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

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Corresponding Author: Dr. Philip E. Silkoff, MD

Corresponding Author's Institution:

First Author: Philip E. Silkoff, MD

Order of Authors: Philip E. Silkoff, MD; Susan Flavin, PhD; Robert Gordon, MS; Matthew J Loza, PhD; Peter J Sterk, MD; Rene Lutter, MD; Zuzana Diamant, MD; Ronald B Turner, MD; Brian J Lipworth, MD; David Proud, PhD; Dave Singh, MD; Andreas Eich, MD; Vibeke Backer, MD; James E Gern; Christian Herzmann, MD; Scott A Halperin, MD; Tjeert T Mensinga, MD; Alfred M Del Vecchio, PhD; Frederic Baribaud, PhD; Elliot S Barnathan, MD; Sebastian L Johnston, MD

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Abstract: Background
Human rhinoviruses (HRV) commonly precipitate asthma exacerbations. TLR3, an innate pattern-recognition receptor, is triggered by HRV thereby increasing inflammation that may worsen asthma.
Objective.
To assess the impact of an inhibitory IgG4 kappa monoclonal antibody to TLR3, CNTO3157, on experimental HRV-16 inoculation in healthy and asthmatic subjects.
Methods
In this double-blind randomized parallel-group study at multiple sites in North America and Europe, healthy and mild-moderate stable asthmatic subjects were enrolled between 2012-2014. Healthy and 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 the 10-day post-inoculation period.
Results
In asthmatic subjects (N=63), CNTO3157 provided no protection against FEV1 decline with LS mean [SE]: CNTO3157 (n=30) = -7.08 [8.15] % and placebo (n=25) groups = -5.98 [8.56] %), or symptoms after 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 a 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.

Re: JACI-D-16-01607, TLR3 Inhibition in Rhinovirus-Induced Experimental Asthma Exacerbations: A Randomized Controlled Study

Dear Dr. Silkoff:

Your manuscript, referenced above, has been reviewed. The reviewers feel that extensive revisions are needed, as indicated in the posted comments. Any revision you may choose to submit must incorporate changes, and these must thoroughly and satisfactorily address each of the criticisms made by the referees. Please note: An invitation to resubmit a manuscript does not constitute a commitment to publish, since a revision may still not achieve a priority rating sufficient to warrant acceptance.

We ask that you submit your revision by Apr 13 2017 12:00AM or correspond with the Editorial Office to disclose your plans for your manuscript.

EDITOR'S SPECIFIC COMMENTS:

Reviewer #1: This study investigates an inhibitory anti-TLR3 mAb in asthma, and evaluates the effects of this mAb on experimental viral challenge in humans in a clinical trial. Unfortunately, the manuscript is written in a very difficult way to understand, figures should be improved and discussion should focus more on their own data and challenges and hurdles.

RESPONSE:

- We have improved the figures as suggested and revised the manuscript extensively. The discussion section has been expanded to discuss hurdles and challenges.

The authors studied the impact of an inhibitory IgG4 kappa monoclonal antibody to TLR3, CNTO3157 on experimental HRV16 inoculation in healthy and asthmatic subjects. I have some major comments, which may help to improve the manuscript.

The study was designed as double blind randomized parallel group study at multiple sites in North America and Europe, healthy (n=13) and mild-moderate stable asthmatic subjects (n=63) were enrolled after HRV inoculation. In this study, anti-TLR3 Ab suppressed symptoms of viral infection in healthy subjects, but was ineffective in attenuating the impact of a HRV16 challenge on lung function, asthma control, and symptoms in asthma. It is interesting why the authors provide very little immunological data; nowadays there are so many easy to use techniques that allow measuring a large set of inflammatory molecules with a very little amount of patient material. It is a major handicap why the authors did not measure at all the IFN family of anti-viral cytokines.

RESPONSE:

- Various IFN classes were tested for the sputum samples but were not reliably detected (almost all samples were below the lower limit of detection (LLOQ). In a previous optimization study for RV16 infection in healthy individuals (NCT# NCT01466738), IFNs were not reliably detected in nasal lavage and serum samples and therefore were not included for the current study.
- We included results from an exploratory analysis of a broad panel of nasal lavage analytes (SomaLogic SOMAscan platform), which confirmed and extended the findings of the targeted, hypothesis-driven analyses (IP-10, CRP). Included in the panel, but not reported in the submitted manuscript, were acute phase proteins, IFN-induced chemokines (e.g. CXCL11, CXCL12), type 2 inflammation-associated proteins (e.g., Periostin, IL1 R4/IL33R, mast cell tryptase, IgE, IL-5, etc.). Results for some of the type 2-inflammatiory cytokines were already presented in Figure E3.
- We believe that inclusion of more of these biomarker exploratory results could overwhelm the focus of the paper on clinical outcomes and pre-specified, hypothesis-driven biomarker outcomes. However, we have added an online supplement table, Table E2, which is now referred to in the nasal lavage biomarker section with the following text: “From exploratory analyses of nasal lavage analytes measured using the SomaLogic SOMAscan v3 platform, analytes increased in asthma patients after RV16 infection in the placebo and CNTO 3157 treatment groups 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.”

1. First of all, the manuscript is written in a very difficult to understand style and instead of giving little introduction for the readers a link was given in many parts, which is not helpful for the readers of the journal. The authors should summarize the findings and still give the link in these places.

RESPONSE:

- A single link to the full study protocol is provided in the methods section but the essential details of the study design are presented in the main manuscript. The online repository contains further details about methodologies and results that were not essential communication of the study results, or possible to include in the main manuscript for space considerations.

2. CNTO3157 is not a scientific name it should be used anti-TLR3 Ab in the abstract. The last paragraph in the abstract should be conclusion.

RESPONSE:

- Many early development compounds are referred to by a company internal nomenclature in the published literature. Janssen did not assign a name to CNTO3157 as this compound had yet proven to be efficacious. We respectfully

prefer to keep the CNTO3157 name as this will distinguish this from other internal or external anti-TLR3 antibodies.
- The abstract now has a conclusion.

2. Human in vitro data with CNTO3157 should be clearly discussed; the manuscript gives the impression that the authors started a clinical trial without any preclinical evidence.

RESPONSE:
- 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 infection. We did not include these in the manuscript to keep this focused and some of the data is as of yet unpublished. We have added a background section to the online supplement that includes references to published internal data regarding preclinical studies around TLR3 or relevance to asthma and viral-induced inflammation, and referred to this in the last paragraph of the introduction.

3. This below sentence in the abstract should be rewritten. It is not clear. "Two severe and three out of four moderate asthma non-serious asthma exacerbations occurred on CNTO3157. There were no other significant safety signals."

RESPONSE:
- We have rewritten this sentence in the abstract.

4. In the abstract if primary outcome was FEV1 than it should be mentioned first in the results.

RESPONSE: We have moved the primary endpoint to the beginning of the abstract.

5. The title should be reconsidered and represent the findings of the study, inhibition is not the correct word.

RESPONSE:
- We have changed this to “blockade” of TLR3.

6. In the methodology, an effective dose was selected for CNTO3157 in the first 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 presumed to be due to incomplete occupation of the receptor by CNTO3157. The authors mention here that 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. There is no data regarding the asthmatic individuals. It will be good to include the measurement of these cytokines and also other Th2 related cytokines at least in whole blood stimulated with poly I: C in placebo and CNTO3157 group.

RESPONSE:
- In the first-in-human study, whole blood ex vivo stimulation assays demonstrated that four weekly doses of 10 mg/kg CNTO 3157 in asthmatic subjects produced a

significant pharmacodynamics (PD) response relative to placebo, as measured by inhibition of TLR3-mediated gene expression and cytokine release. In addition, four weekly doses of 10 mg/kg CNTO 3157 in asthmatic subjects resulted in a significantly greater PD response compared with four weekly doses of 3 mg/kg CNTO 3157 for cytokine release (internal data but unpublished results).

- From the assay optimization experiments, type 2 inflammation-associated cytokines were not identified that gave a robust, consistent performance for induction with poly I:C. This was not unexpected given that type 2-phenotype cells are found at very low proportions in whole blood. Please see the above response with respect to measurement of type 2-inflammation associated analytes in nasal lavage samples from the current study (Figure E3).

7. In the biomarker assessment the authors should make it clear to the readers what the biomarker is. Do they mean that measurement of RV load is the biomarker?

RESPONSE:

- It was not clear what the reviewer meant here by “biomarker assessment”.

8. In Table 1, patient characteristics need more detailed information and also some more explanation. For example, "A higher proportion of subjects in the placebo group (70%) vs. 58% of subjects on CNTO3157 reported ICS use at enrolment." That means that patients in the placebo group were more severe than CNTO3157 group. This can be a problem.

RESPONSE:

- It is not clear what additional patient characteristics the reviewer would like to see. We believe we have presented the essential characteristics.
- Analysis of the primary endpoint for multiple sensitivity analyses including ICS-use did not impact the outcome, so it does not appear that the imbalance in ICS use was a confounding factor.

9. In the table p-values are missing. It should be also listed here whether patients are having other diseases and related medications (comorbidities).

RESPONSE:

- We have added p values to Table 1.
- On review of the comorbid conditions, this was a very heathy population and few subjects reported common medical conditions. For example, arterial hypertension was reported in 1.6%, no subjects had diabetes mellitus, 12.7% reported psychiatric conditions, and there were no other conditions present in >5% of the population. For this reason, we do not think that adding comorbid conditions or concomitant medications to the manuscript will add anything to the understanding of the results.

10. Patients eosinophil counts seems not to high, did they select especially very mild eosinophilic asthma patients, for sure the patients were not neutrophilic if this cause another problem.

RESPONSE: As this was the first time a TLR3 blocking antibody was administered in asthmatic subjects in the context of a viral challenge, we intentionally excluded severe

asthmatics or those with recent exacerbations. This may explain the low peripheral eosinophil counts. As we observed an excess of exacerbations in those on active treatment, this was in retrospect a wise precaution.

10. Results need clear subsection titles.

RESPONSE:

- We have added subsection titles.

12. Figure qualities and data demonstration can be substantially improved.
In figure 8, part 2 whether CRP and IL-6 were measured from the same donor, it will be good to show the link in between the time points to see the kinetics of these molecules.
13. In figure 10, this figure needs more explanation to be clear. Donor numbers for each time point is different, for example on day 2 there is only 2 dots, again if they are the same donors it will be good to see the time kinetic. The information about statistical analyses should go to the figure legends.
14. In the discussion part, the authors should focus on the discussion of their own findings not the hypothetical and findings of other authors.

RESPONSE:

- We have now provided high-resolution, re-formatted Figures 7, 8 and 9 . Figures 7 and 9 now have lines connecting between time points by subject, as requested by reviewer and we have revised the figure legends.
- We have revised the discussion to focus more on the results obtained here as suggested.

15. Considering all efficacy endpoints, and the excess of asthma exacerbations in the CNTO3157 group, the author's claim that CNTO3157 compared with placebo was ineffective in attenuating and may have augmented the respiratory manifestations of HRV16 in asthma. The reasons for the unsuccessful outcome of this study are that HRV16 also interacted with other receptors like RIG-I and MDA5 and there they also show elevated Th2 cytokines particularly IL-5. In the study, they did not measure eosinophils neither in blood or nasal lavage. The other explanation for the unsuccessful outcome that blockade of TLR3, by reducing interferon signaling, could conceivably have left viral replication unchecked resulting in worsening inflammation. Again they did not measure also IFN family of anti-viral cytokines during the study.

Reviewer #2: The study reports the efficacy of an inhibitory IgG4 kappa monoclonal antibody to TLR3 on experimental HRV16 infection in healthy and asthmatic subjects. The topic is important and current. Study uses RCT design. The paper is very well written and includes both clinical and biomarker data, both are interesting.

1. Rhinovirus infection has close association with allergic sensitization, but sensitization levels is not reported. Do authors have data on allergic sensitization measured? Eosinophil counts indicates that probably most of the study subjects were non-allergic. Do clinical and biomarker data look different in allergic subjects or those higher blood eosinophil count.

RESPONSE:

- A sensitivity of the primary endpoint in Part 2 (asthmatic subjects) for eosinophil count did not impact the primary endpoint.

2. Would be also interesting to know whether any demographics including ICS use, disease severity etc. were associated with the efficacy (clinical or biomarker) of the drug.

RESPONSE:

- As reported in the methods section, pre-specified and post-hoc sensitivity analyses around the primary endpoint including Pre-BD FEV_1 (< Median, ≥ Median), Exhaled nitric oxide (FENO); <Median ≥ Median) Blood eosinophils (< Median, ≥ Median) ACQ7 symptom score (> 1.5, ≤ 1.5) and ICS use (Yes, No) did not impact the primary endpoint.

Although the data are unique, I am not sure if this paper interests a general reader of JACI. The result was null, and the setting is not clinically feasible, i.e. drug administered 3 days before inoculation of rhinovirus, and allergy status is lacking.

RESPONSE:

- We respectfully differ. The mechanisms underlying acute exacerbations in asthma are relevant to the readership of the JACI, and although this study was negative, the search is on for novel approaches to reduce the impact of respiratory viral infections. We believe that our work, despite not demonstrating efficacy, contributes to the understanding of the role of the TLR3 axis.
- We agree that prophylactic treatment is less clinically feasible but a) this prophylactic study for an anti-TLR3 if proven positive would have led to a program for maintenance therapy of this mAB in severe asthmatics who frequently exacerbate. We also discussed using this treatment on a regular basis during the winter exacerbation season.

Reviewer #3: In this double blind, randomized parallel group study, the authors investigated the efficacy of CNTO3157, a TLR3 antagonist mAb, in attenuating the impact of a HRV16 challenge on asthma control, asthma symptoms, and lung function. This was a negative study that did not demonstrate efficacy of TLR3 antagonist mAb, and it only suppressed cold symptoms in healthy subjects. Though negative findings, the authors are commended to bring forth this novel, potentially therapeutic agent into clinical implication. I have a few suggestions for authors to consider to improve this manuscript:

1. One of the major drawbacks of the study was the patient selection. It looks the study involved a rather homogeneous asthma population with stable, well-controlled asthma. The authors do not disclose much detail regarding the characteristics of these subjects, and Table 1 only has scarce clinical information on the subjects. The sample size was rather small as well. Inclusion of a more diverse asthmatic subjects who have different clinical phenotypes (atopic, younger or older in age, etc) may lead to different outcome. The authors may need to consider broadening the subject inclusion criteria.

RESPONSE:

- This was the first ever treatment of an anti-TLRs antibody in the context of experimental rhinovirus challenge, and we were concerned about recruiting a more symptomatic severe asthma population. Based on FENO, blood eosinophils and IgE at baseline, we estimate that X% were atopic. Pediatric studies would have followed much later if we had demonstrated efficacy in adult subjects.

2. Is it possible that experimental model of HRV infection may have affected the outcome of the study? Natural HRV infection could have responded differently to TLR3 antagonist mAb. This could be included in the Discussion section.

RESPONSE:

- In a previous study of this RV16 strain in healthy volunteers (NCT01466738), the nasal symptoms were comparable to those seen in natural colds, so we believe this strain was able to cause respiratory symptoms. The 5 asthma exacerbations reported after inoculation in Part 2 support this contention.

3. Was there any statistically significant difference between treatment groups for Part 2?

RESPONSE:

- We did not observe any statistical significant difference in Part 2 but numerical trends for worse outcomes in those on active treatment as discussed.

4. Figure 3: The authors present data on change over the 10-day post-inoculation from pre-inoculation baseline in mean CSAS and CCSS for Part 1. There was a significant attenuation of both symptom scales on CNTO3157 vs placebo, but p-values are missing.
5. Line 227: enrollment is misspelled
6. Line 358: HRV16 is misspelled

RESPONSE: we have addressed points 4, 5 and 6.

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

[1]Philip E Silkoff MD, [1]Susan Flavin PhD, [1]Robert Gordon MS, [1]Mathew J Loza PhD, [2]Peter J Sterk MD, [3]Rene Lutter MD, [4]Zuzana Diamant MD, [5]Ronald B Turner MD, [6]Brian J Lipworth MD, [7]David Proud PhD, [8]Dave Singh MD, [9]Andreas Eich MD, [10]Vibeke Backer MD, [11]James E Gern MD, [12]Christian Herzmann MD, [13]Scott A Halperin MD, [14]Tjeert T Mensinga MD, [1]Alfred M Del Vecchio PhD, [1]Patrick Branigan MS, [1]Lani San Mateo PhD, [1]Frédéric Baribaud PhD, [1]Elliot S Barnathan MD, and [15]Sebastian L Johnston MD

[1]Janssen Research & Development LLC, 1400 McKean Rd, Spring House, PA, 19477, USA
[2]Dept. Respiratory Medicine F5-259, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, 1105AZ Amsterdam, the Netherlands. Email: p.j.sterk@amc.nl
[3] Depts. of Respiratory Medicine and Experimental Immunology, K0-150, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, 1105AZ Amsterdam, the Netherlands. Email: r.lutter@amc.uva.nl
[4] Department of Respiratory Medicine & Allergology, Institute for Clinical Science, Skane University Hospital, Klinikgatan 18, S-221 81, Lund, Sweden, and QPS Netherlands, Hanzeplein 1, Entrance 53, 9713GZ Groningen, The Netherlands. Email: z.diamant@gems.demon.nl
[5] Department of Pediatrics, University of Virginia School of Medicine, P.O. Box 800386, Charlottesville, VA 22908, USA. Email: RBT2N@hscmail.mcc.virginia.edu
[6]Scottish Centre for Respiratory Research, Ninewells Hospital and Medical School, University of Dundee, DD19SY, United Kingdom. Email: b.j.lipworth@dundee.ac.uk
[7]Department of Physiology & Pharmacology, Snyder Institute for Chronic Diseases, University of Calgary Cumming School of Medicine, Calgary, Alberta, T2N 4Z6, Canada. Email: dproud@ucalgary.ca
[8]Centre for Respiratory Medicine and Allergy, Medicines Evaluation Unit, University Hospital of South Manchester Foundation Trust, University of Manchester, Southmoor Road, Manchester M23 9QZ, United Kingdom. Email: dsingh@meu.org.uk
[9] IKF Pneumologie Frankfurt, Clinical Research Center Respiratory Diseases, 60596 Frankfurt, Germany. Email: eich@ikf-pneumologie.de
[10] Department of Respiratory Medicine, Bispebjerg Bakke 23, DK-2400 Copenhagen, Denmark. Email: backer@dadlnet.dk
[11] School of Medicine and Public Health, University of Wisconsin-Madison K4/918 CSC, 600 Highland Ave, Madison WI 53792-9988, USA. Email: gern@medicine.wisc.edu
[12] Research Center Borstel, Center for Clinical Studies, 23845 Borstel, Germany, Email: cherzmann@fz-borstel.de
[13] Canadian Center for Vaccinology, Dalhousie University and the IWK Health Centre, Halifax, Nova Scotia, B3K 6R8, Canada. Email:scott.halperin@dal.ca
14 QPS Netherlands, Hanzeplein 1, Entrance 53, 9713 GZ Groningen, The Netherlands. Email: tjeert.mensinga@qps.com
[15] Airway Disease Infection Section, National Heart and Lung Institute, Imperial College London, Norfolk Place, London W2 1PG, United Kingdom. Email: s.johnston@imperial.ac.uk

Corresponding author: Philip E Silkoff. Dr. Silkoff ~~is now an employee of Third Pole Inc..~~ was an employee of Janssen R&D at the time of the study. Address: 715, Bryn Mawr Ave, Penn Valley, PA, 19072, USA. Telephone +16106671698. Email philsilkoff@gmail.com

Abstract

~~**Summary**~~

Background

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

Objective.

