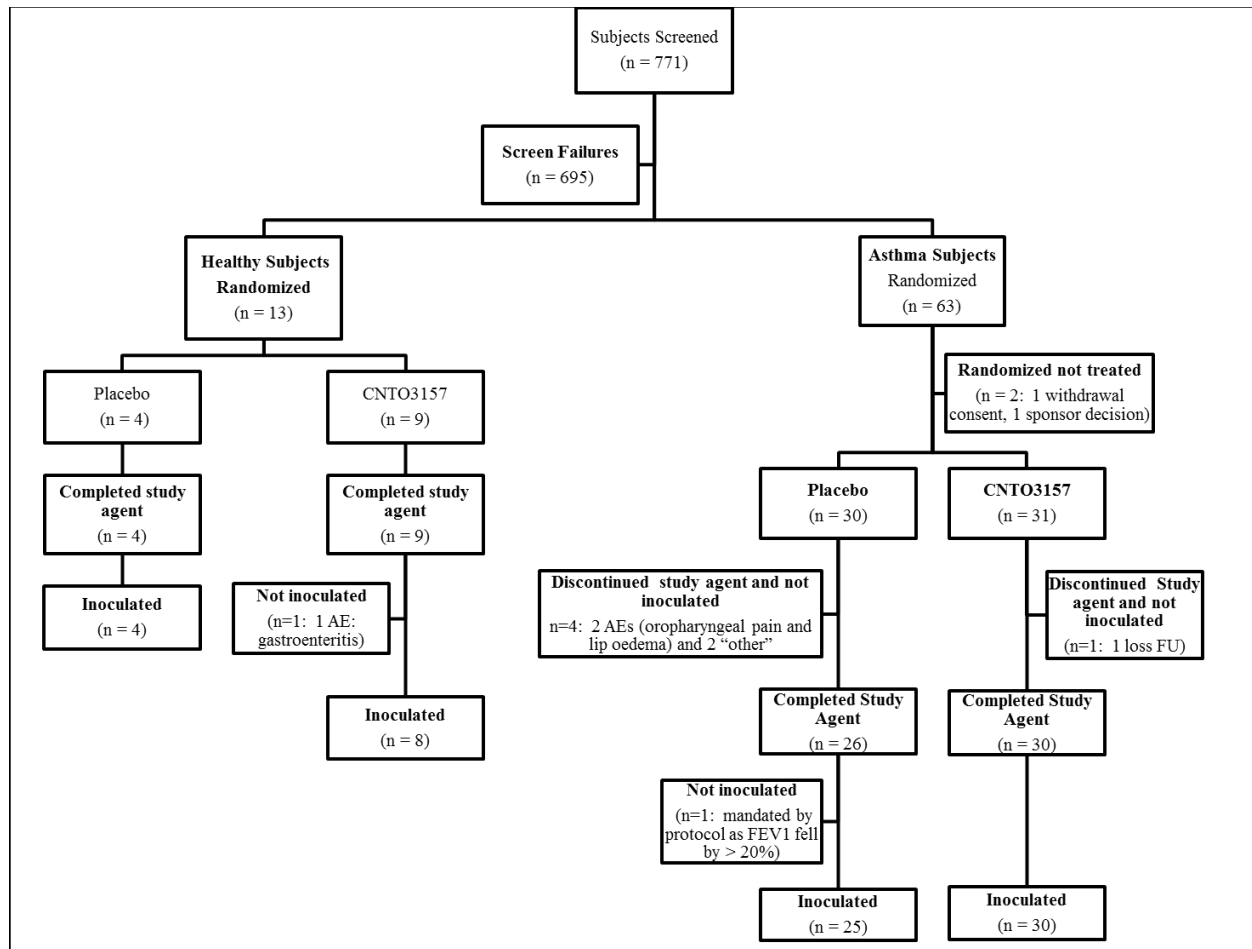


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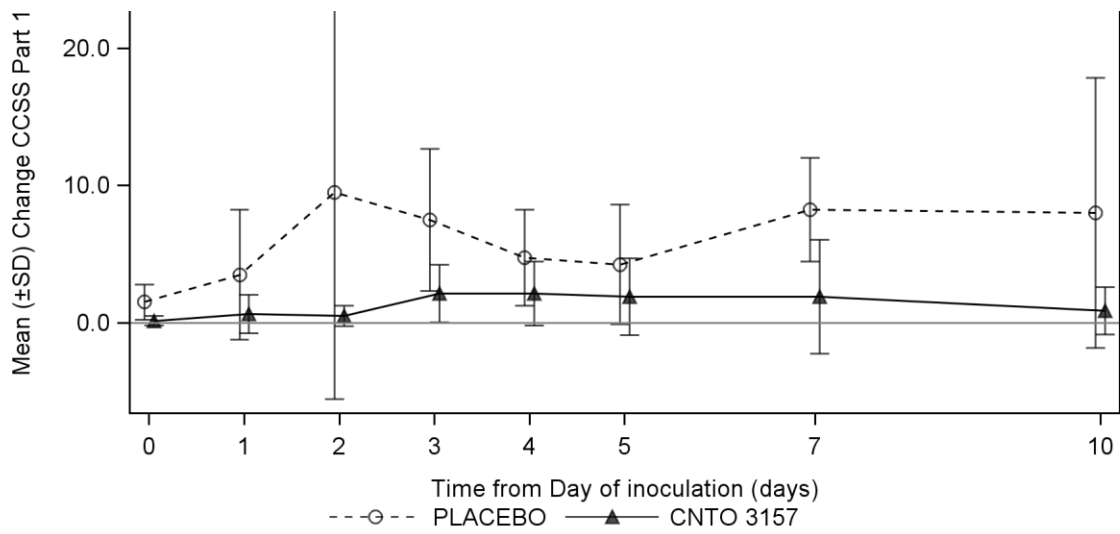
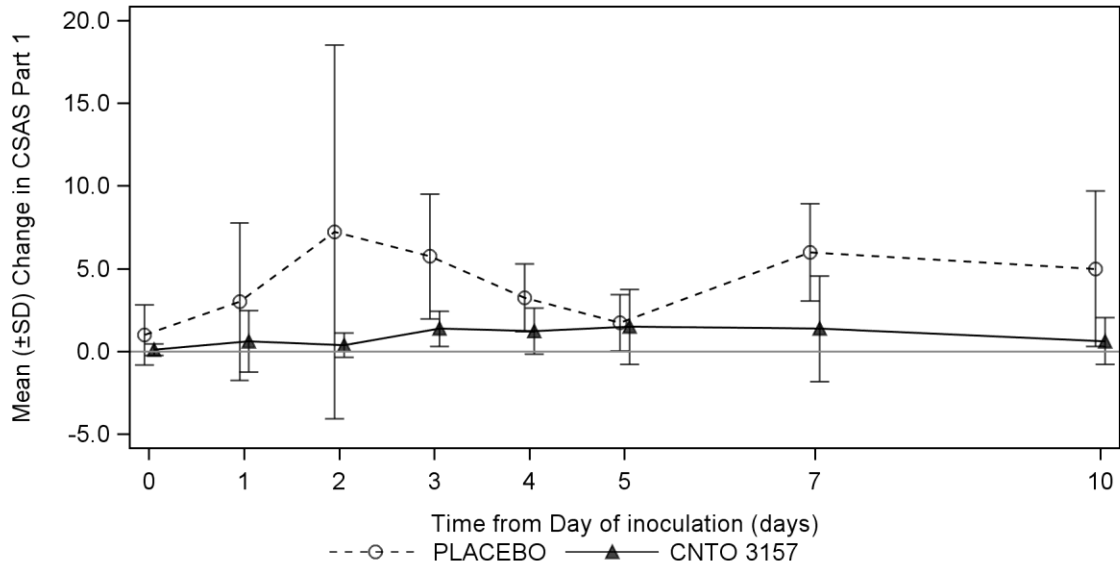