~~We assessed~~To assess the impact of an inhibitory IgG4 kappa monoclonal antibody to TLR3, CNTO3157, on experimental ~~HRV16~~HRV-16 inoculation in healthy and asthmatic subjects.

~~**Findings**~~**Methods**

In this ~~double~~ double-blind randomized ~~parallel~~ parallel-group study at multiple sites in North America and Europe, healthy ~~(n=13)~~ and mild-moderate stable asthmatic subjects ~~(n=63)~~ were enrolled between 2012-2014. ~~Thirteen h~~Healthy and asthmatic subjects subjects received ~~a~~ single or multiple dose~~s~~ of CNTO 3157 or placebo ~~but one subject on CNTO 3157 was not inoculated.~~ , respectively, ~~Asthmatic subjects received multiple doses of CNTO3157 (n=31) or placebo (n=30)~~ and ~~30/31 CNTO 3157 and 26/30 placebo subjects~~ were then inoculated with ~~HRV16~~HRV-16 within 72 hours. All subjects were ~~then~~ monitored for respiratory symptoms, lung function, ~~with sampling for~~and nasal viral load ~~and biomarkers~~. The primary endpoint was maximal decline in forced expired volume in 1 second (FEV_1) during the 10-day post-inoculation period. ~~Subjects who received at least one dose of study agent were included in the analysis. Subjects were recruited between 2012-2014.~~

Results

~~In contrast, in~~In asthmatic subjects (N=63), CNTO3157 provided no protection against FEV_1 decline with LS mean [SE]: CNTO3157 (n=30) = -7.08 [8.15] % and placebo (n=25) groups = -5.98 [8.56] %), or symptoms after inoculation. In healthy subjects (N=12), ~~a single dose of~~ 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. ~~In contrast, in asthmatic subjects, CNTO3157 provided no protection against FEV_1 decline with LS~~

~~mean [SE]: CNTO3157 (n=30) = -7.08 [8.15] % and placebo (n=25) groups = -5.98 [8.56] %), or symptoms after inoculation.~~ ~~Two severe and three out of four moderate asthma non-serious~~All of the severe and three of the four non-serious asthma exacerbations occurred on CNTO3157. ~~There were no other significant safety signals.~~

~~Interpretation~~Conclusion

In summary, CNTO3157 ~~, at doses previously effective in blocking TLR3,~~ was ineffective in attenuating the impact of a HRV-16 challenge on lung function, asthma control, and symptoms in asthma but suppressed cold symptoms in healthy subjects~~, but was ineffective in attenuating the impact of a HRV16 challenge on lung function, asthma control, and symptoms in asthma~~. 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

Respiratory viral infections account for the majority of asthma exacerbations. Inhibition of pro-inflammatory pathways could attenuate the respiratory manifestations of such infections. Blockade of TLR3, a major viral-sensing receptor, was however ineffective in reducing the impact of a rhinovirus infection on asthma symptoms and lung function. The unmet need for other effective anti-inflammatory approaches remains.

Key words: asthma, viral infection, inflammation, TLR3

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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	elecrocardiogram
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

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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 reviewed [3]. 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/-ß), and Type III interferons (IFN-λ1-4), 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 vitro [7], 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 mice [9-11]. We hypothesized that ~~inhibition~~ 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 ~~HRV16~~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 ~~HRV16~~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) FEV_1 ≥65 % predicted. Low to medium dose inhaled corticosteroids (ICS) (based on the National Heart, Lung, and Blood Institute Guidelines [13] 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 ~~HRV16~~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 ~~herpes simplex 1 (~~HSV-1~~)~~ encephalitis [14].

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~~0~~** 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 ~~RV16~~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 ~~inhibition~~ 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 ~~HRV16~~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)~~0~~** 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 FEV_1 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 ~~HRV16~~HRV-16, ~~HRV16~~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 ~~HRV16~~HRV-16. Detailed biomarker analyses can be found in **Section E2** of the online repository.

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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 (≥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 FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16. Based on Message et al [17], for 80% power to detect a relative reduction in FEV_1 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 ~~HRV16~~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 (~~HRV16~~HRV-16 negative and HSV-1 positive). **Figure ~~1~~ 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 ~~adverse event (~~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 ~~adverse events (~~AE~~)~~s, 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.

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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 ~~2~~3**.

Part 2: asthmatic subjects

Demographical characteristics

~~Part 2:~~ The mean age of the asthmatic subjects was 38.9 years (range 18 to 65 years), and subjects were primarily (65.1%) male **~~(Table 1)~~**. 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 ~~HRV16~~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) FEV_1 (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 FEV_1 measurements for 10 consecutive days following inoculation with ~~HRV16~~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 ~~HRV16~~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 FEV_1 (< 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 ~~3~~ 4 shows the percentage change in pre-BD FEV_1 from pre-inoculation baseline to 21 days post-inoculation for the primary analysis set. The fall in FEV_1 was approximately 50% of expected based on powering assumptions as detailed in the methods section. ~~**Figure 4** shows a scatter plot of individual data points by post-inoculation day for the primary endpoint, confirming marked variability.~~

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 FEV_1, 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~~5**. Following ~~HRV16~~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~~ 6 shows the percentage change from baseline for pre-BD FEV_1 during the treatment phase before ~~HRV16~~HRV-16 inoculation in Part 2 from the mITT analysis set, to evaluate the impact of CNTO3157 vs placebo on lung function after ~~HRV16~~HRV-16 inoculation. There were no significant or clinically meaningful differences for pre-BD FEV_1 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

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in subjects treated with CNTO3157. More detailed safety information is presented in Section E6 and Table E1 in the online repository.

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~~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.~~

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~~Table 3 (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. A full AE profile can be found in Table E1 in the online repository.~~

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~~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.~~

Viral load and infectivity

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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 ~~7~~E1**).

IFN-induced chemokines CXCL10 and CXCL11 were up-regulated in both the placebo and CNTO3157 groups in Part 2. **Figure ~~8~~ E2 (online supplement)** shows the AUC and maximal value for CXCL10 in Part 1 and Part 2 after inoculation with ~~HRV16~~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 ~~HRV16~~HRV-16 in CNTO3157 but not on placebo, including IgE, IL-5 and soluble IL-33 receptor (IL-1 R4) (See **Figure ~~9~~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 ~~on~~ 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 ~~HRV16~~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 ~~HRV16~~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 asthma [18-20] in some reports, but has been reported to be greater in uncontrolled asthma with a lower FEV_1 [21]. Our assumptions for this study were that ~~HRV16~~HRV-16 challenge would result in a decline of 13% in pre-BD FEV_1 and that CNTO3157 would attenuate this decline by 50% based on Message et al [17].

To increase the probability of seeing a moderate FEV_1 decline, we allowed not only mild but also moderate persistent asthmatics on ICS therapy, and allowed subjects with a pre-BD FEV_1 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 FEV_1 decline (the maximal decline for pre-BD FEV_1 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 ~~HRV16~~HRV-16 in asthma.

Our primary hypothesis for the unsuccessful outcome of this study is that ~~HRV16~~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 ~~HRV16~~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

production [24]. 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 load [16]. In another report, a subset of asthmatic subjects infected with ~~HRV16~~HRV-16 (61%) had increased levels of secreted IL-25, a cytokine that can also augment Type-2 inflammation in the nasal mucosal fluid [27]. We observed a similar Type-2-associated response to infection with ~~HRV16~~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 infants [28], and may represent a protective host response mechanism similar to that described in models of allergic asthma [29] and lipopolysaccharide-induced acute lung injury in mice [30].

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 review[31]. 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 TLR3in 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 FEV_1 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, PEFR, 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 ~~inhibition~~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 ~~HRV16~~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 Ingleheim, 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

PA, Johnston SL, Holgate ST, Davies DE. The use of Interferon Lambda for the treatment and prevention of virally-induced exacerbation in asthma and chronic pulmonary obstructive disease. UK patent application No. 0518425.4, 9 September 2005. licensed, a patent Wark PA, Johnston SL, Holgate ST, Davies DE. Anti-Virus Therapy for Respiratory Diseases. US Patent Application – 11/517,763, Patent No.7569216, National Phase of PCT/GB2005/050031, 04 August 2009. licensed, a patent Wark PA, Johnston SL, Holgate ST, Davies DE. Interferon-beta for Anti-Virus Therapy for Respiratory Diseases. European Patent Number 1734987, 5 May 2010. licensed, a patent Wark PA, Johnston SL, Holgate ST, Davies DE. Anti-Virus Therapy for Respiratory Diseases (IFNb therapy) Hong Kong Patent Number 1097181, 31 August 2010. licensed, a patent Wark PA, Johnston SL, Holgate ST, Davies DE. Anti-Virus Therapy for Respiratory Diseases (IFNb therapy). Japanese Patent Number 4807526, 26 August 2011. licensed, a patent Wark PA, Johnston SL, Holgate ST, Davies DE. Interferon-beta for Anti-Virus Therapy for Respiratory Diseases. New Hong Kong - Divisional Patent Application No. 11100187.0, 10 January 2011. licensed, and a patent Burdin N, Almond J, Lecouturieir, V, Girerd-Chambaz Y, Guy, B, Bartlett N, Walton R, McLean G, Glanville N, Johnston SL. Induction of cross-reactive cellular response against rhinovirus antigens European Patent Number 13305152, 4 April 2013. Pending; **RBT** reports personal fees from Janssen Research and Development, during the conduct of the study; grants from Janssen Research and Development, grants from Danisco Sweeteners OY, other from Pfizer, other from PrEP Biopharm, other from GlaxoSmithKline, outside the submitted work; **BJL** reports personal fees from Teva, grants and personal fees from Chiesi, personal fees from Dr Reddy , personal fees from Sandoz, personal fees from Boehringer Ingelheim, grants and personal fees from Meda , other from Napp, outside the submitted work; **DP** reports personal fees from Janssen, during the conduct of the study; personal fees from AstraZeneca, personal fees from Pfizer, personal fees from Procter & Gamble, grants from AstraZeneca, grants from MedImmune, outside the submitted work; **RL** has nothing to disclose; **JG** received consulting fees from Janssen related to this study and multiple other consultancy fees unrelated to this study that do not constitute a conflict of interest for the subject matter of this article.

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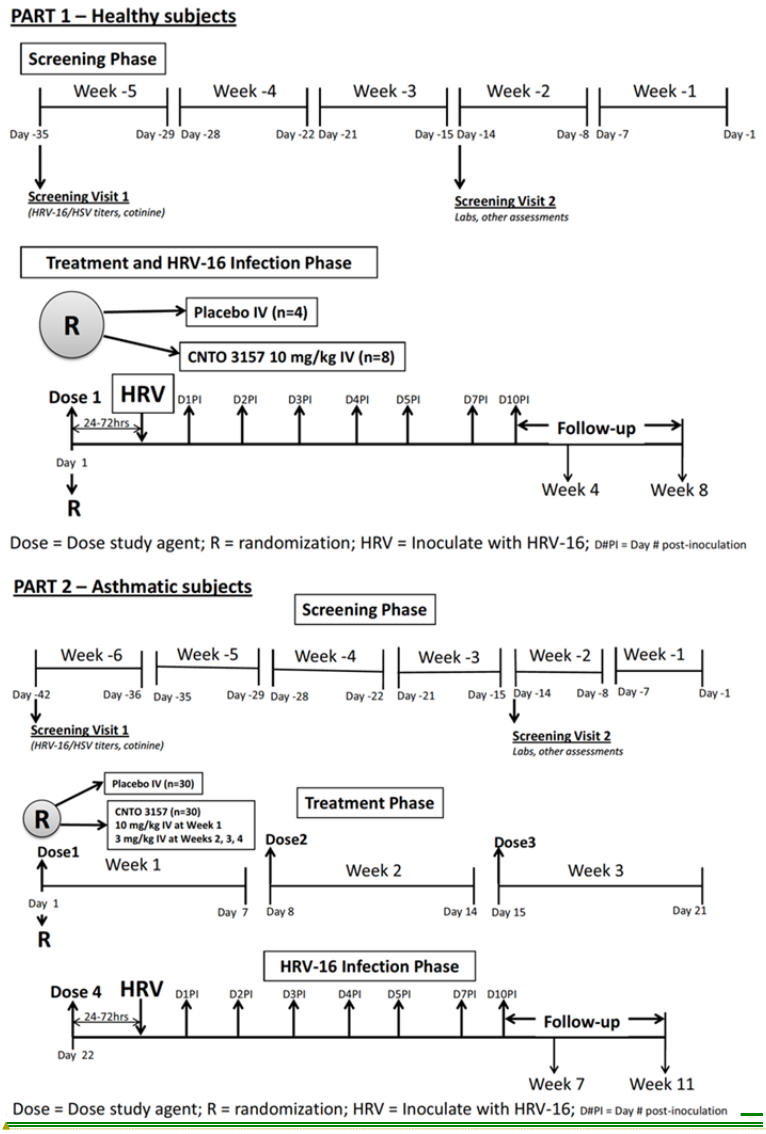


Figure legends

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

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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.

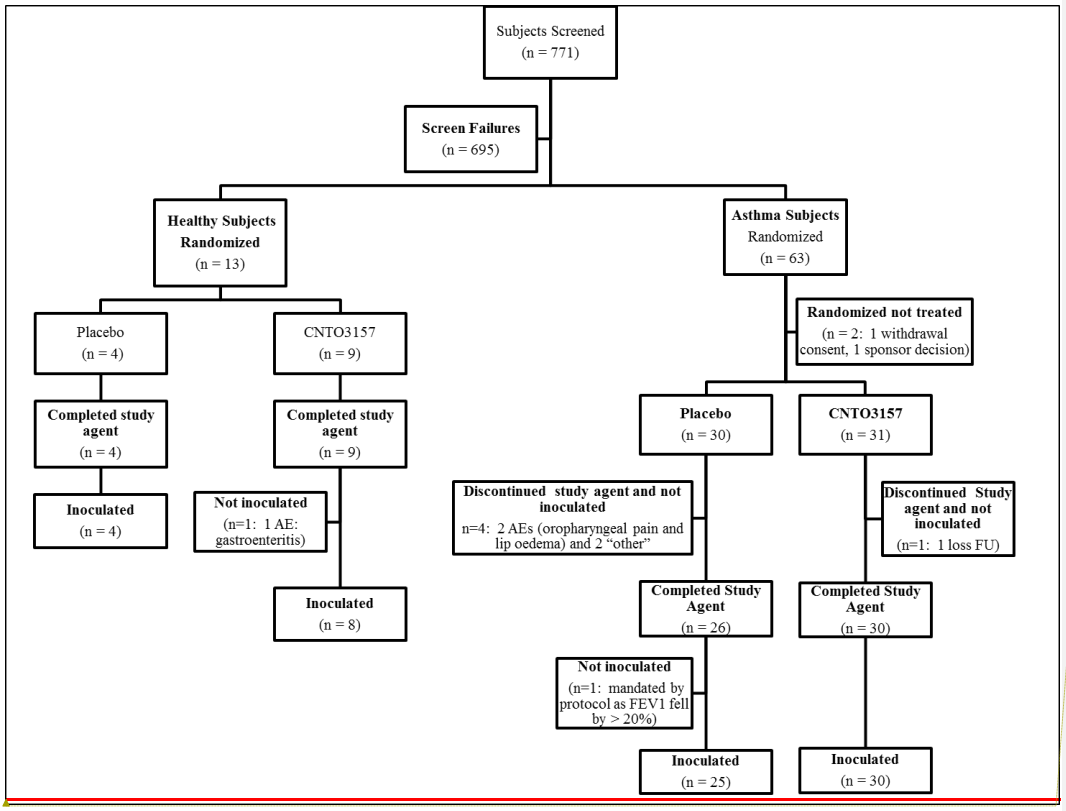


Figure ~~1~~2: Disposition of participants for Part 1 (healthy subjects) and Part 2 (mild-to moderate persistent asthma). Where reasons for discontinuation are recorded as "other", there is no documented reason in the database. AE= adverse events.