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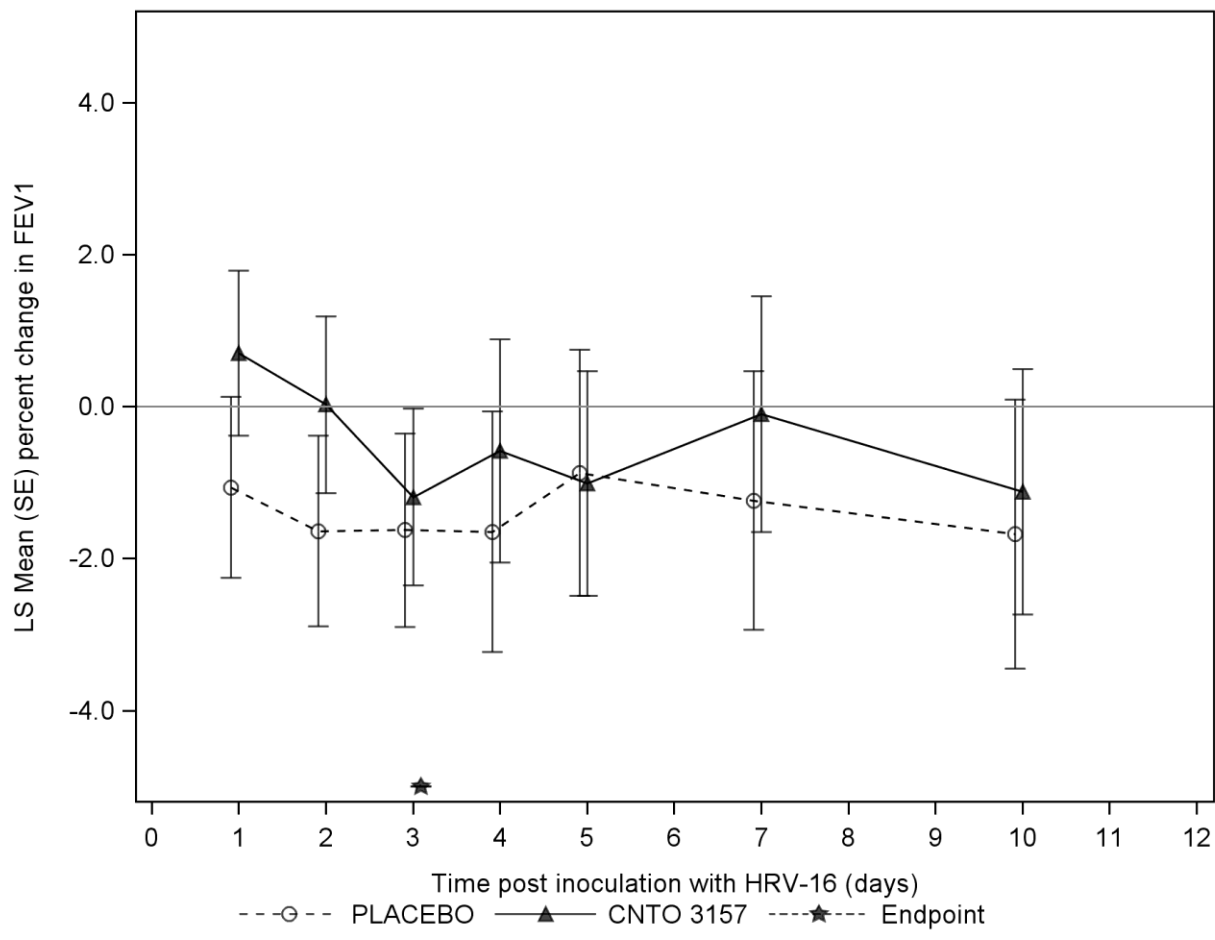


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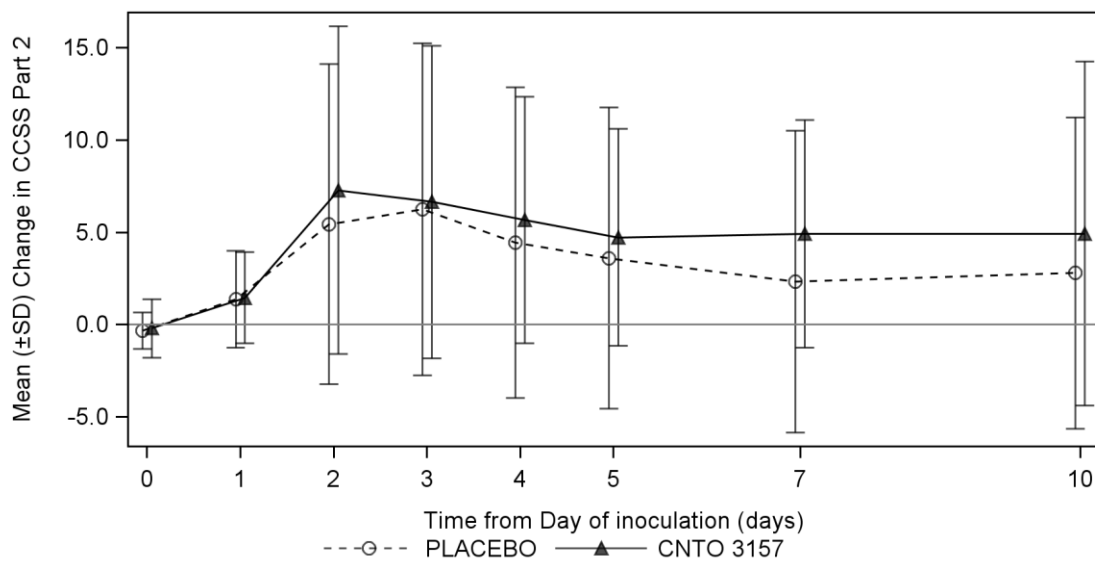
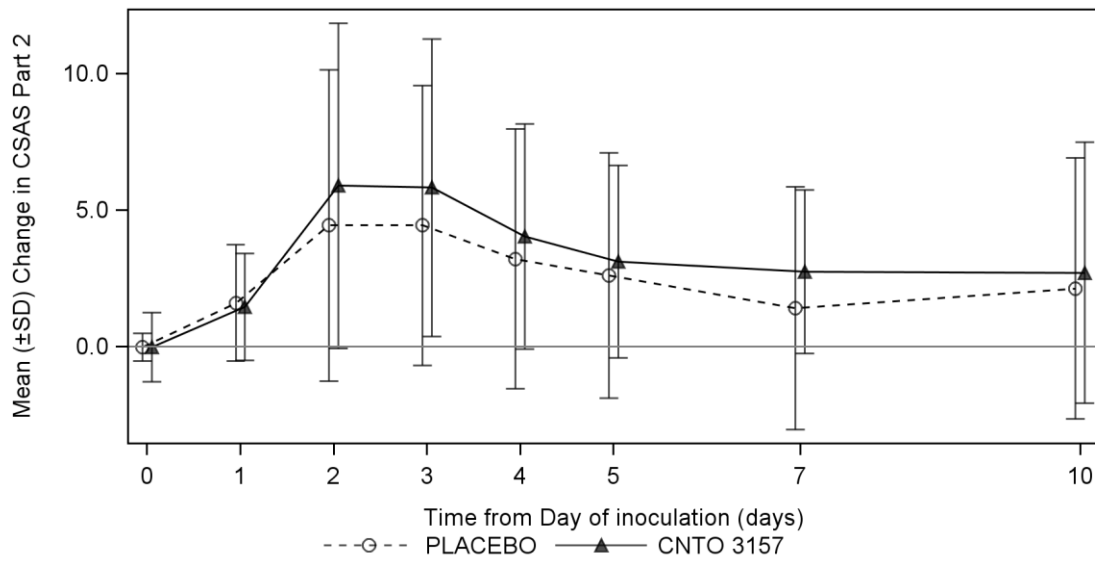
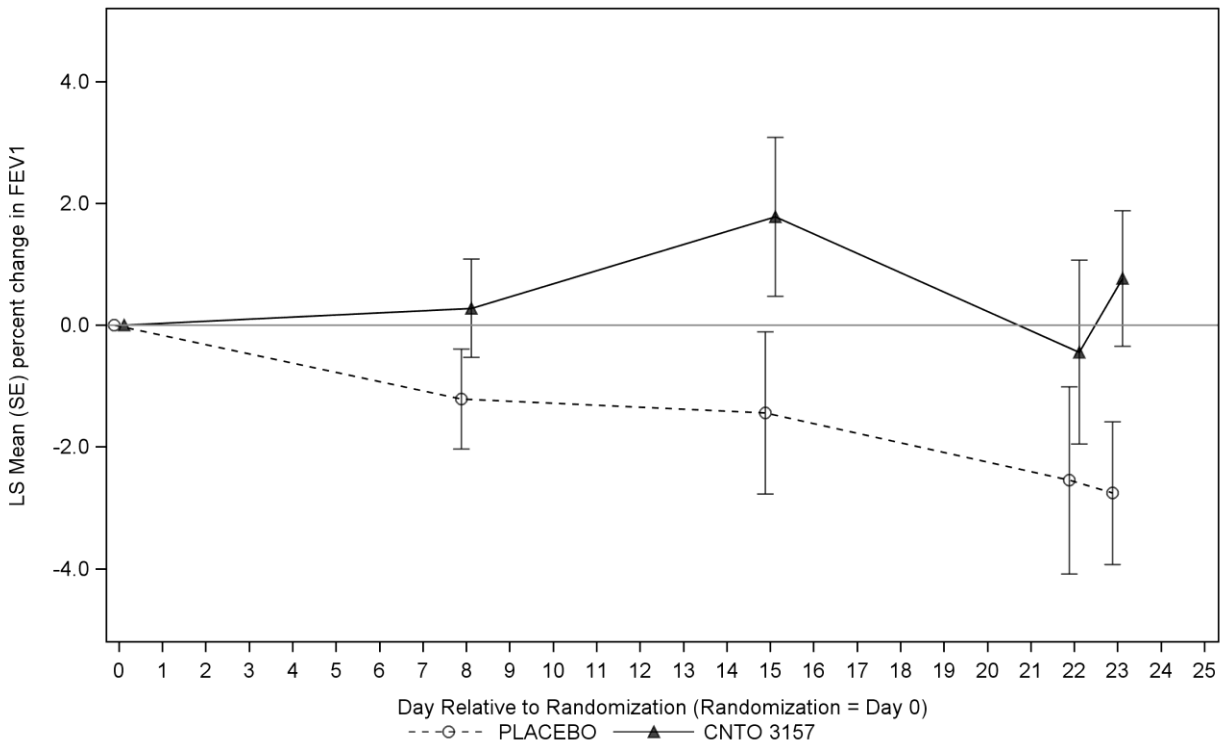