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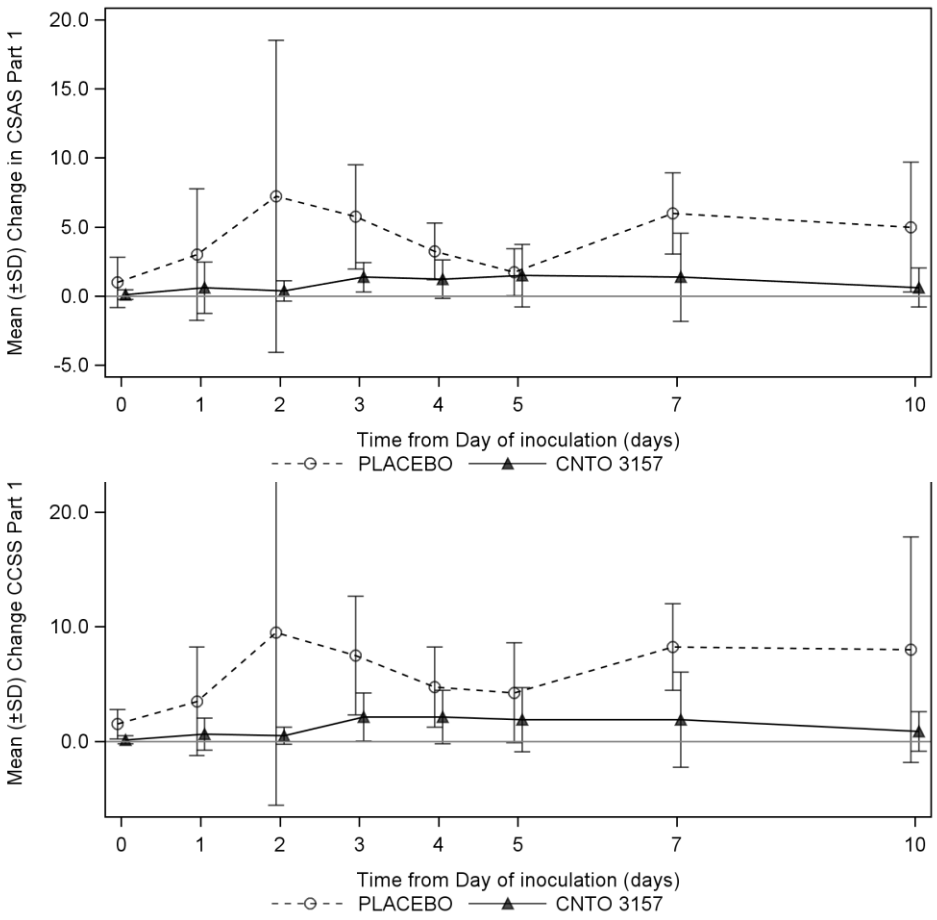


Figure ~~23~~3: Change over the 10-day post-inoculation from pre-inoculation baseline in mean (±SD) CSAS and CCSS (symptom scales) for Part 1 (healthy subjects), where there was a significant attenuation of both symptom scales on CNTO3157 (n=8) vs placebo (n=4).

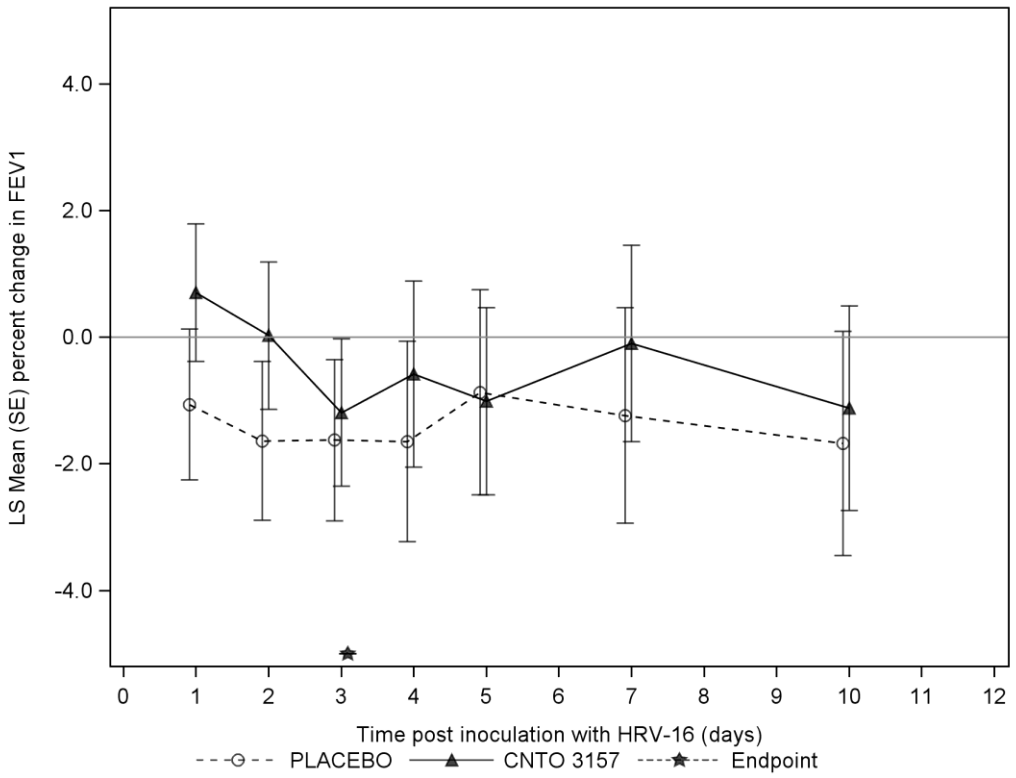
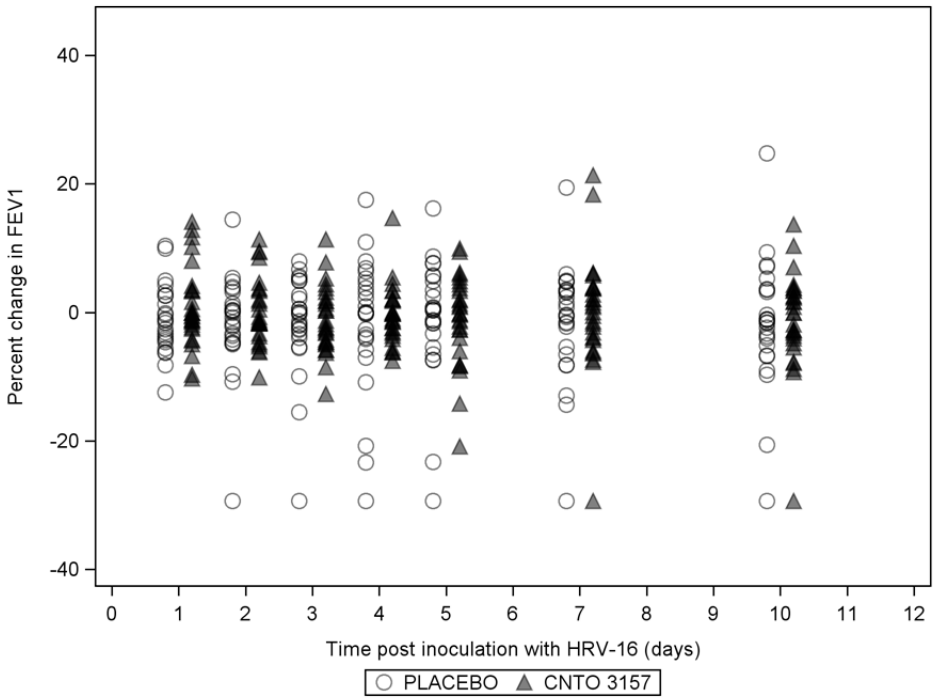


Figure ~~3~~4: The primary endpoint, % change from pre-inoculation baseline in LS mean (SE) Pre-BD FEV1 (mITT ~~HRV16~~HRV-16 analysis set) for CNTO3157 and placebo in Part 2 (persistent asthmatic subjects). There was no significant difference for maximal fall or AUC day 1-Day 10 post-inoculation between CNTO3157 and placebo.

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~~**Figure 4**: For the primary endpoint, a scatter plot of individual subject data for the % change from screening baseline CNTO3157 and placebo in Part 2 during the post-inoculation phase. This demonstrates the marked variability in FEV1 post-inoculation~~

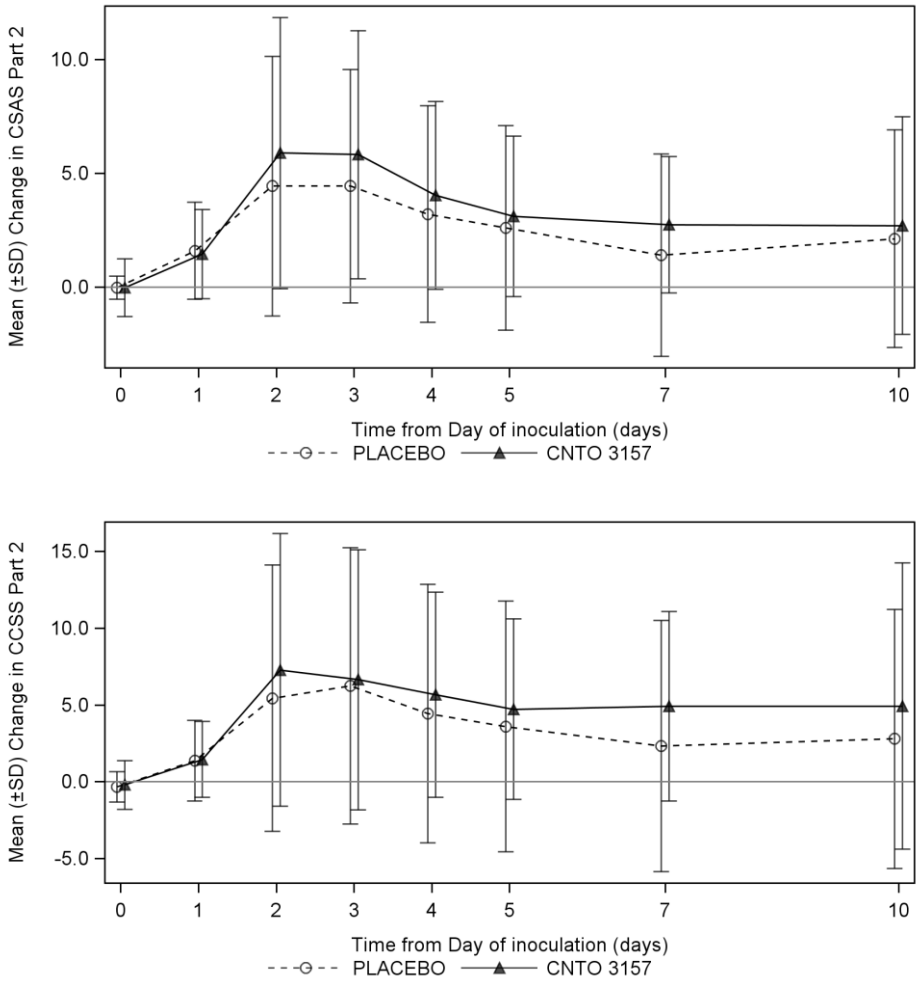
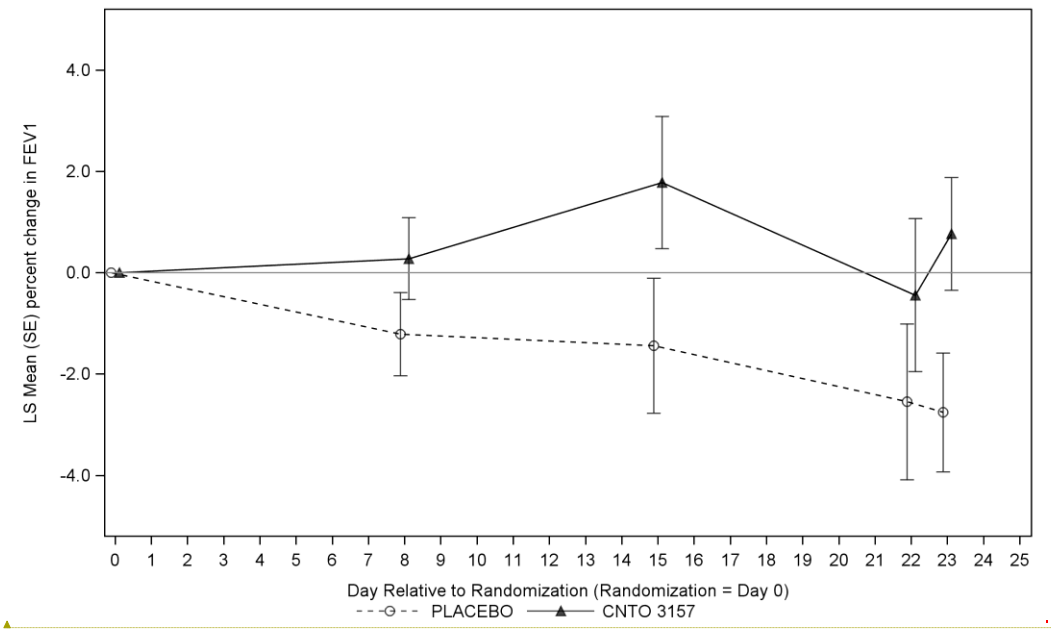


Figure ~~5~~5: Change in mean (SD) CSAS and CCSS post HRV inoculation from pre-inoculation baseline over the 10-day post-inoculation period for Part 2 (persistent asthmatic subjects) where both symptom scales were numerically higher on CNTO3157.

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Figure ~~6~~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 ~~HRV16~~HRV-16 inoculation). This demonstrates the effect of ~~inhibition~~ blockade of TLR3 compared to placebo on lung function. While there was a numerical difference between CNTO 3167 and placebo, this was not significant.

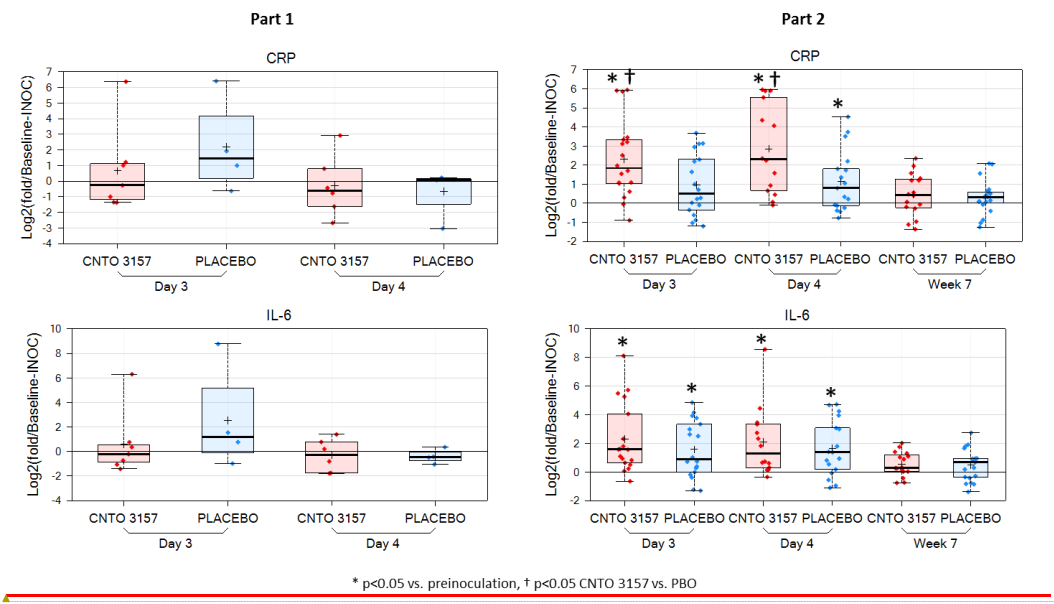
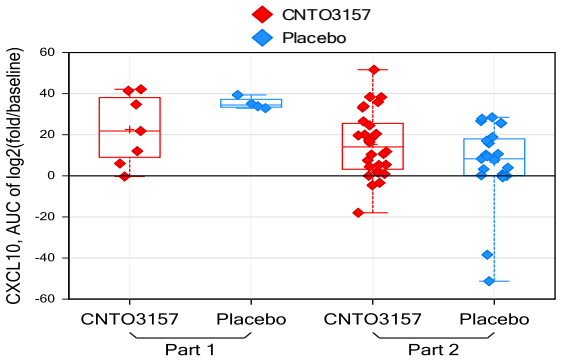
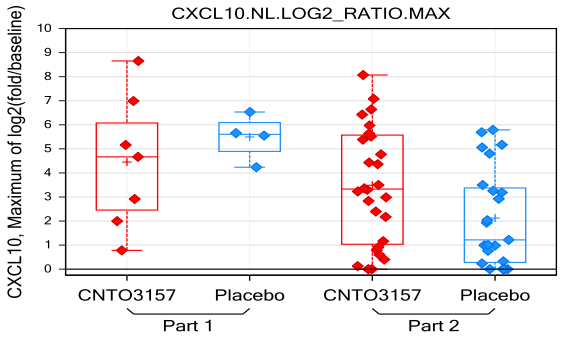


Figure 7: Acute phase reactants (CRP, and IL6) in nasal lavage by day post-inoculation expressed as Log2 fold change post inoculation (INOC) versus pre-inoculation baseline. In Part 2 (persistent asthma), There were significant within-group elevations in CRP on CNTO3157 on Days 3 and 4 post-inoculation compared to baseline and on placebo for Day 4, and significant CRP elevations on CNTO3157 compared to placebo on Days 3 and 4 post-inoculation ($p<0.05$). Both CNTO3157 and placebo showed significant elevations in nasal IL-6 on Days 3 and 4 post-inoculation compared to pre-inoculation baselines ($p<0.05$).

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CXCL10 Area Under the Curve Over Time Relative to Individual Baseline: Nasal



CXCL10 Maximal Response Relative to Individual Baseline: Nasal

~~**Figure 8**: AUC (top panel) and maximal (bottom panel) nasal lavage CXCL10 (IP10) levels post-inoculation in Parts 1 and 2. In Part 1 there was greater suppression of AUC and maximal IP10 post-inoculation on CNTO3157 compared to placebo, whereas in Part 2, there were numerically greater symptoms accompanied by a greater elevation of maximal and AUC IP10 (right panels) on CNTO3157 relative to placebo~~

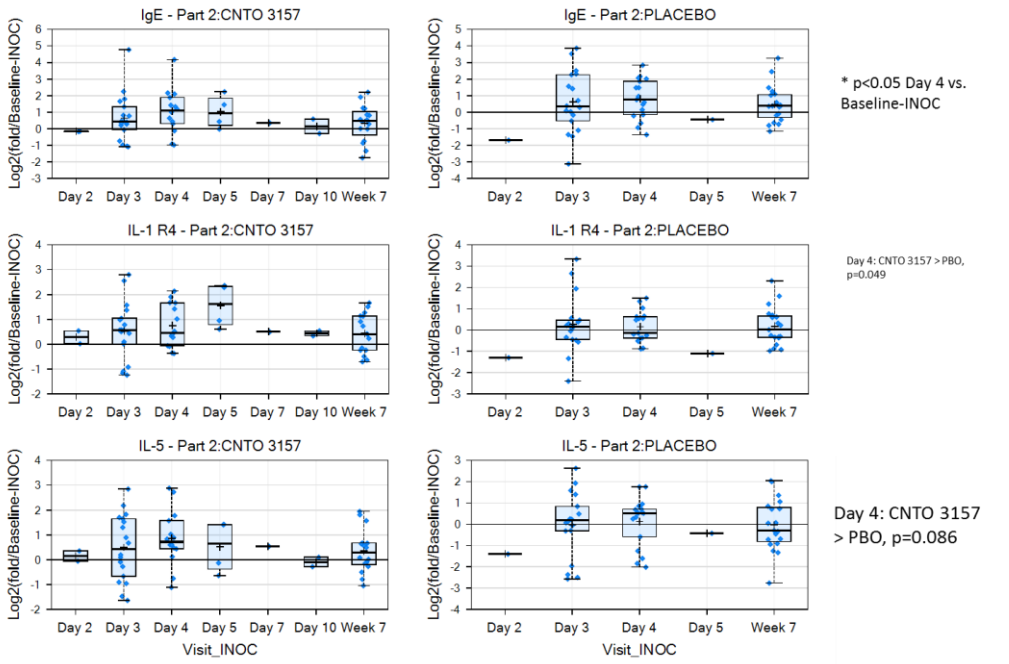
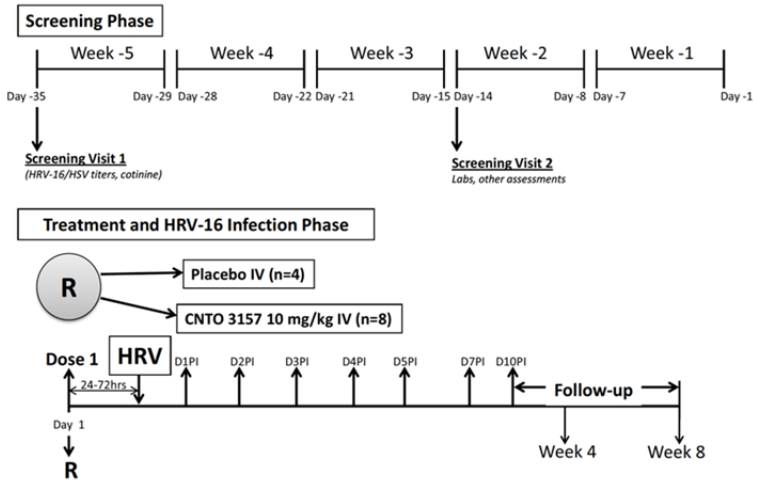


Figure 9: Th2 cytokines in nasal lavage for Part 2 by Day post-inoculation

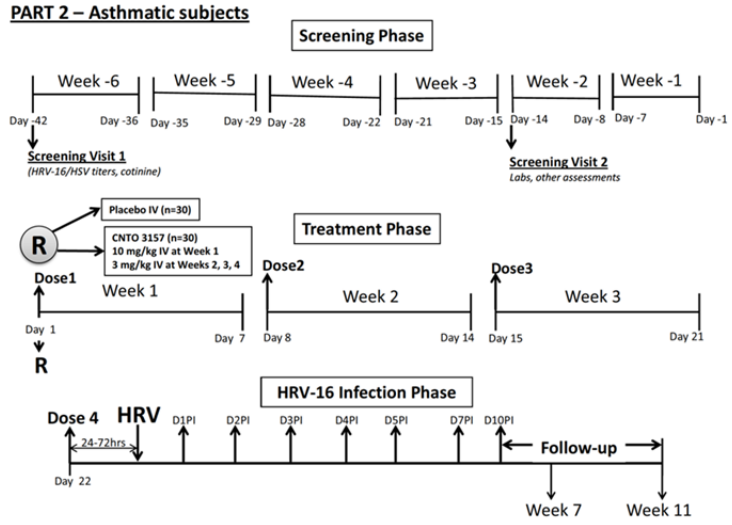
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PART 1 – Healthy subjects



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

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~~**Figure 10**: 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 HRV16 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 HRV16 within 72 hours and monitored closely for 10 days post inoculation with safety follow-up visits at weeks 7 and 11.~~

Table 1: Demographic and Disease characteristics for Part 2

	Placebo	CNTO3157
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)
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; FEV_1: 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.

Comment [PES1]: Matt, can you add p values, and do you know where there is a list of comorbid diseases and medications?

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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

Table 3: Number of Subjects With 1 or More Treatment-Emergent Adverse Events by in Part 2; Safety Analysis Set

	placebo				CNTO3157			
	High Dose Period	Low Dose Period	Virus → End	Treat → End	High Dose Period	Low Dose Period	Virus → End	Treat → End
n	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 CNTO3157 and HRV16

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

[1]Philip E Silkoff MD, [1]Susan Flavin PhD, [1]Robert Gordon MS, [1]Mathew J Loza PhD, [2]Peter J Sterk MD, [3]Rene Lutter MD, [4]Zuzana Diamant MD, [5]Ronald B Turner MD, [6]Brian J Lipworth MD, [7]David Proud PhD, [8]Dave Singh MD, [9]Andreas Eich MD, [10]Vibeke Backer MD, [11]James E Gern MD, [12]Christian Herzmann MD, [13]Scott A Halperin MD, [14]Tjeert T Mensinga MD, [1]Alfred M Del Vecchio PhD, [1]Patrick Branigan MS, [1]Lani San Mateo PhD, [1]Frédéric Baribaud PhD, [1]Elliot S Barnathan MD, and [15]Sebastian L Johnston MD

[1]Janssen Research & Development LLC, 1400 McKean Rd, Spring House, PA, 19477, USA
[2]Dept. Respiratory Medicine F5-259, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, 1105AZ Amsterdam, the Netherlands. Email: p.j.sterk@amc.nl
[3] Depts. of Respiratory Medicine and Experimental Immunology, K0-150, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, 1105AZ Amsterdam, the Netherlands. Email: r.lutter@amc.uva.nl
[4] Department of Respiratory Medicine & Allergology, Institute for Clinical Science, Skane University Hospital, Klinikgatan 18, S-221 81, Lund, Sweden, and QPS Netherlands, Hanzeplein 1, Entrance 53, 9713GZ Groningen, The Netherlands. Email: z.diamant@gems.demon.nl
[5] Department of Pediatrics, University of Virginia School of Medicine, P.O. Box 800386, Charlottesville, VA 22908, USA. Email: RBT2N@hscmail.mcc.virginia.edu
[6]Scottish Centre for Respiratory Research, Ninewells Hospital and Medical School, University of Dundee, DD19SY, United Kingdom. Email: b.j.lipworth@dundee.ac.uk
[7]Department of Physiology & Pharmacology, Snyder Institute for Chronic Diseases, University of Calgary Cumming School of Medicine, Calgary, Alberta, T2N 4Z6, Canada. Email: dproud@ucalgary.ca
[8]Centre for Respiratory Medicine and Allergy, Medicines Evaluation Unit, University Hospital of South Manchester Foundation Trust, University of Manchester, Southmoor Road, Manchester M23 9QZ, United Kingdom. Email: dsingh@meu.org.uk
[9] IKF Pneumologie Frankfurt, Clinical Research Center Respiratory Diseases, 60596 Frankfurt, Germany. Email: eich@ikf-pneumologie.de
[10] Department of Respiratory Medicine, Bispebjerg Bakke 23, DK-2400 Copenhagen, Denmark. Email: backer@dadlnet.dk
[11] School of Medicine and Public Health, University of Wisconsin-Madison K4/918 CSC, 600 Highland Ave, Madison WI 53792-9988, USA. Email: gern@medicine.wisc.edu
[12] Research Center Borstel, Center for Clinical Studies, 23845 Borstel, Germany, Email: cherzmann@fz-borstel.de
[13] Canadian Center for Vaccinology, Dalhousie University and the IWK Health Centre, Halifax, Nova Scotia, B3K 6R8, Canada. Email:scott.halperin@dal.ca
14 QPS Netherlands, Hanzeplein 1, Entrance 53, 9713 GZ Groningen, The Netherlands. Email: tjeert.mensinga@qps.com
[15] Airway Disease Infection Section, National Heart and Lung Institute, Imperial College London, Norfolk Place, London W2 1PG, United Kingdom. Email: s.johnston@imperial.ac.uk

Corresponding author: Philip E Silkoff. Dr. Silkoff was an employee of Janssen R&D at the time of the study. Address: 715, Bryn Mawr Ave, Penn Valley, PA, 19072, USA. Telephone +16106671698. Email philsilkoff@gmail.com

Abstract

Background

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

Objective.

To assess the impact of an inhibitory IgG4 kappa monoclonal antibody to TLR3, CNTO3157, on experimental HRV-16 inoculation in healthy and asthmatic subjects.

Methods

In this double-blind randomized parallel-group study at multiple sites in North America and Europe, healthy and mild-moderate stable asthmatic subjects were enrolled between 2012-2014. Healthy and 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 (FEV_1) during the 10-day post-inoculation period.

Results

In asthmatic subjects (N=63), CNTO3157 provided no protection against FEV_1 decline with LS mean [SE]: CNTO3157 (n=30) = -7.08 [8.15] % and placebo (n=25) groups = -5.98 [8.56] %), or symptoms after 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 a 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

Respiratory viral infections account for the majority of asthma exacerbations. Inhibition of pro-inflammatory pathways could attenuate the respiratory manifestations of such infections. Blockade of TLR3, a major viral-sensing receptor, was however ineffective in reducing the impact of a rhinovirus infection on asthma symptoms and lung function. The unmet need for other effective anti-inflammatory approaches remains.

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	elecrocardiogram
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 reviewed [3]. 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/-ß), and Type III interferons (IFN-λ1-4), 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 vitro [7], 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 mice [9-11]. 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) FEV_1 ≥65 % predicted. Low to medium dose inhaled corticosteroids (ICS) (based on the National Heart, Lung, and Blood Institute Guidelines [13] 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 encephalitis [14].

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 FEV_1 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 (≥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 FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16. Based on Message et al [17], for 80% power to detect a relative reduction in FEV_1 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) FEV_1 (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 FEV_1 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 FEV_1 (< 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 FEV_1 from pre-inoculation baseline to 21 days post-inoculation for the primary analysis set. The fall in FEV_1 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 FEV_1, 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 FEV_1 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 FEV_1 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 asthma [18-20] in some reports, but has been reported to be greater in uncontrolled asthma with a lower FEV_1 [21]. Our assumptions for this study were that HRV-16 challenge would result in a decline of 13% in pre-BD FEV_1 and that CNTO3157 would attenuate this decline by 50% based on Message et al [17].

To increase the probability of seeing a moderate FEV_1 decline, we allowed not only mild but also moderate persistent asthmatics on ICS therapy, and allowed subjects with a pre-BD FEV_1 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 FEV_1 decline (the maximal decline for pre-BD FEV_1 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 production [24]. 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 load [16]. 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 fluid [27]. 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 infants [28], and may represent a protective host response mechanism similar to that described in models of allergic asthma [29] and lipopolysaccharide-induced acute lung injury in mice [30].

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 review[31]. 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 TLR3in 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 FEV_1 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, PEFR, 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 Ingleheim, 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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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 (±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	CNTO3157
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)
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)

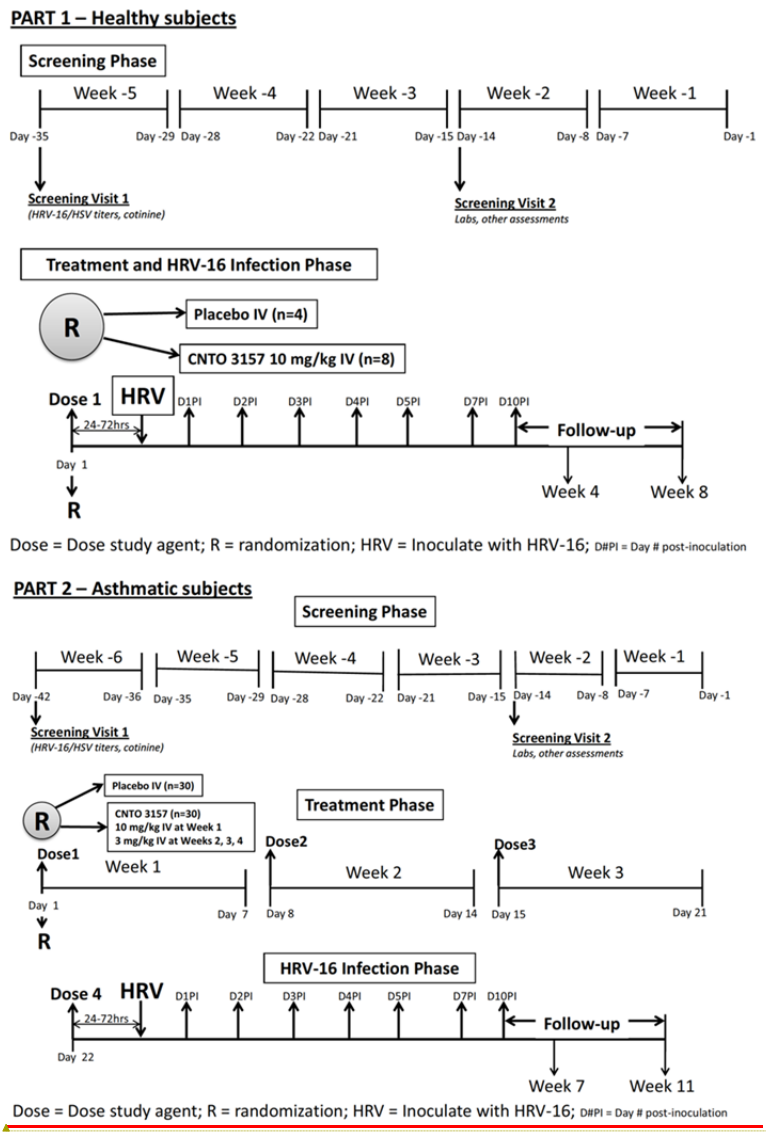
BMI: body mass index; SD: standard deviation; pre-BD: pre-bronchodilator; FEV_1: forced expired volume in 1 second; FENO: fractional concentration of exhaled nitric oxide; ACQ: asthma control questionnaire; ICS: inhaled corticosteroids. There were no significant between-group differences for demographic 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

Figure No.1

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Figure No.2

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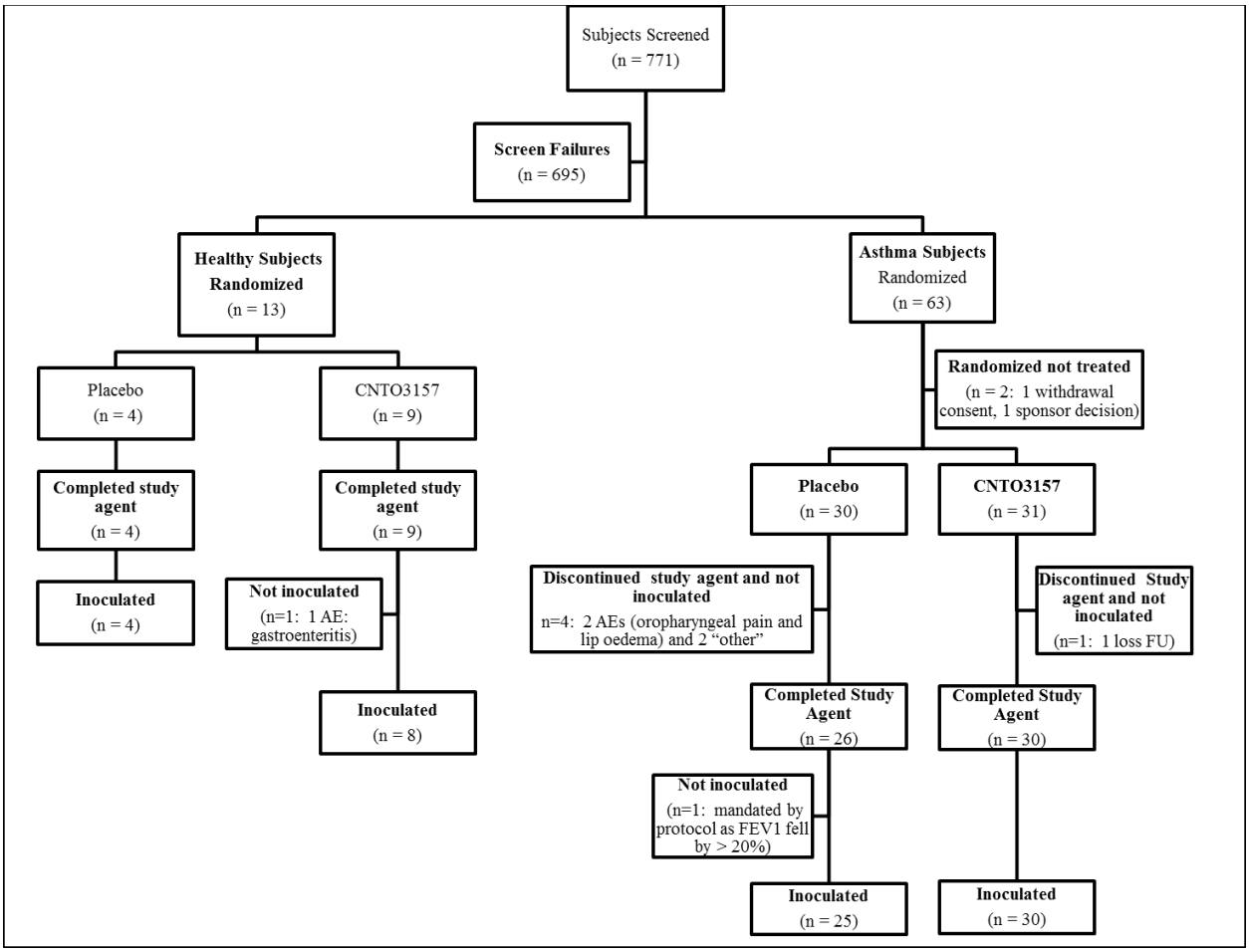