Figure No.6

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Online Supplement

Background

Extensive human in vitro and murine in vivo experiments supported the hypothesis that CNTO 3157 would be an effective agent to suppress the inflammatory effects induced by rhinovirus infection¹⁻⁶.

E1: Dosing rationale

The PK profile (data not shown) from the Ph1 first in human multiple ascending dose study demonstrated increasing trough concentrations with each the second, third and fourth dose given at weekly intervals, presumed to be due to incomplete occupation of the receptor by CNTO3157. In addition, there was complete inhibition of cytokine release in whole blood stimulated with poly I:C (IL1b, IL6, IL12p40 and IL12p70) at 7 days post dosing at 3mg/kg in healthy volunteers (data not shown). In light of these data, a higher dose, 10mg/kg, was selected in Part 1, and a loading dose of 10mg/kg, followed by 3 additional weekly doses of 3mg/kg, were selected for Part 2 to increase the certainty of TLR3 blockade, and to inhibit cytokine release.

E2: Biomarker analytical methods and analytes measured.

Nasal lavage, serum, and sputum CXCL10

Nasal wash, serum and sputum CXCL10. CXCL10 protein levels were assessed using the VeriPlex Human Interferon 9-Plex ELISA (PBL Assay Sciences, Piscataway, NJ). Additional protein markers were assessed using the aptamer-based SomaScan assay (Somalogic, Boulder, CO).

HRV16 antibody titers.

HRV16 neutralizing serum antibody titers were determined in a cell-based assay using MRC-5 cells. Briefly, MRC-5 cells were cultured under standard conditions in MRC-5 growth medium (EMEM with 10% heat inactivated FBS, 1M HEPES, L-Glutamine, NEAA and antibiotics). Test sera were diluted in duplicate in a 96-well plate followed by addition of an equal volume of virus with an expected titer of $3.3 \log_{10} \text{TCID}_{50}/\text{mL}$ ($2 \times 10^3 \text{TCID}_{50}/\text{mL}$) and incubated for 30 minutes at 33°C. Dissociated MRC-5 cells were then added to the virus/sera and allowed to incubate for 5 days at 33°C when the plates were visually inspected by light microscopy to determine viral cytopathic effect (CPE). Cells within an individual well were considered to be HRV-16 antibody negative with CPE > 50%. The neutralizing antibody titer was then calculated using the Reed Muench formula. In each assay a control consisting of a pool

of human sera of known neutralizing antibody titer and a commercially sourced anti-HRV-16 antiserum (ATCC) were used to confirm the assay was performing within specifications and to validate the results obtained with the test sera. Virology assays including the determination of HRV-16 and HSV1 neutralizing antibody titers, HRV-infectivity and RVP analysis were performed at hVIVO, London, UK(Formerly Retroscreen Virology).

HRV-16 Infectivity Assay.

Replication of HRV-16 in nasal wash samples was determined in a cell-based assay using MRC-5 cells. Briefly, MRC-5 cells were prepared for culture in a 96-well flat bottom plate and incubated for 1-2 days until 60-90% confluent. Nasal wash samples were added in quadruplicate to the 96-well plate containing MRC-5 cells, titrated using a 0.5 log₁₀ dilution series and incubated at 33°C for 5 days. Plates were then examined for viral CPE to determine the presence or absence of virus in each well. Virus titers were calculated using the Karber formula. A stock virus generated from the GMP challenge virus used in the study was used as a positive control.

HSV screening. The HerpeSelect HSV-1 IgG ELISA (Focus Diagnostics) was used to determine the presence of HSV-1 antibodies in serum with interpretation of the test results in accordance with the manufacturer's instructions.

Respiratory Viral Panel (RVP). Throat swabs were used for a respiratory viral panel screen by multiplex qPCR. The viral panel screen tested for the presence of HRV-16 RNA, Influenza A RNA, Influenza B RNA, RSV RNA, Para Influenza 1, 2 and 3 RNA, Metapneumovirus RNA and adenovirus DNA.

Section E3

HRV challenge virus

The strain of HRV16 used was isolated via nasal lavage from a subject in a clinical study at the University of Virginia. Good Manufacturing Practice guidelines were followed to manufacture the virus (Meridian Life Sciences, Memphis, TN, USA) and regulatory approval was obtained for its use in human subjects (investigational new drug number 014757). The HRV16 strain was demonstrated to cause a classical upper respiratory infection without safety concerns at total doses of approximately 100 and 1000 TCID₅₀/mL in 2 cohorts of healthy volunteer characterization study (data not shown).

For the study reported here, HRV16 at a total dose of approximately 1000 TCID₅₀/mL, in a volume of approximately 1.0 mL, was administered by instillation with a pipette (divided into 2 instillations per naris). A confirmed infection with HRV16

was defined as a positive culture from nasal lavage at any time in the 5 days post-inoculation, and/or a 4-fold serological conversion to HRV16 assessed at the week 8 or 11 visits.

Before inoculation, nasal lavage was cultured for the presence of viruses including HRV but also other viruses e.g. influenza.

Section E4.

Pharmacokinetics and Immunogenicity

The pharmacokinetic (PK) profiles of CNTO3157 in Part 1 and Part 2 were similar to the profiles seen in the first in human study (NCT01195207) for similar doses and dosing regimens (data not shown). No apparent differences in serum CNTO3157 concentrations over 7 days following the first dose of 10 mg/kg administered by IV infusion were observed when comparing healthy subjects in Part 1 with asthmatic subjects in Part 2. Also, there were no apparent changes in serum CNTO3157 concentration-time profiles after the inoculation of HRV16. All subjects treated with CNTO3157 in Part 1 were negative for antidrug antibodies (ADA) while only 1 subject in Part 2 (3.3% of all subjects) tested positive for ADA with no impact on this subject's PK profile.

Section E5. Viral load and biomarkers.

Viral load

HRV16 replication was determined in a cell-based assay and represented as log tissue culture infective dose (TCID)₅₀ post-inoculation. The replication profile was not significantly different between the treatment groups (data not shown).

Of note, 1 subject in Part 1 (on CNTO3157) and 2 subjects in Part 2 (one on CNTO3157 and 1 on placebo) were positive for HRV16 at the pre-inoculation visit, and 1 subject in Part 1 on CNTO3157 was positive for influenza B at Day 10 post-inoculation. These subjects were excluded from biomarker analyses but not excluded from the clinical analysis.

Nasal lavage

In Part 1, the cystatins, (CST)-1, -2, -4, and -5 were upregulated on CNTO3157 to a greater degree than placebo, while CD27, IL-37, cathepsin V, and carbonic anhydrase 6 were up-regulated on CNTO3157 alone. IFN-induced chemokines

CXCL10/IP-10 and CXCL11/ITAC were up-regulated in both placebo and CNTO3157 groups, demonstrating an attenuated rise but incomplete inhibition of IFN activity by CNTO3157 compared with placebo.

Section E6

Safety Part 2: The AEs are presented in 4 phases: 1) high dose (10mg/kg or placebo), 2) low dose (3mg/kg administered 3 times at weekly intervals), 3) from virus inoculation to end of study, and 4) from randomization to end of study. No deaths, serious AEs, or other significant AEs occurred in Part 2 of the study. The vast majority of AEs were mild in severity. If judged to be related, the majority of adverse events were reported as very likely related to HRV16.

Table E1 (abbreviated) presents asthmatic subjects with 1 or more treatment-emergent adverse events (TEAEs) that occurred in at least 5% of subjects. Of note, the highest number of subjects with at least 1 AE was in the virus-end (of study) phase as might be expected. For the treatment phase, more of the reported AEs occurred in the low-dose period (Weeks 2, 3 and 4 during which subjects received CNTO3157 3mg/kg) than in the high-dose period (following the 10 mg/kg infusion). Slightly more subjects in the CNTO3157 group reported respiratory AEs than in the placebo group. There was no imbalance between the CNTO3157 and placebo groups for infections including oral herpes.

Six subjects (5 in the CNTO3157 group and 1 in the placebo group) met the protocol-defined criteria of non-serious moderate or severe exacerbations during Part 2 of the study. The single subject in the placebo group had a protocol-defined moderate asthma exacerbation during the treatment period consisting of multiple events characterized by decreases in peak expiratory flow rate (PEFR) and increased rescue medication use.