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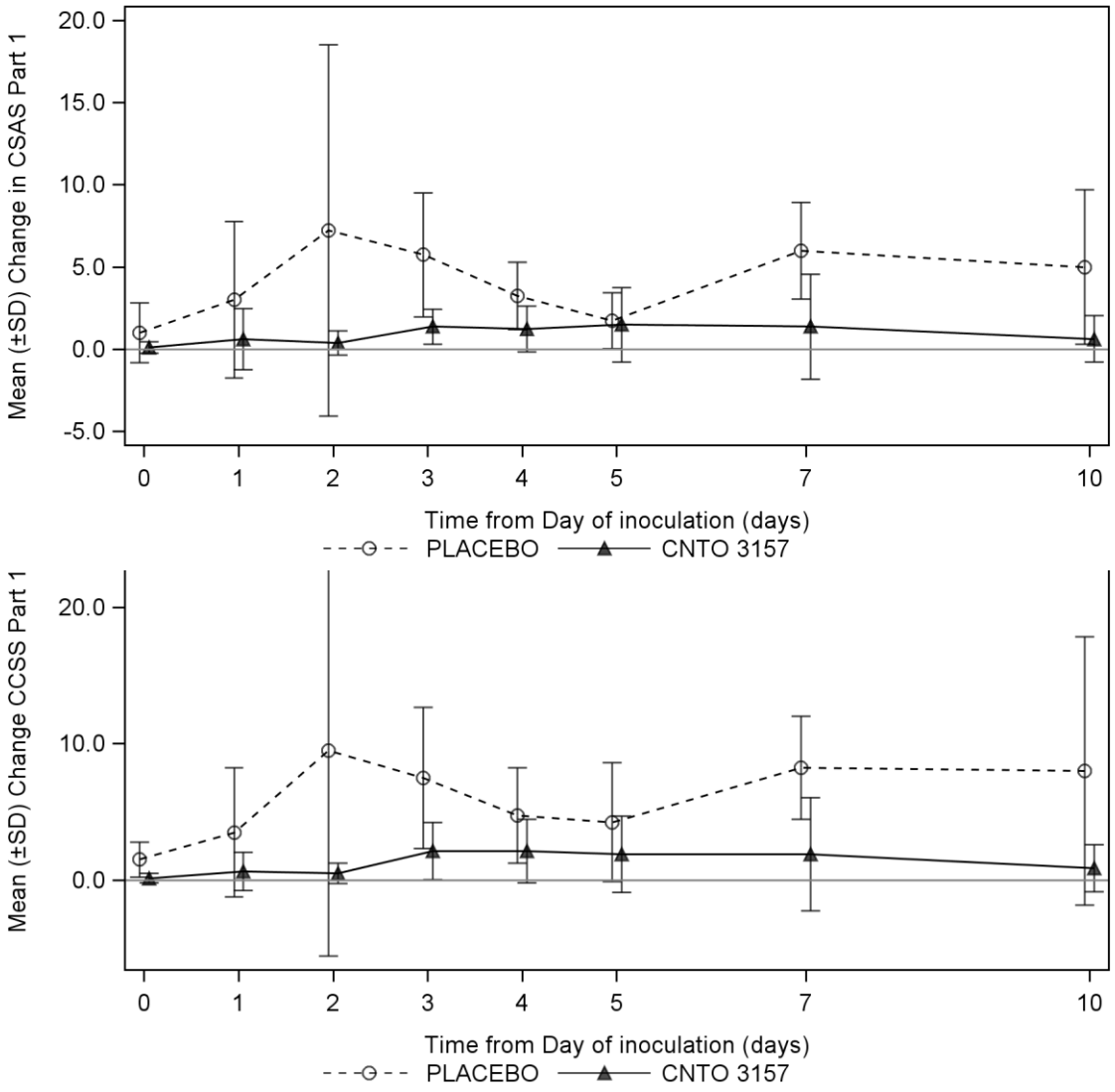


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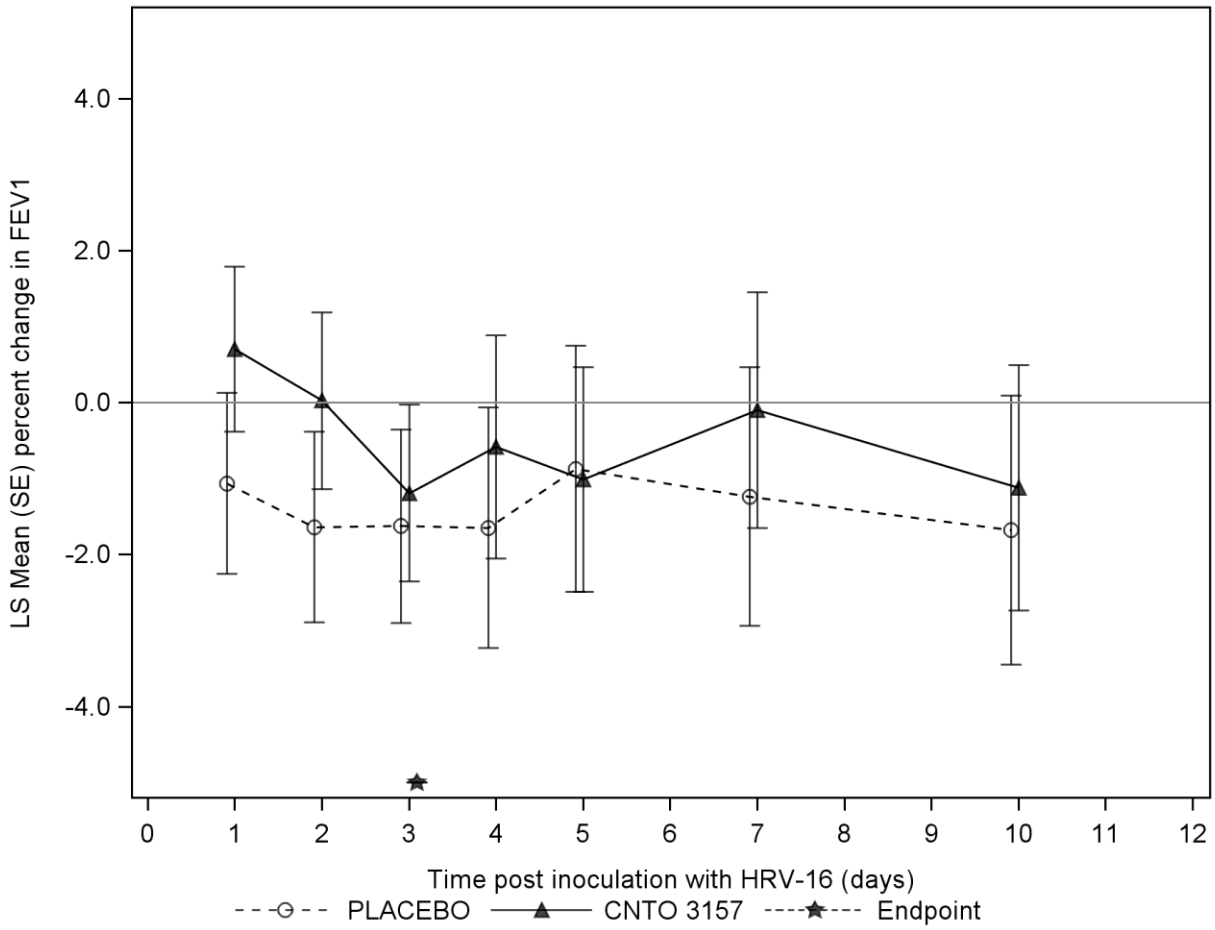
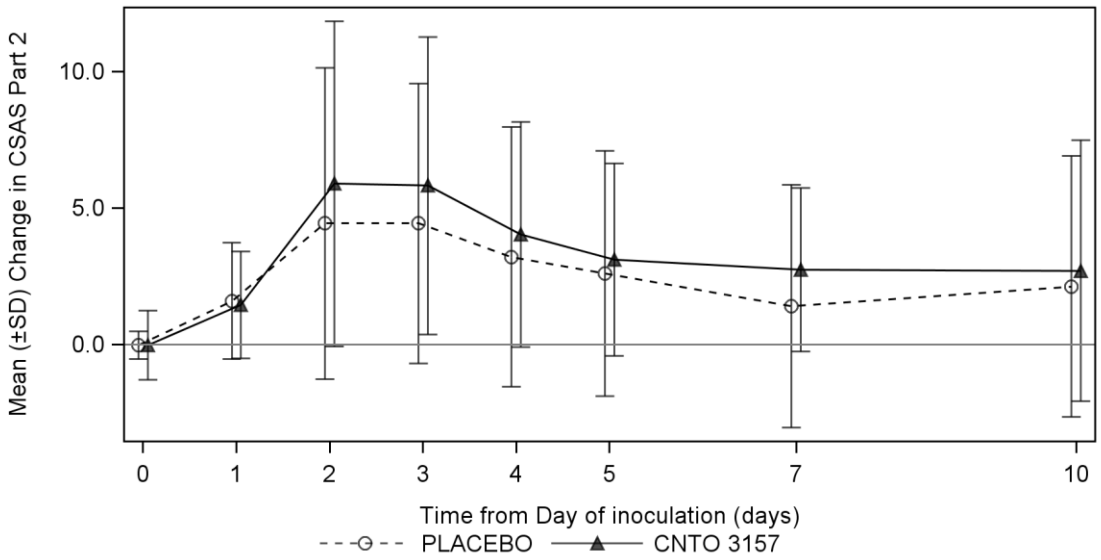


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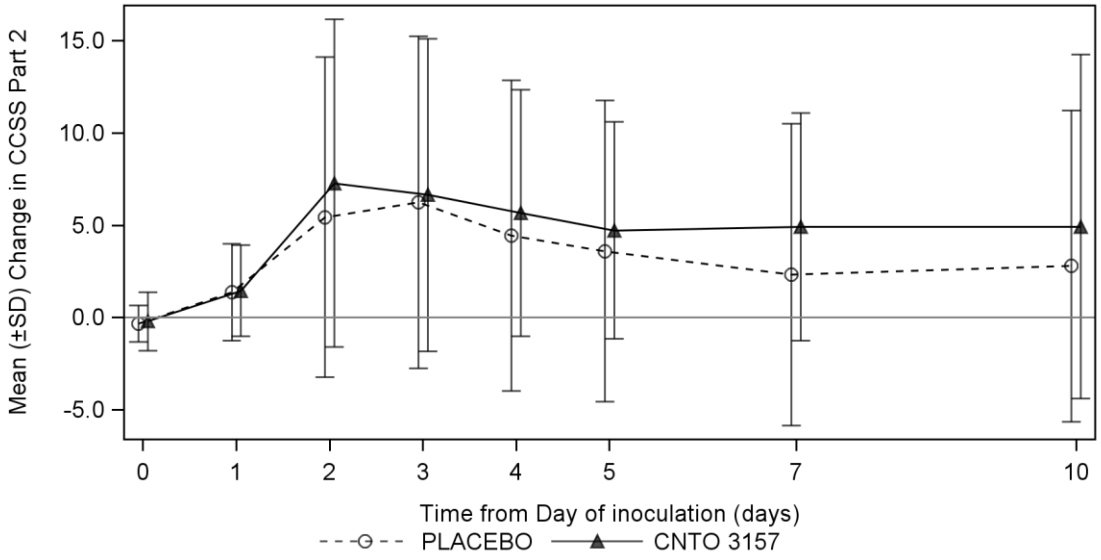
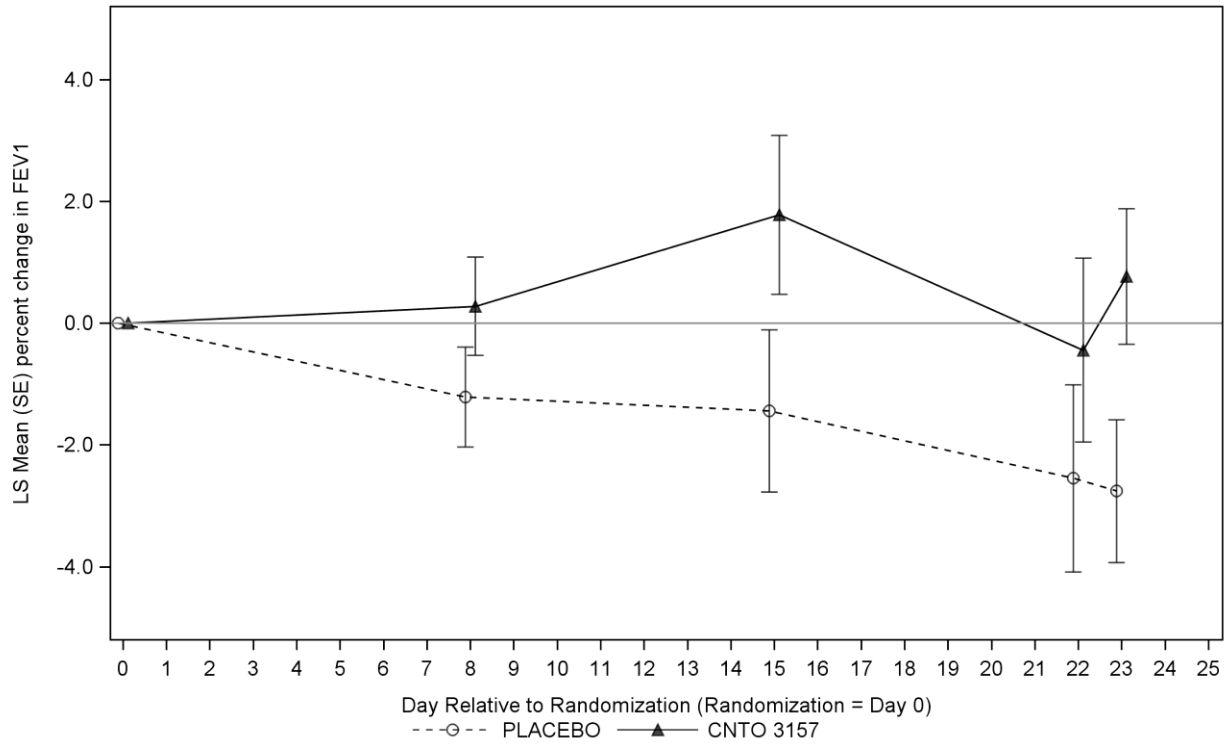


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Online Supplement

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 infection[1-6].

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 $\log_{10}$ $TCID_{50}$/mL ($2x10^3$ $TCID_{50}$/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 $\log_{10}$ 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 $TCID_{50}$/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 $TCID_{50}$/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

	PBO				CNTO3157			
	High Dose Period	Low Dose Period	Virus → End	Treat → End	High Dose Period	Low Dose Period	Virus → End	Treat → End
n	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 CNTO3157 and HRV16