Five CNTO3157 treated subjects (17%) had asthma exacerbations post-inoculation. Two of the 5 subjects had protocol-defined severe exacerbations (use of systemic steroids). The 2 severe exacerbations occurred on Days 3, and 13 post-inoculation while the 3 moderate exacerbations occurred on Days 2, and at Weeks 7 and 11 post-inoculation. All of the post-inoculation asthma exacerbations occurred in subjects treated with CNTO3157.

Section E7: Biomarkers.

Table E2 presents the nasal lavage analytes in Part 2 asthma that were significantly elevated post viral challenge.

Figures E1, E2 and E3 present the biomarkers and are discussed in the results section of the main paper.

Figure E1. Changes in acute phase reactants in nasal lavage. For (A) study part 1 in healthy control subjects and (B) part 2 in persistent asthma subjects, relative changes in nasal lavage levels of IL-6 (top panels) and CRP (bottom panels), expressed as \log_2 -transform of within-subject ratios of post-inoculation (INOC) visit over pre-inoculation baseline levels (y-axis), are displayed for each subject by time point post-inoculation (x-axis). * $p < 0.05$ for change from baseline within-treatment group; † $p < 0.05$ CNTO 3157 vs. placebo, at indicated time point.

Figure E2. Changes in CXCL10 levels in nasal lavage. (A) Area-under-curve (AUC) and (B) maximum value for relative changes in nasal lavage levels of CXCL10 (expressed as \log_2 -transform of within-subject ratios of post-inoculation (INOC) visit over pre-inoculation baseline levels), from day of inoculation to day 10 post-inoculation with RV16, stratified by study part and treatment group. * $p < 0.05$ CNTO 3157 vs. placebo for study part 2.

Figure E3. Changes in Th2 cytokines in nasal lavage. For study Part 2, relative changes in nasal lavage levels of (A) IgE, (B) soluble IL-1R4 (IL-33R), and (C) IL-5, expressed as \log_2 -transform of within-subject ratios of post-inoculation (INOC) visit over pre-inoculation baseline levels (y-axis), are displayed for each subject by time point post-inoculation (x-axis). * $p < 0.05$ for change from baseline within-treatment group, at indicated time point.

Table E1: Number of Subjects With 1 or More Treatment-Emergent Adverse Events by in Part 2; Safety Analysis Set