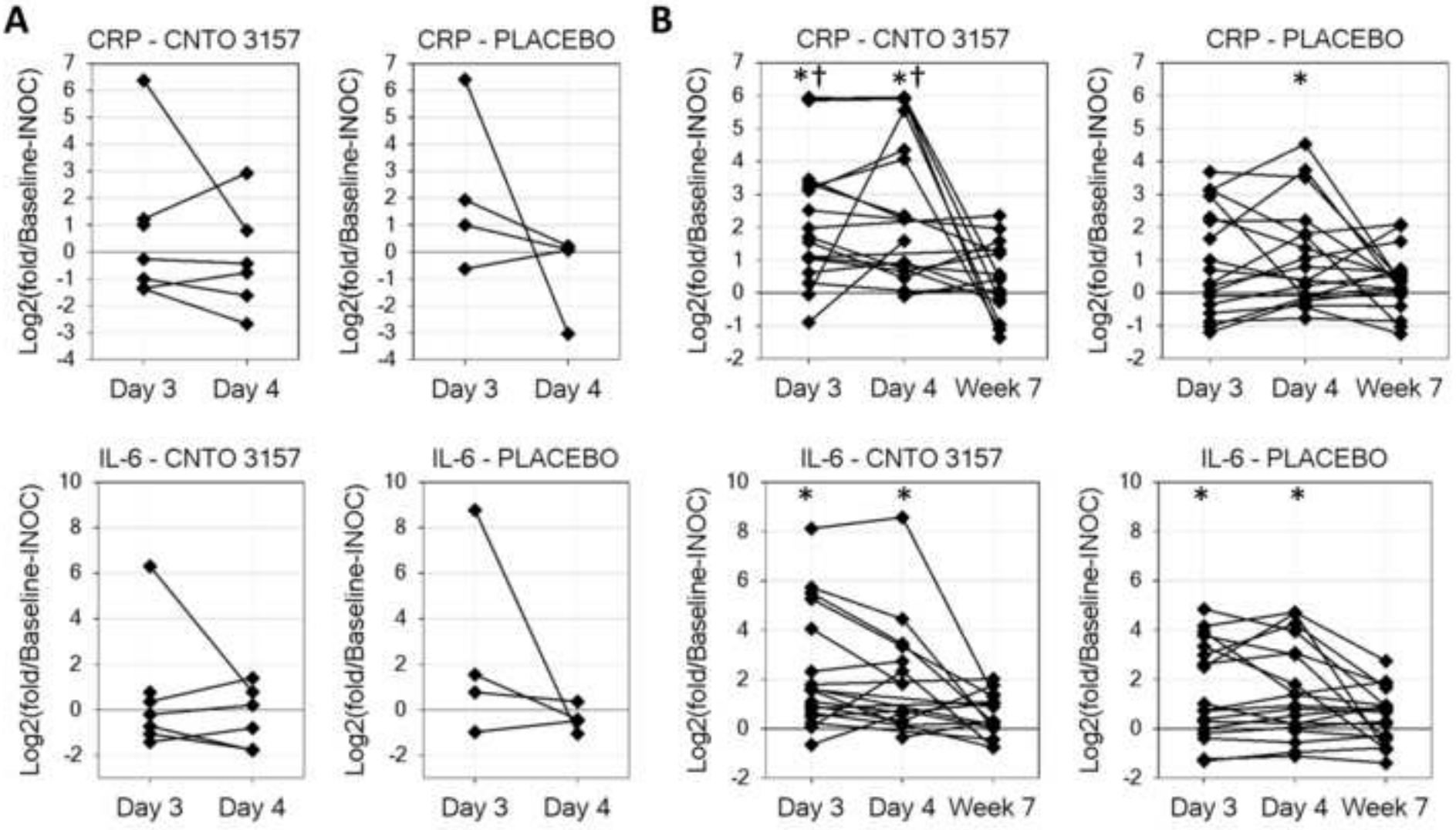
Table E2: Part 2 asthma, day 4 post-RV16 inoculation up-regulated nasal lavage analytes*			
6Ckine	Coagulation Factor IXab	**IgG**	Proteinase-3
a1-Antitrypsin	Coagulation Factor XI	**ILT-2**	PSME1
a2-Antiplasmin	Dkk-4	ILT-4	resistin
a2-HS-Glycoprotein	ECM1	**LBP**	RTN4
Afamin	**Elastase**	LYVE1	SAA
Angiotensinogen	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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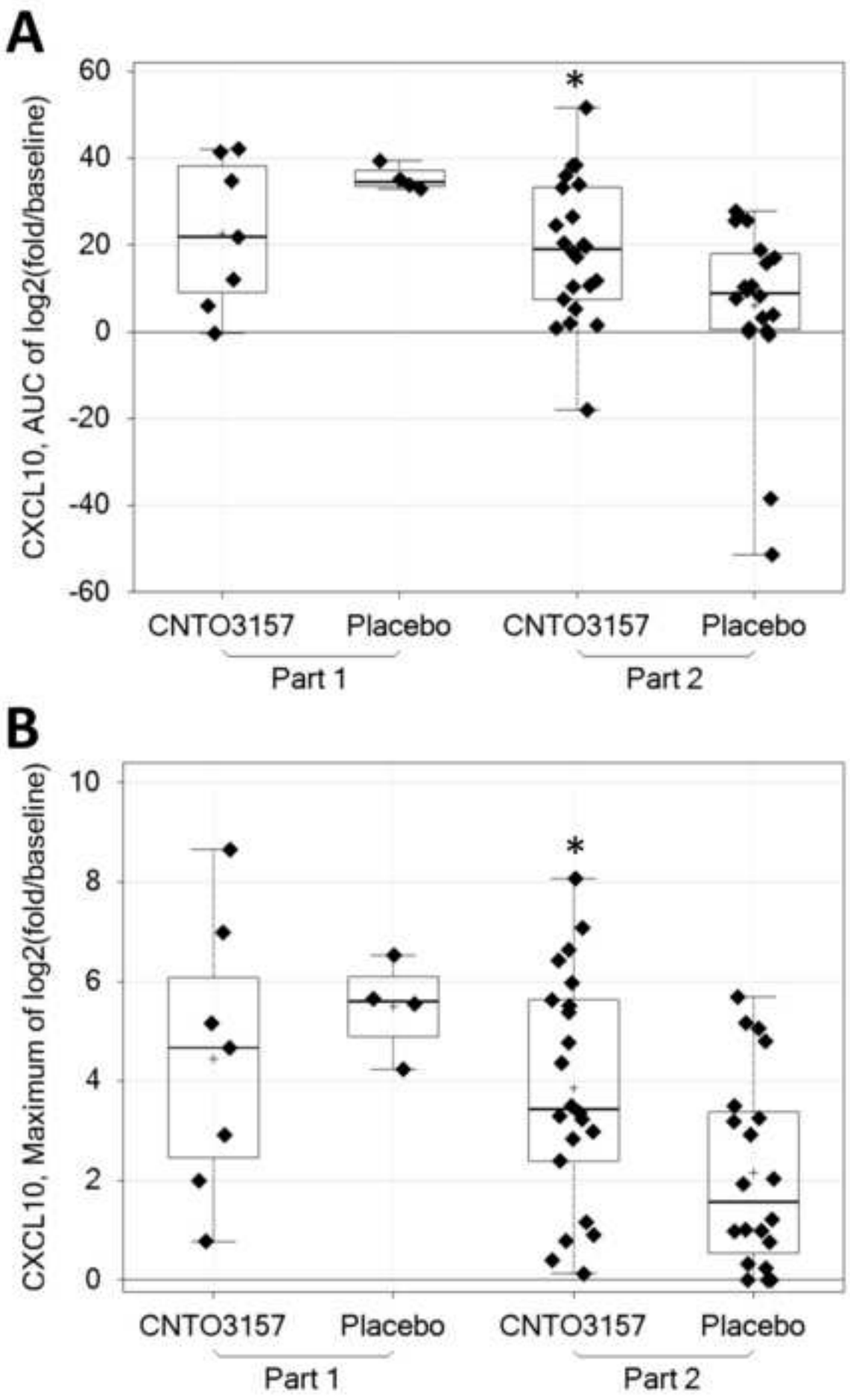
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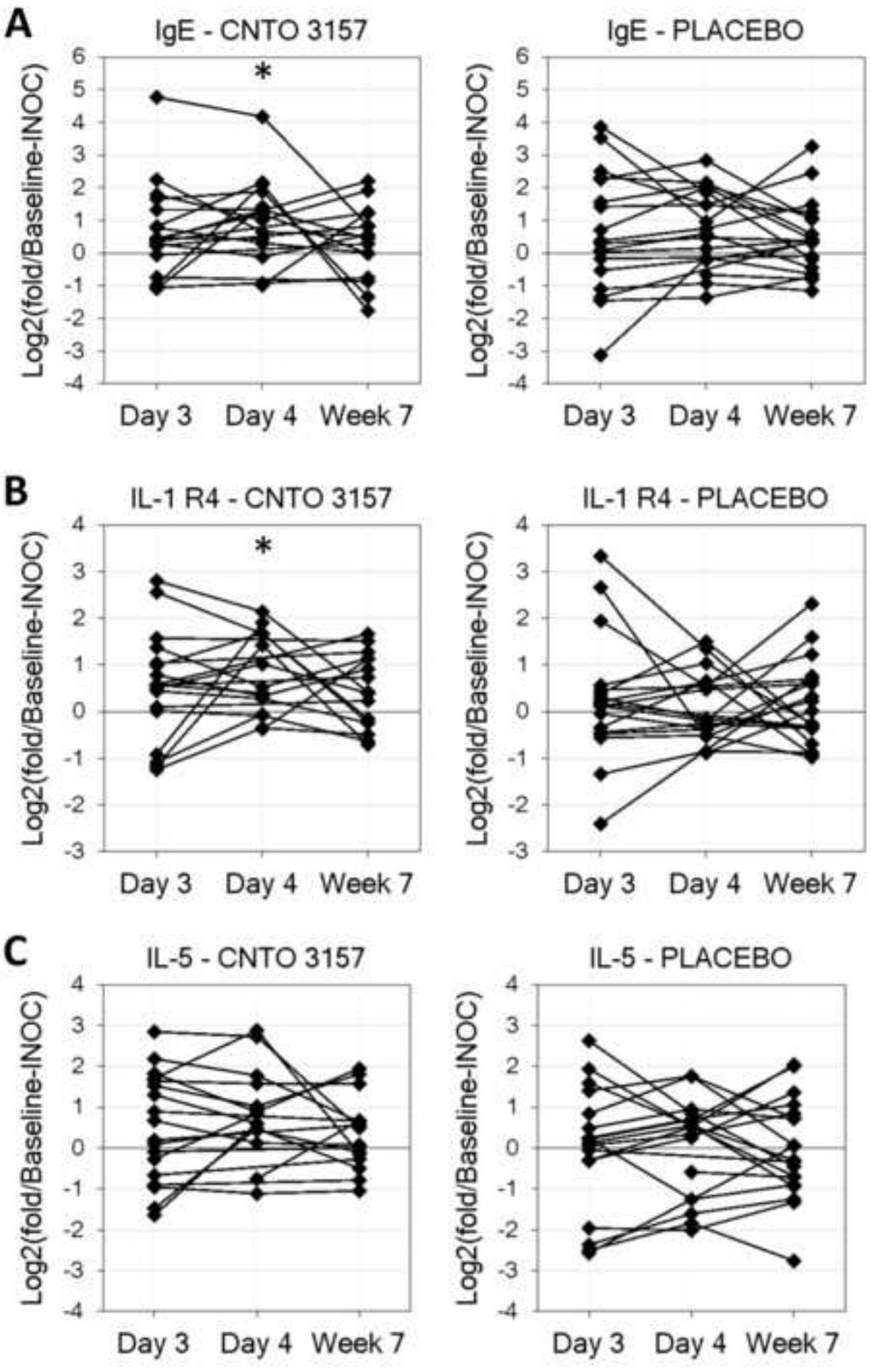
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Janssen Research & Development*

Clinical Protocol

A Phase 1, Randomized, Double-blind, Placebo-controlled Study Evaluating CNTO 3157 in Healthy Normal and Asthmatic Subjects Inoculated with Human Rhinovirus Type 16

Protocol CNTO3157ASH1002; Phase 1b

CNTO 3157

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EudraCT NUMBER: 2011-005369-19

Issue/Report Date: 18 Apr 2012
Prepared by: Janssen Research & Development, LLC
Document No.: EDMS-ERI-30584521

Compliance: This study will be conducted in compliance with this protocol, Good Clinical Practice, and applicable regulatory requirements.

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INVESTIGATOR AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study agent, the conduct of the study, and the obligations of confidentiality.

Coordinating Investigator (where required):

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Signature: _____ Date: _____

(Day Month Year)

Principal (Site) Investigator:

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Telephone Number: _____

Signature: _____ Date: _____

(Day Month Year)

Sponsor's Responsible Medical Officer:

Name (typed or printed): Jerome A. Boscia, MD

Institution: Janssen Research & Development, LLC

Signature: FOR JEROME BOSCIA Date: 17 APR 2012

(Day Month Year)

Note: If the address or telephone number of the investigator changes during the course of the study, written notification will be provided by the investigator to the sponsor, and a protocol amendment will not be required.

TABLE OF CONTENTS

INVESTIGATOR AGREEMENT **2**

TABLE OF CONTENTS **3**

LIST OF ATTACHMENTS **6**

LIST OF IN-TEXT TABLES AND FIGURES **6**

SYNOPSIS **8**

TIME AND EVENTS SCHEDULES **14**

1. PART 1: HEALTHY SUBJECTS **14**

2. PART 2: ASTHMATIC SUBJECTS **17**

ABBREVIATIONS **21**

1. INTRODUCTION **23**
1.1. Overall Rationale for the Study 27

2. OBJECTIVES AND HYPOTHESIS **29**
2.1. Objectives 29
2.1.1. Part 1 29
2.1.1.1. Primary 29
2.1.1.2. Secondary 29
2.1.1.3. Exploratory 29
2.1.2. Part 2 29
2.1.2.1. Primary 29
2.1.2.2. Secondary 29
2.1.2.3. Exploratory 29
2.2. Hypothesis 30
2.2.1. Part 1 30
2.2.2. Part 2 30

3. STUDY DESIGN AND RATIONALE **30**
3.1. Overview of Study Design 30
3.1.1. Part 1 31
3.1.1.1. Study Design Rationale – Part 1 (Healthy Adult Subjects) 31
3.1.1.2. Dose Rationale – Part 1 32
3.1.2. Part 2 33
3.1.2.1. Study Design Rationale – Part 2 (Adult Asthmatic Subjects) 33
3.1.2.2. Dose Rationale – Part 2 34
3.2. Randomization and Blinding – Part 1 and Part 2 36

4. SUBJECT SELECTION **36**
4.1. Part 1 37
4.1.1. Inclusion Criteria 37
4.1.2. Exclusion Criteria 39
4.1.3. Randomization Criteria 42
4.2. Part 2 42
4.2.1. Inclusion Criteria 42
4.2.2. Exclusion Criteria 45
4.2.3. Randomization Criteria 48
4.3. Eligibility Criteria for Sputum Induction 48

5. TREATMENT ALLOCATION AND BLINDING 49

6. DOSAGE AND ADMINISTRATION 50

7. TREATMENT COMPLIANCE 51

8. PRESTUDY AND CONCOMITANT THERAPY 51
8.1. Part 1 - Healthy Adult Subjects 51
8.2. Part 2 - Asthmatic Subjects 52
8.2.1. Treatment of Asthma Exacerbations 54

9. STUDY EVALUATIONS 54
9.1. Study Procedures in Part 1 55
9.1.1. Screening Phase (Part 1) 55
9.1.1.1. Screening Visit 1 55
9.1.1.2. Screening Visit 2 56
9.1.1.3. Screen Failure/Rescreening 56
9.1.2. Double-Blind Treatment Phase (Part 1) 56
9.1.3. Follow-Up Phase (Part 1) 58
9.2. Study Procedures in Part 2 58
9.2.1. Screening Phase (Part 2) 59
9.2.1.1. Screening Visit 1 59
9.2.1.2. Screening Visit 2 59
9.2.1.3. Repeat Testing During Screening (Part 2 only) 60
9.2.1.4. Screen Failure/Rescreening 60
9.2.2. Double-Blind Treatment Phase (Part 2) 60
9.2.2.1. Day 1/Day of Randomization 60
9.2.2.2. Week 2 to Week 4 61
9.2.2.3. Intranasal Inoculation with HRV-16 61
9.2.2.4. Post-HRV-16 Inoculation Assessment Phase 62
9.2.3. Follow-Up Phase (Part 2) 62
9.3. Early Termination 62
9.4. Efficacy 63
9.4.1. Evaluations 63
9.4.1.1. Pulmonary Function Testing at Clinic Visits 63
9.4.1.2. Fractional Exhaled Nitric Oxide (FENO) 64
9.4.1.3. Asthma Control Questionnaire (ACQ) 64
9.4.1.4. Electronic Data Collection Devices 64
9.4.1.5. Total Nasal and Ocular Symptom Score (TNOSS) 65
9.4.1.6. Subject Cold Question 65
9.4.1.7. Cold Symptom Assessment Score 65
9.4.1.8. Cold and Chest Symptom Scale 65
9.4.2. Endpoints 66
9.4.2.1. Part 1 66
9.4.2.2. Part 2 66
9.5. Pharmacokinetics and Immunogenicity 67
9.5.1. Evaluations 67
9.5.2. Analytical Procedures 68
9.5.3. Pharmacokinetic Parameters 68
9.5.3.1. Part 1 68
9.5.3.2. Part 2 68
9.5.4. Antibodies to CNTO 3157 68
9.6. Pharmacokinetic/Pharmacodynamic Evaluations 69
9.7. Biomarkers 69
9.7.1. Nasal Lavage and Brushings 69
9.8. Pharmacogenomic (DNA) Evaluations 69
9.9. Safety Evaluations 70
9.10. Sample Collection and Handling 72

10. SUBJECT COMPLETION/WITHDRAWAL 72
10.1. Completion 72
10.1.1. Part 1 72
10.1.2. Part 2 72
10.2. Discontinuation of Study Agent and/or HRV-16 Inoculation 73
10.2.1. Subject Stopping Criteria – Study Agent 73
10.2.2. Subject Stopping Criteria - HRV-16 Inoculation 73
10.3. Withdrawal From the Study 74
10.4. Suspension/Termination of Study Treatments 75
10.4.1. Part 1 75
10.4.2. Part 2 75
10.5. Study Interruption/Stopping Criteria 75

11. STATISTICAL METHODS 76
11.1. Statistical Design 76
11.2. Hypothesis 77
11.2.1. Part 1 77
11.2.2. Part 2 77
11.3. Primary Analysis 77
11.3.1. Part 1 77
11.3.2. Part 2 77
11.4. Sample Size Determination 78
11.4.1. Part 1 78
11.4.2. Part 2 79
11.5. General Statistical Methods 79
11.6. Pharmacokinetic and Immunogenicity of CNTO 3157 Analysis 80
11.7. Biomarkers Analysis 80
11.8. Pharmacogenomic Analyses 80
11.9. Medical Resource Utilization 80
11.10. Safety Analyses 80
11.11. Data Monitoring Committee 82

12. ADVERSE EVENT REPORTING 82
12.1. Definitions 82
12.1.1. Adverse Event Definitions and Classifications 82
12.1.2. Attribution Definitions 84
12.1.3. Severity Criteria 84
12.2. Special Reporting Situations 84
12.3. Procedures 85
12.3.1. All Adverse Events 85
12.3.2. Adverse Events During or Following Study Agent Infusion 86
12.3.3. Study Agent Infusion Site Reactions 87
12.3.4. Delayed Hypersensitivity (Serum Sickness-Like) Reactions 87
12.3.5. Serious Adverse Events 87
12.3.6. Pregnancy 88
12.4. Contacting Sponsor Regarding Safety 88

13. PRODUCT QUALITY COMPLAINT HANDLING 88
13.1. Procedures 89
13.2. Contacting Sponsor Regarding Product Quality 89

14. STUDY AGENT INFORMATION 89
14.1. Study Agents 89
14.1.1. Physical Description of Study Agent(s) 89
14.1.2. Labeling of Study Agent(s) 89
14.1.3. Preparation, Handling, and Storage of Study Agent(s) 89
14.1.4. Accountability for Study Agents(s) 90
14.2. HRV-16 Challenge Agent 90

14.2.1. Physical Description of HRV-16 90
14.2.2. Packaging of HRV-16 91
14.2.3. Labeling of HRV-16 91
14.2.4. Preparation, Handling, and Storage of HRV-16 91

15. STUDY-SPECIFIC MATERIALS 91

16. ETHICAL ASPECTS 91
16.1. Study-Specific Design Considerations 91
16.2. Regulatory Ethics Compliance 92
16.2.1. Investigator Responsibilities 92
16.2.2. Independent Ethics Committee or Institutional Review Board 92
16.2.3. Informed Consent 93
16.2.4. Privacy of Personal Data 94
16.2.5. Long-Term Storage of Samples for Future Research 95
16.2.6. Country Selection 95

17. ADMINISTRATIVE REQUIREMENTS 95
17.1. Protocol Amendments 95
17.2. Regulatory Documentation 96
17.2.1. Regulatory Approval/Notification 96
17.2.2. Required Prestudy Documentation 96
17.3. Subject Identification, Enrollment, and Screening Logs 97
17.4. Source Documentation 97
17.5. Case Report Form Completion 97
17.6. Data Quality Assurance/Quality Control 98
17.7. Record Retention 98
17.8. Monitoring 99
17.9. Study Completion/Termination 99
17.9.1. Study Completion 99
17.9.2. Study Termination 100
17.10. On-Site Audits 100
17.11. Use of Information and Publication 101

REFERENCES 103

LAST PAGE 113

LIST OF ATTACHMENTS

Attachment 1: Blood Volume Sampling (Part 1) 106
Attachment 2: Blood Volume Sampling (Part 2) 107
Attachment 3: Candidate Gene List for Part 1 of Pharmacogenomics 108
Attachment 4: Cold Symptom Assessment Score 109
Attachment 5: Cold and Chest Symptom Scale 111

LIST OF IN-TEXT TABLES AND FIGURES

TABLES

Table 1: Concomitant Medications in Part 1 (Healthy Adult Subjects) 52
Table 2: Concomitant Medications in Part 2 (Asthmatic Subjects) 53
Table 3: Power to detect a significant treatment difference in maximum percent decrease from baseline in prebronchodilator FEV_1 79

FIGURES

Figure 1: Schematic diagram of Part 1 of the CNTO3157ASH1002 study. 31
Figure 2: Schematic diagram of Part 2 of the CNTO3157ASH1002 study. 34
Figure 3: Mean (SD) concentration-time profiles following q1w IV infusion (30min) of 3 mg/kg of CNTO 3157 in asthmatic subjects (Part II, lowest quantifiable concentration in a sample = 0.008 µg/mL) 35

SYNOPSIS

A Phase 1, Randomized, Double-blind, Placebo-controlled Study Evaluating CNTO 3157 in Healthy Normal and Asthmatic Subjects Inoculated with Human Rhinovirus Type 16

CNTO 3157 is a fully human, sequence-adapted, IgG4κ monoclonal antibody (mAb) that binds the extracellular domain (ECD) of human Toll-like receptor 3 (TLR3).

Exacerbations of asthma are frequently due to respiratory viral infections, in particular those caused by human rhinoviruses (HRVs). While the underlying mechanisms driving viral-induced exacerbations of asthma are not fully understood, it is possible that activation of TLR3 by viral RNA could trigger or enhance immune-mediated pathologies associated with exacerbations of asthma.

OBJECTIVES AND HYPOTHESIS

Part 1

Primary

The primary objective of Part 1 of this study is to determine the safety and tolerability of a single IV administration of CNTO 3157 compared with placebo by examining the effects of pre-treatment on the respiratory manifestations of inoculation of healthy adult subjects with HRV-16.

Secondary

The secondary objectives are to assess the PK, PD, and immunogenicity of a single IV administration of CNTO 3157 in healthy adult subjects inoculated with HRV-16.

Exploratory

The exploratory objectives are to determine the impact of CNTO 3157 on host immune response biomarker profiles of healthy subjects inoculated with HRV-16 as assessed by proteomic profiling of serum and/or plasma, gene expression profiling in whole blood, nasal lavage samples, viral titers, and measurement of the fractional concentration of exhaled nitric oxide (FENO).

Part 2

Primary

The primary objective of Part 2 of this study is to determine the efficacy of pretreatment with CNTO 3157 compared with placebo in attenuating the respiratory manifestations of inoculation with HRV-16 in adult subjects with mild to moderate asthma.

Secondary

The secondary objectives are to assess the safety, tolerability, PK, PD, and immunogenicity of multiple IV administrations of CNTO 3157 compared with placebo in adult subjects with mild to moderate asthma inoculated with HRV-16.

Exploratory

The exploratory objectives are to determine the impact of CNTO 3157 on host immune response biomarker profiles of subjects as assessed by proteomic profiling of serum and/or plasma, gene expression profiling in whole blood, measurement of induced sputum biomarkers, nasal lavage samples, nasal brushings, viral titers, and measurement of FENO. In addition, the effect of four weekly doses of CNTO 3157 on asthma control prior to HRV-16 inoculation will be evaluated using standard assessments.

Hypothesis

Part 1: There is no hypothesis for Part 1 of the study.

Part 2: The hypothesis is that CNTO 3157 is superior to placebo in attenuating the respiratory manifestations of HRV-16 inoculation as measured by the maximum percent decrease relative to baseline in prebronchodilator FEV_1 assessed over 10 days following HRV-16 inoculation of asthmatic subjects.

OVERVIEW OF STUDY DESIGN

The purpose of this Phase 1, 2-part, randomized, multi-center, double-blind, parallel-design, placebo controlled study is to evaluate the safety and efficacy of pretreatment with CNTO 3157 in healthy adult and asthmatic adult subjects before and after intranasal inoculation with HRV-16.

In Part 1 and Part 2, the severity of an upper respiratory tract infection due to inoculation with HRV-16 will be assessed, and in Part 2, efficacy will be assessed using standard assessments to evaluate asthma treatments (eg, FEV_1, PEFR, ACQ). There is no primary efficacy analysis in Part 1. The study (Part 1 and Part 2) will be completed when the last subject in Part 2 completes the last visit (Week 11).

An independent Data Monitoring Committee will be commissioned for this study.

SUBJECT SELECTION

Part 1

Part 1 will enroll approximately 12 healthy, adult subjects ages 18 to 65 years, inclusive, who meet all inclusion criteria and none of the exclusion criteria.

Part 2

Part 2 will enroll approximately 60 adult asthmatic subjects ages 18 to 65 years, inclusive, who meet all inclusion criteria and none of the exclusion criteria. Subjects in Part 2 will have a physician diagnosis of asthma for at least 6 months prior to screening that is stable asthma based on physician assessment at Screening Visit 1. Subjects will:

- have an ACQ symptom score <1.5 at Screening Visit 2.
- have a prebronchodilator forced expiratory volume in the first second (FEV_1) ≥65% of predicted normal value at Screening Visit 2.
- have a history of worsening of asthma symptoms (eg, increased use of rescue medication, increased wheeze or shortness of breath, requirement for corticosteroid use) during naturally acquired upper respiratory tract infections ("a common cold").

Subjects in Part 2 are allowed to use low or medium dose ICS (≤500 µg/day fluticasone or equivalent) with or without LABA and with or without other certain controller therapies (eg, leukotriene receptor antagonists [LTRA]).

DOSAGE AND ADMINISTRATION

Subjects in Part 1 will receive a single IV infusion over a period of not less than 30 minutes of either placebo or CNTO 3157 10 mg/kg on Day 1 of Week 1 prior to (within 24 to 72 hours) inoculation with HRV-16.

Subjects in Part 2 will receive either 4 IV infusions of placebo at Week 1, Week 2, Week 3, and Week 4 (30 subjects), or 1 IV infusion of CNTO 3157 10 mg/kg at Week 1 followed by 3 infusions of 3 mg/kg of CNTO 3157 at Week 2, Week 3, and Week 4 (30 subjects). These infusions will also be administered

over a period of not less than 30 minutes. After the last infusion (within 24 to 72 hours) at Week 4, subjects will be inoculated with HRV-16.

EFFICACY EVALUATIONS/ENDPOINTS

Part 1

Part 1 is designed to assess the safety and tolerability of CNTO 3157 initially in healthy adult subjects inoculated with HRV-16 prior to testing the combination of CNTO 3157 and HRV-16 infection in asthmatic subjects. There are no planned efficacy assessments in Part 1. However, spirometry (including FEV_1, etc), FENO, and cold and chest symptom assessments will be collected for the purposes of monitoring safety.

The endpoints for Part 1 are:

- AUC of change from baseline in Cold Symptom Assessment Score, Cold and Chest Symptom Scale, and log-transformed FENO from the day of HRV-16 inoculation through 10 days following HRV-16 inoculation.
- Change from baseline in Cold Symptom Assessment Score, Cold and Chest Symptom Scale, and log-transformed FENO over time.
- Change from baseline over time in FEV_1, percent-predicted FEV_1, FVC, FEV_{25-75}, and FEV_1/FVC

Part 2

Efficacy in Part 2 will be assessed using standard assessments to evaluate asthma treatments (eg, FEV_1, ACQ). Although these standard assessments are primarily meant to assess efficacy, changes in these assessments will also be used to monitor safety throughout the study.

Efficacy evaluations will include spirometry (including FEV_1, etc.), collection of patient reported outcomes (ACQ), daily asthma symptom diaries collected on a handheld electronic device (including the number of nocturnal awakenings, rescue medication use, impact on activities, and PEF), and assessment of nasal and ocular symptoms using TNOSS. These evaluations are widely accepted as standard endpoints for demonstrating therapeutic efficacy in terms of reduction of signs and symptoms of asthma. TNOSS will be used to evaluate the potential effect of CNTO 3157 on nasal and ocular symptoms which often co-exist in subjects with asthma.

The primary endpoint is the maximum percent decrease relative to baseline in the prebronchodilator FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16. The baseline is defined as the average of all prebronchodilator FEV_1 measurements prior to study agent administration.

The major secondary endpoints in Part 2 of the study are:

- AUC of the percent change from baseline in clinic assessed prebronchodilator FEV_1 through 10 days following HRV-16 inoculation
- Change from baseline in ACQ reported approximately 10 days following inoculation with HRV-16 (D10PI)
- AUC of the change from baseline in Cold Symptom Assessment Scores from the day of HRV-16 inoculation through 10 days following HRV-16 inoculation

- AUC of the change from baseline in Cold and Chest Symptom Scale from the day of HRV-16 inoculation through 10 days following HRV-16 inoculation
- AUC of change from baseline in morning (AM) PEFR through 10 days following inoculation with HRV-16

To further evaluate the treatment effect of CNTO 3157 in the asthmatic population, analyses are planned for the following other secondary efficacy endpoints:

Pulmonary function tests:

- The maximum decrease from baseline in the prebronchodilator FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16
- AUC of the change from baseline in clinic assessed prebronchodilator FEV_1, percent predicted FEV_1, FVC, FEV_{25-75}, and FEV_1/FVC through 10 days following inoculation with HRV-16
- Change from baseline over time in prebronchodilator and postbronchodilator parameters: percent-predicted FEV_1, FEV_1, FVC, FEV_{25-75}, and FEV_1/FVC
- Change from baseline in morning/evening PEFR over time

Symptoms and other measures:

- AUC of change from baseline in average total asthma symptom diary score through 10 days following inoculation with HRV-16
- Change from baseline in TNOSS
- Change from baseline in Cold Symptom Assessment Score, Cold and Chest Symptom Scale, average total asthma symptom diary score, average number of nocturnal awaking, and average rescue medication use over time
- Number of symptom-free days through 10 days following inoculation with HRV-16
- Time to the maximum decrease relative to baseline in prebronchodilator FEV_1 assessed 10 days following HRV-16 inoculation
- Change from baseline in log-transformed FENO over time

Exploratory Endpoints

To account for the potential separate effects of study agent and viral inoculation upon efficacy assessments (eg, pre-bronchodilator FEV_1), exploratory endpoints defined as changes and/or percent changes relative to the assessment immediately prior to the HRV-16 inoculation will be explored.

PHARMACOKINETIC, PHARMACODYNAMIC, AND IMMUNOGENICITY EVALUATIONS

Serum concentration of CNTO 3157 will be analyzed for all subjects with all available samples. The serum concentration-time data of CNTO 3157 will be analyzed for all evaluable subjects for PK parameters. All calculations will be based on actual sampling times. PK parameters and concentrations will be summarized by treatment group among PK evaluable subjects.

Serum samples will be screened for antibodies binding to CNTO 3157 and the titer of confirmed positive samples will be reported.

PK/PD analysis may be conducted to evaluate the relationship between exposure to CNTO 3157 and appropriate efficacy/safety outcomes (eg, change in FEV_1, ACQ).

BIOMARKER EVALUATIONS

Whole blood, serum, nasal lavage and nasal brushing (Part 2 only) samples for biomarker analyses will be collected at specified times during the study. All nasal lavage samples will be evaluated for the presence/absence of HRV-16, HRV-16 titers, and other biomarkers which may include, but are not limited to, IL-6, CXCL8/IL-8, and CXCL10/IP-10. RNA from whole blood will be analyzed for transcriptome profiling. In addition, FENO will be measured at specified times during the study, including change from baseline in log-transformed FENO over time.

PHARMACOGENOMIC (DNA) EVALUATIONS

Pharmacogenomic blood samples will be collected from those subjects who give separate consent to allow for pharmacogenomic research (where local regulations permit).

SAFETY EVALUATIONS

Safety assessments include vital signs, general physical examination, adverse events (AEs), concomitant medication review, electrocardiograms (ECGs), pregnancy testing, laboratory testing, and urine testing. Any clinically significant abnormalities persisting at the end of the study/early discontinuation will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

STATISTICAL METHODS

In both Part 1 and part 2, randomization will be performed by IVRS/IWRS using a permuted block method. There is no stratification in the randomization.

There is no primary efficacy analysis in Part 1. The primary analysis will be performed on the primary endpoint in Part 2 on the modified intent-to-treat (mITT) population, which includes all randomized subjects who receive at least 1 dose of study agent, have at least 1 measurement prior to study agent infusion, are inoculated with HRV-16, and have at least one post-inoculation prebronchodilator FEV_1 measurement. Since all subjects to be included in the primary analysis have at least one post-inoculation measurement; therefore, no subject will have a missing primary endpoint assessment.

An analysis of covariance (ANCOVA) model with treatment group as a fixed factor and baseline prebronchodilator FEV_1 as a covariate will be used in the primary efficacy analysis. The baseline value is defined as the average of all prebronchodilator FEV_1 measurements before the first infusion at Week 1. If significant non-normality is observed, appropriate non-parametric tests will be used to evaluate the differences between treatments.

Part 2 and hence the study will be considered positive if the primary analysis achieves statistical significance at a significance level of 0.1 (2 sided), and CNTO3157 shows better treatment effect than placebo.

In addition to the primary analysis, sensitivity analyses will also be performed. Sensitivity analysis 1 is similar to the primary analysis, but only includes subjects with all the pre bronchodilator FEV_1 10 days immediately following inoculation with HRV-16. Sensitivity analysis 2 directly compares the treatment effect among those who are infected after inoculation with HRV-16, a similar analysis will be conducted on the subgroup of the infected population.

Sample Size Determination

The sample size calculation is based on the primary endpoint, the maximum percent decrease relative to baseline in the prebronchodilator FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16. To have 80% power to detect a clinically significant relative reduction of 50% (from 13% 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) are required. The assumption of a 13% reduction for placebo and the 10% SD are based upon a previous study in which a similar asthmatic population was inoculated with HRV-16. In that study, the mean maximum percent decrease relative to baseline in prebronchodilator percent predicted FEV1 was 12.83% (n=9, SD=9.71%). The significance level of 0.1 was chosen as this is an early phase, proof of concept study.

General Statistical Methods

Demographic and baseline disease characteristic data will be summarized by treatment group in each study part. Descriptive statistics (eg, mean, median, standard deviation, interquartile range, minimum, and maximum) will be used to summarize continuous variables. Counts and percentages will be used to summarize categorical variables. Graphic data displays (eg, box plots) may also be used to present data. Categorical data will be analyzed using chi-square test, CMH chi-square test, or logistic regression. Continuous responses will be analyzed using the same statistical method as in the primary efficacy analysis. Nonparametric methods will be adopted when the normality assumption is violated. Survival analysis techniques will be used for endpoints defined by time to an event. Log-rank test will be used to compare endpoints defined by time to an event.

For efficacy analysis, data will be analyzed according to the assigned treatment group. Unless otherwise stated, efficacy analyses in Part 1 and Part 2 will be based on a mITT population.

Safety, PK, and PD analyses in Part 1 and Part 2 will include all subjects treated with study agent and will be summarized based on the actual treatment received. Some safety, PK, and PD analyses may also be performed on the population inoculated with HRV-16.

Pharmacokinetic and Immunogenicity of CNTO 3157 Analysis

Individual subject serum concentration-time data of CNTO 3157 will be analyzed for all subjects with all available samples. All calculations will be based on actual sampling times.

In Part 1, PK parameters to be calculated will include, but are not limited to AUCt, AUCinf, Cmax, Vz, CL and T1/2 following the single dose administration.

In Part 2, PK parameters to be calculated will include, but are not limited to AUC(t1-t2), Cmax,n, Cmin,n, and T1/2 following multiple dose administration.

Biomarker Analysis

Changes in the concentration of individual biomarkers from baseline to the selected posttreatment timepoints will be summarized. Associations between baseline levels and changes from baseline in select biomarkers and clinical response will be explored.

Safety Analysis

Safety data, including but not limited to, AEs, SAEs, infections, serious infections, mortality, changes in laboratory assessments, changes in vital signs, and incidence of formation of antibodies to CNTO 3157 will be summarized. Treatment-emergent AEs will be summarized by treatment group and MedDRA system organ class and preferred terms.

TIME AND EVENTS SCHEDULES

1. Part 1: Healthy Subjects

Study Week (Study Days)	Week-5 to Week -1 (Study Days -35 to -1)		Week 1 (Study Days 1 to 7)							Week 2 (Study Days 8 to 14)		Week 4	Week 8
Study Phase	**Screening**		**Treatment Phase[l]**									**Follow-up**	
Days from Randomization	**Study Day -35**	**Study Day -14**	**Study Day 1**					**Study Day 2[i]**	**Study Days 3 to 7**	**Study Day 9**	**Study Day 12**	**Study Days 22 to 28[b]**	**Study Days 50 to 56[b]**
	Screening Visit 1[c]	**Screening Visit 2**	**Pre Dose[g]**	**Dose**	**5 min post infusion**	**2 hr post infusion**	**4 hr post infusion**	**HRV**	**HRV-16 assessments (Day 1 to Day 10 post inoculation [D1PI to D10PI])**			**Follow-up Visits**	
Study Procedures													
Administrative/Screening													
Informed consent	X												
Inclusion/Exclusion criteria review	X[k]	X	X										
Medical history and demographics		X											
Serum pregnancy test[h]		X											
Urine cotinine test	X												
Urine drug screening		X											
Study agent Administration													
Randomization			X[g]										
Administer study agent				X									
Study agent accountability				X									
Inoculation with HRV-16[e]								X[e]					
Viral challenge agent accountability								X					
Safety Assessments													
Physical examination[f]		X											
Nasal examination		X											
Brief physical examination			X					X	X	X	X	X	X
Vital signs		X	X[f]	X	X	X	X	X	X	X	X	X	X
12-lead ECG		X	X				X						

Study Week (Study Days)	Week-5 to Week -1 (Study Days -35 to -1)		Week 1 (Study Days 1 to 7)							Week 2 (Study Days 8 to 14)		Week 4	Week 8
Study Phase	**Screening**		**Treatment Phase[l]**									**Follow-up**	
Days from Randomization	**Study Day -35**	**Study Day -14**	**Study Day 1**					**Study Day 2 [i]**	**Study Days 3 to 7**	**Study Day 9**	**Study Day 12**	**Study Days 22 to 28[b]**	**Study Days 50 to 56[b]**
	Screening Visit 1[c]	**Screening Visit 2**	**Pre Dose[g]**	**Dose**	**5 min post infusion**	**2 hr post infusion**	**4 hr post infusion**	**HRV**	**HRV-16 assessments (Day 1 to Day 10 post inoculation [D1PI to D10PI])**			**Follow-up Visits**	
Efficacy Assessments													
Cold and Chest Symptom Scale			X					X	X	X	X	X	X
Cold Symptom Scores			X					X	X	X	X	X	X
FENO[m]			X						X	X	X	X	X
Spirometry		X	X						X[d]	X		X	X
Subject Cold Question									X	X			
Clinical Laboratory Assessments													
Antibodies to HRV-16	X							X		X		X	X
Antibodies to HSV-1	X												
Other viral serology (HBV, HCV, HIV)		X											
Hematology, Chemistry		X								X			X
Urinalysis		X											
Screening for other respiratory viruses								X[i]			X		
Pharmacokinetics/ Immunogenicity													
Serum CNTO 3157 concentration			X		X			X	X[a]	X	X	X	X
Antibodies to CNTO 3157			X									X	X
Pharmacodynamics													
Nasal lavage for HRV titers and biomarkers		X	X						X	X	X	X	
Pharmacogenomics													
Pharmacogenomic consent			X										
Pharmacogenomic sampling			X										X

Study Week (Study Days)	Week-5 to Week -1 (Study Days -35 to -1)		Week 1 (Study Days 1 to 7)						Week 2 (Study Days 8 to 14)		Week 4	Week 8
Study Phase	**Screening**		**Treatment Phase[l]**								**Follow-up**	
Days from Randomization	**Study Day -35**	**Study Day -14**	**Study Day 1**					**Study Day 2[i]**	**Study Days 3 to 7**	**Study Day 9**	**Study Day 12**	**Study Days 22 to 28[b]**	**Study Days 50 to 56[b]**
	Screening Visit 1[c]	**Screening Visit 2**	**Pre Dose[g]**	**Dose**	**5 min post infusion**	**2 hr post infusion**	**4 hr post infusion**	**HRV**	**HRV-16 assessments (Day 1 to Day 10 post inoculation [D1PI to D10PI])**			**Follow-up Visits**	
Biomarkers													
Serum biomarkers		X	X						X	X	X	X	
Whole blood biomarkers			X						X	X	X	X	
Ongoing Subject Review													
Concomitant therapy	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X

a: Only on Day 3, 5, and 7 post inoculation.
b: Study visits in the Follow-up period include a window of ±4 days.
c: Screening Visit 1 must occur within 5 weeks of inoculation with HRV-16. Only subjects without a neutralizing serum antibody titer to HRV-16 (to allow successful challenge) will be permitted to proceed to Screening Visit 2.
d: Spirometry to be performed on Day 5 (D3PI).
e: To be performed after all other visit assessments.
f: Includes height and body weight. Body weight should be taken as part of vital signs on day of dosing to calculate dose of study agent.
g: At the Day 1 visit, subjects must not have had an upper respiratory illness in the screening period or signs or symptoms of other acute illnesses, moderate or severe rhinorrhea, a Cold Symptom Assessment Score ≥2 or any other major illness during the screening period in order to be eligible for randomization.
h: To be done only for female subjects of child-bearing potential. Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the subject's participation in the study.
i: Sample must be collected prior to inoculation with HRV-16 (see Section 9.1.2).
k: Review includes only those inclusion/exclusion criteria which can be assessed at Screening Visit 1 (eg, age).
l: Inoculation with HRV-16 may occur 24 to 72 hours after the first dose of study agent on Study Day 1 visit.
m: FENO testing must precede any other pulmonary procedures.