n	PBO				CNT03157			
	High Dose Period	Low Dose Period	Virus → End	Treat → End	High Dose Period	Low Dose Period	Virus → End	Treat → End
	30	25	25	30	31	30	30	31
Subjects with 1 or more AEs	13 (43.3%)	10 (40.0%)	16 (64.0%)	25 (83.3%)	8 (25.8%)	13 (43.3%)	21 (70.0%)	25 (80.6%)
System organ class/preferred term								
Respiratory, thoracic and mediastinal disorders	3 (10.0%)	2 (8.0%)	7 (28.0%)	11 (36.7%)	2 (6.5%)	1 (3.3%)	11 (36.7%)	12 (38.7%)
Asthma	2 (6.7%)	0	1 (4.0%)	3 (10.0%)	0	0	5 (16.7%)	5 (16.1%)
Cough	1 (3.3%)	0	1 (4.0%)	2 (6.7%)	2 (6.5%)	0	2 (6.7%)	3 (9.7%)
Wheezing	0	0	0	0	1 (3.2%)	1 (3.3%)	2 (6.7%)	3 (9.7%)
Dyspnea	0	0	1 (4.0%)	1 (3.3%)	0	0	2 (6.7%)	2 (6.5%)
Oropharyngeal pain	0	1 (4.0%)	1 (4.0%)	2 (6.7%)	0	0	2 (6.7%)	2 (6.5%)
Epistaxis	0	0	2 (8.0%)	2 (6.7%)	0	0	1 (3.3%)	1 (3.2%)
Rhinitis allergic	0	0	0	0	0	0	1 (3.3%)	1 (3.2%)
Rhinorrhoea	0	0	1 (4.0%)	1 (3.3%)	0	0	1 (3.3%)	1 (3.2%)
Asthma exercise induced	0	1 (4.0%)	0	1 (3.3%)	0	0	0	0
Dysphonia	0	0	1 (4.0%)	1 (3.3%)	0	0	0	0
Nasal congestion	0	0	1 (4.0%)	1 (3.3%)	0	0	0	0
Nervous system disorders	2 (6.7%)	6 (24.0%)	5 (20.0%)	11 (36.7%)	1 (3.2%)	4 (13.3%)	7 (23.3%)	10 (32.3%)
Headache	2 (6.7%)	5 (20.0%)	5 (20.0%)	10 (33.3%)	0	3 (10.0%)	6 (20.0%)	8 (25.8%)
Dizziness	0	0	0	0	0	1 (3.3%)	1 (3.3%)	2 (6.5%)
Syncope	0	0	0	0	1 (3.2%)	0	0	1 (3.2%)
Migraine	0	1 (4.0%)	0	1 (3.3%)	0	0	0	0
General disorders and administration site conditions	0	2 (8.0%)	3 (12.0%)	4 (13.3%)	3 (9.7%)	2 (6.7%)	5 (16.7%)	9 (29.0%)
Infections and infestations	1 (3.3%)	0	8 (32.0%)	9 (30.0%)	1 (3.2%)	0	7 (23.3%)	7 (22.6%)
Nasopharyngitis	0	0	4 (16.0%)	4 (13.3%)	1 (3.2%)	0	3 (10.0%)	4 (12.9%)
Oral herpes	1 (3.3%)	0	1 (4.0%)	2 (6.7%)	0	0	2 (6.7%)	2 (6.5%)
Rhinitis	0	0	2 (8.0%)	2 (6.7%)	0	0	2 (6.7%)	2 (6.5%)
Otitis media	0	0	0	0	0	0	1 (3.3%)	1 (3.2%)
Upper respiratory tract infection	0	0	1 (4.0%)	1 (3.3%)	0	0	1 (3.3%)	1 (3.2%)
Injury, poisoning and procedural complications	1 (3.3%)	2 (8.0%)	6 (24.0%)	8 (26.7%)	0	2 (6.7%)	6 (20.0%)	7 (22.6%)
Musculoskeletal and connective tissue disorders	1 (3.3%)	0	2 (8.0%)	3 (10.0%)	0	2 (6.7%)	4 (13.3%)	6 (19.4%)
Gastrointestinal disorders	2 (6.7%)	1 (4.0%)	4 (16.0%)	6 (20.0%)	0	1 (3.3%)	4 (13.3%)	5 (16.1%)
Skin and subcutaneous tissue disorders	3 (10.0%)	1 (4.0%)	0	4 (13.3%)	0	3 (10.0%)	0	3 (9.7%)
Eye disorders	1 (3.3%)	0	0	1 (3.3%)	0	0	2 (6.7%)	2 (6.5%)
Ear and labyrinth disorders	0	0	0	0	1 (3.2%)	0	0	1 (3.2%)
Psychiatric disorders	0	0	0	0	0	1 (3.3%)	0	1 (3.2%)
Immune system disorders	2 (6.7%)	0	0	2 (6.7%)	0	0	0	0
Investigations	1 (3.3%)	0	0	1 (3.3%)	0	0	0	0

Note: Percentages calculated with the number of randomized, treated subjects in each study phase as the denominator. Incidence is based on the number of subjects experiencing at least one AE, not the number of events. Adverse events are coded using the MedDRA version 15.1. The table has been abbreviated to focus on system organ classes of greater relevance to CNT03157 and HRV16

Table E2: Part 2 asthma, day 4 post-RV16 inoculation up-regulated nasal lavage analytes*			
6Ckine	Coagulation Factor IXab	IgG	Proteinase-3
a1-Antitrypsin	Coagulation Factor XI	ILT-2	PSME1
a2-Antiplasmin	Dkk-4	ILT-4	resistin
a2-HS-Glycoprotein	ECM1	LBP	RTN4
Afamin	Elastase	LYVE1	SAA
Angiotensinogen	EMR2	M-CSF R	SAP
Antithrombin III	Factor B	MDC	sCD163
Apo A-I	Factor H	MMP-1	SHBG
Apo E	Factor I	MMP-9	SHP-2
BGH3	FCG3B	Notch 1	SIG14
C3	FETUB	Nucleoside diphosphate kinase A	Siglec-7
C4	Fibrinogen	OLR1	sL-Selectin
C5	Fibronectin	OMD	SREC-I
C5a	Gro-b/g	PCI	Tenascin
C5b, 6 Complex	hnRNP A/B	PCSK9	Thyroxine-Binding Globulin
Calpastatin	HSP 70	PF-4	TNF sR-II
CaMKK alpha	IGFBP-3	Properdin	TSG-6
Cathepsin S	IGFBP-5	Protein C	TSP4
CLM6			

* For CNTO 3157 treatment group, analytes passing significance filter of FDR<0.05 and fold(Day4/baseline)>2 listed; bolded analytes pass filter of nominal p-value<0.05 for day3 vs. baseline in CNTO 3157 treatment group

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