2. Part 2: Asthmatic Subjects

Study Week (Study Days)	Week-5 to Week -1 (Study Days -35 to -29)		Week 1 (Study Days 1 to 7)					Week 2 (Study Days 8 to 14)	Week 3 (Study Days 15 to 21)	Week 4 (Study Days 22to 28)			Week 5 (Study Days 29 to 35)		Week 7	Week 11
Study Phase	**Screening**		**Treatment Phase**												**Follow-up**	
Days from Randomization	Study Day -35	Study Day -14	Day 1					Day 8[n]	Day 15[n]	Day22[n]	Day23[o]	Days 24 to 28	Day 30	Day 33	Study Days 43 to 49[b]	Study Days 71 to 77[b]
	Screening Visit 1[a]	Screening Visit 2	Pre Dose[m]	Dose	5 min post infusion	2 hr post infusion	4 hr post infusion				HRV	HRV-16 assessments (Day 1 to Day 10 post inoculation [D1PI to D10PI])			Follow-up Visits	
Study Procedures																
Administrative/ Screening																
Informed consent	X															
Inclusion/Exclusion criteria review	X	X	X													
Medical history and demographics		X[k]														
Serum pregnancy test[l]		X														
Urine cotinine test	X															
Urine drug screening		X														
Study agent Administration																
Review Randomization criteria			X													
Randomization			X[m]													
Administer study agent				X				X	X	X						
Study agent accountability				X				X	X	X						
Inoculation with HRV-16											X[c]					
Viral challenge agent accountability											X					

Study Week (Study Days)	**Week-5 to Week -1 (Study Days -35 to -29)**		**Week 1 (Study Days 1 to 7)**					**Week 2 (Study Days 8 to 14)**	**Week 3 (Study Days 15 to 21)**	**Week 4 (Study Days 22to 28)**			**Week 5 (Study Days 29 to 35)**		**Week 7**	**Week 11**
Study Phase	**Screening**		**Treatment Phase**												**Follow-up**	
Days from Randomization	**Study Day -35**	**Study Day -14**	**Day 1**					**Day 8[n]**	**Day 15[n]**	**Day22[n]**	**Day23[o]**	**Days 24 to 28**	**Day 30**	**Day 33**	**Study Days 43 to 49[b]**	**Study Days 71 to 77[b]**
	Screening Visit 1[a]	**Screening Visit 2**	**Pre Dose[m]**	**Dose**	**5 min post infusion**	**2 hr post infusion**	**4 hr post infusion**				**HRV**	**HRV-16 assessments (Day 1 to Day 10 post inoculation [D1PI to D10PI])**			**Follow-up Visits**	
Safety Assessments																
Physical examination[i]		X														
Nasal examination		X														
Brief physical examination			X					X	X	X	X	X	X	X	X	X
Vital signs		X	X[l]	X	X	X	X	X[l]	X[l]	X[l]	X	X	X	X	X	X
12-lead ECG		X	X				X			X[j]						
Efficacy Assessments																
ACQ		X	X					X	X	X				X	X	X
Distribute Diary device		X[q]														
Daily Diary review		X[p]	X					X	X	X	X	X	X	X	X	X
Spirometry (pre)		X	X					X	X	X	X	X	X	X	X	X
Spirometry (post)		X								X	X		X			
TNOSS			X							X						
FENO[r]			X					X	X	X	X	X	X	X	X	X
Cold and Chest Symptom Scale			X					X	X	X	X	X	X	X	X	X
Cold Symptom Scores			X					X	X	X	X	X	X	X	X	X
Subject Cold Question												X	X			
Clinical Laboratory Assessments																
Antibodies to HRV-16	X										X[d]				X	
Antibodies to HSV-1	X															
Other serology (HBV, HCV, HIV)		X														
Screening for other respiratory viruses											X[d]			X		
Hematology, Chemistry		X											X			X
Urinalysis		X														

Study Week (Study Days)	**Week-5 to Week -1 (Study Days -35 to -29)**		**Week 1 (Study Days 1 to 7)**					**Week 2 (Study Days 8 to 14)**	**Week 3 (Study Days 15 to 21)**	**Week 4 (Study Days 22to 28)**			**Week 5 (Study Days 29 to 35)**		**Week 7**	**Week 11**
Study Phase	**Screening**		**Treatment Phase**												**Follow-up**	
Days from Randomization	**Study Day -35**	**Study Day -14**	**Day 1**					**Day 8[n]**	**Day 15[n]**	**Day22[n]**	**Day23[o]**	**Days 24 to 28**	**Day 30**	**Day 33**	**Study Days 43 to 49[b]**	**Study Days 71 to 77[b]**
	Screening Visit 1[a]	**Screening Visit 2**	**Pre Dose[m]**	**Dose**	**5 min post infusion**	**2 hr post infusion**	**4 hr post infusion**				**HRV**	**HRV-16 assessments (Day 1 to Day 10 post inoculation [D1PI to D10PI])**			**Follow-up Visits**	
Pharmacokinetics/ Immunogenicity																
Serum CNTO 3157 concentration			X		X			X[e]	X[e]	X[e]	X[d]	X[f]	X	X	X	X
Antibodies to CNTO 3157			X							X[g]					X	X
Pharmacodynamics																
Nasal lavage for HRV titers and biomarkers		X	X					X	X	X	X[d]	X	X	X	X	
Nasal brushing			X									X[h]				
Pharmacogenomics																
Pharmacogenomic consent			X													
Pharmacogenomic sampling			X													X
Biomarkers																
Serum biomarkers		X	X					X	X	X	X	X	X	X	X	X
Whole blood biomarkers			X							X		X	X	X	X	X
Sputum induction		X								X			X			

Study Week (Study Days)	Week-5 to Week -1 (Study Days -35 to -29)		Week 1 (Study Days 1 to 7)					Week 2 (Study Days 8 to 14)	Week 3 (Study Days 15 to 21)	Week 4 (Study Days 22to 28)			Week 5 (Study Days 29 to 35)		Week 7	Week 11
Study Phase	**Screening**		**Treatment Phase**												**Follow-up**	
Days from Randomization	Study Day -35	Study Day -14	Day 1					Day 8[n]	Day 15[n]	Day22[n]	Day23[o]	Days 24 to 28	Day 30	Day 33	Study Days 43 to 49[b]	Study Days 71 to 77[b]
	Screening Visit 1[a]	Screening Visit 2	Pre Dose[m]	Dose	5 min post infusion	2 hr post infusion	4 hr post infusion				HRV	HRV-16 assessments (Day 1 to Day 10 post inoculation [D1PI to D10PI])			Follow-up Visits	
Ongoing Subject Review																
Concomitant therapy	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a: Screening Visit 1 must occur within 8 weeks of inoculation with HRV-16. Only subjects without a neutralizing serum antibody titer to HRV-16 (to allow successful challenge) will be permitted to proceed to Screening Visit 2.
b: Study visits in the Follow-up period include a window of ±4 days.
c: To be performed after all other visit assessments.
d: Sample must be collected prior to inoculation with HRV-16.
e: Two samples are to be collected: 1 sample collected prior to start of infusion, and 1 sample collected at 5 minutes post infusion.
f: Only on Day 24 (D1PI), Day 26 (D3PI), and Day 28 (D5PI).
g: One sample should be taken prior to start of infusion.
h: Nasal brushing will be performed 48 hours post inoculation with HRV-16.
i: Includes height and body weight. Body weight should be taken as part of vital signs on day of dosing to calculate dose of study agent.
j: To be performed 4 hours post infusion and collected in triplicate.
k: The subject must answer "yes" to the question: *"Do you have worsening of your symptoms of asthma (eg, shortness of breath wheezing, cough phlegm) when you get a cold?"*.
l. To be done only for female subjects of child-bearing potential. Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the subject's participation in the study.
m: At the Day 1 visit, subjects must not have had an upper respiratory illness in the screening period or signs or symptoms of other acute illnesses, moderate or severe rhinorrhea, a Cold Symptom Assessment Score ≥2 or any other major illness during the screening period in order to be eligible for randomization.
n: Study visits include a window of ±2 days.
o: Inoculation with HRV-16 may occur 24 to 72 hours after the fourth dose of study agent (Study Day 22 visit).
p: Site staff will review the use of the electronic diary with the subject at this visit.
q: The subject will receive appropriate training on the use of the handheld electronic device and electronic peak flow meter at Screening Visit 2.
r: FENO testing must precede any other pulmonary procedures.

ABBREVIATIONS

ACQ	Asthma Control Questionnaire
ADME	absorption/distribution/metabolism/excretion
AE	adverse event
AUC	area under the serum concentration versus time curve
AUCinf	area under the serum concentration
CCL2	Chemokine (C-C motif) ligand 2
CCL5	Chemokine (C-C motif) ligand 5
CH2	constant heavy domain 2
CL	apparent clearance
Cmax	maximum observed serum concentration
COPD	chronic obstructive pulmonary disease
CXCL8	CXC ligand 8
CXCL10	CXC ligand 10
D1PI	Day 1 Post-Inoculation
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
dsRNA	double-stranded ribonucleic acid
ECD	extracellular domain
ELISA	enzyme-linked immunosorbent assay
Fc	fragment crystallizable
FENO	fractional concentration of exhaled nitric oxide
FEV_1	forced expiratory volume in 1 second
FIH	first-in-human
FVC	forced vital capacity
GLP	Good Laboratory Practices
GMP	Good manufacturing Practices
HRV-16	human rhinovirus type 16
HSV-1	herpes simplex virus type 1
ICH	International Conference on Harmonization
ID	Inoculation Day
Ig	Immunoglobulin
IgG4	Immunoglobulin G4
IgM	immunoglobulin M
IL	Interleukin
IV	Intravenous
KO	knock-out
LABA	long acting β2 agonist
LTRA	Leukotriene receptor antagonists
mAb	monoclonal antibody
MDA-5	melanoma differentiation-associated gene-5
MedDRA	Medical Dictionary for Regulatory Activities
mg/kg	milligram per kilogram
mmHg	millimeters of mercury
NO	nitric oxide
NOAEL	no observable adverse effects level
NSAIDS	non-steroidal anti-inflammatory drugs
PCR	polymerase chain reaction
PD	Pharmacodynamic
PEF_{25-75}	peak expiratory flow (25-75%)
PEFR	peak expiratory flow rate
PK	pharmacokinetic(s)
poly(I:C)	polyinosine:cytosine
PRN	as occasion requires
RIG-I	retinoic acid-inducible gene 1
RSV	respiratory syncytial virus

SABA	short acting β2 agonist
SAE	serious adverse event
SC	subcutaneous
t1/2	half-life
TLR	Toll-like receptor
TLR3	Toll-like receptor 3
Tmax	time to reach maximal concentration
TNOSS	Total Nasal and Ocular Symptom Score
Vz	volume of distribution at the terminal phase
Vz/F	apparent volume of distribution after subcutaneous administration

1. INTRODUCTION

CNTO 3157 is a human monoclonal antibody (mAb) that binds the extracellular domain (ECD) of human Toll-like receptor 3 (huTLR3). CNTO 3157 reduces TLR3-mediated cytokine and chemokine production in a dose-dependent manner in several human and cynomolgus monkey cell types and reduces TLR3-mediated cytokine generation in an in vivo mouse model. A detailed description of the rationale for studying CNTO 3157 in asthma is presented in Section 1.1.

For the most comprehensive nonclinical and clinical information regarding the efficacy and safety of CNTO 3157, refer to the latest version of the Investigator's Brochure for CNTO 3157.

The term "sponsor" used throughout this document refers to the entities listed in the Contact Information page(s), which will be provided as a separate document.

Nonclinical Studies

Pharmacologic Profile

CNTO 3157 is a fully human, sequence-adapted, IgG4κ mAb. CNTO 3157 has a serine 228 to proline (S228P) substitution in the hinge region to stabilize disulfide bond formation between the two IgG4 heavy chains and phenylalanine 235 to alanine (F235A) and leucine 236 to alanine (L236A) substitutions in the constant heavy chain domain 2 (CH2) to minimize Fc-mediated effector function.

One molecule of CNTO 3157 binds 2 molecules of purified recombinant huTLR3 ECD with an affinity of 350 pM. CNTO 3157 binds to human cells transfected with plasmid encoding full-length human TLR3, but not vector plasmid-transfected cells or cells transfected with plasmid encoding human TLR5, a closely-related receptor. CNTO 3157 binds TLR3 on the surfaces of human epithelial cells and becomes internalized within endosomes in a dynamin-dependent manner. Intracellular levels of CNTO 3157 decrease over time suggesting possible endolysosomal degradation.

CNTO 3157 dose-dependently inhibits double-stranded ribonucleic acid (dsRNA)-induced activation of NF-κB response pathways in a recombinant huTLR3 or cynoTLR3 reporter gene assay and the interferon response pathway in the huTLR3 reporter gene assay. CNTO 3157 reduces dsRNA-induced cytokines and chemokines in both human and cynomolgus monkey peripheral blood mononuclear cell and primary human and cynomolgus monkey bronchial epithelial cell cultures. CNTO 3157 did not have detectable effects on the function of other Toll-like receptors (TLRs).

CNTO 3157 also binds recombinant mouse TLR3, reduces signaling by mouse TLR3 in a NF-κB reporter assay system, and interferes with in vivo TLR3 signaling in mice. CNTO 3157 has significantly lower affinity for both FcγRI (~1000x) and FcγRIIIa (~20x) relative to a control IgG1 antibody, suggesting that this mAb may be less likely to induce antibody-dependent

cytotoxicity. CNTO 3157 binding to FcRn is conserved, suggesting potential for FcRn-mediated recycling to prolong serum half-life.

Epitope mapping studies indicate that CNTO 3157 binds to the C-terminal dsRNA ligand binding region of huTLR3 ECD. Based on these data, it is hypothesized that CNTO 3157 prevents formation of the ligand-induced signaling dimer of TLR3 in cells.

Toxicology

The systemic exposure of CNTO 3157 in monkeys following a single IV administration was approximately dose-proportional.

The systemic exposure to CNTO 3157 in 1-month Good Laboratory Practices (GLP) toxicology studies in mice and monkeys increased in an approximately dose-proportional manner with moderate accumulation following repeated IV administration. The exposures were sufficient to allow the evaluation of potential toxicity of CNTO 3157. There was no apparent sex difference in the exposures. The systemic exposure to CNTO 3157 following IV administration in a GLP cardiovascular and respiratory safety evaluation in cynomolgus monkeys was sufficient for the evaluation. In a viral infection safety assessment study in female BALB/c mice, the satellite group demonstrated ample systemic exposure in the high dose groups.

There was no evidence of CNTO 3157-related effects on ECG (PR interval, QRS interval, QT interval, or QTc interval) or effects on respiratory parameters. Monkeys were chair restrained prior to dosing and at least 60 minutes post infusion. During chair restraint and following dose administration, there was a moderate (-59 mmHg, -45% max change versus predose values) but transient (25 to 75 min duration following the initiation of the infusion) decrease in mean blood pressure in 2 of 4 monkeys at the 50 mg/kg dose level. Individual blood pressure reduction ranged from -42 to -59 mmHg (~ 28 to 40% decrease) for systolic blood pressure, -30 to -56 mmHg (~ 28 to 49% decrease) for diastolic blood pressure, and -38 to –59 mmHg (~ 30 to 45% decrease) for mean blood pressure relative to predose infusion values. No adverse clinical signs were observed in the monkeys during this period. It is important to note, that the decrease in blood pressure only occurred during the interval when monkeys were chair restrained for dosing and post dose observation and therefore had initially elevated mean blood pressure (~ 35 mmHg) levels due to stress. No effect on blood pressure was observed after monkeys were returned to their cages. There were no cardiovascular effects (including blood pressure) observed after a single 15 minute IV infusion at 10 mg/kg (no observable adverse events level [NOAEL]). Mean serum concentrations of CNTO 3157 at 1.4 hours post dose were 243 µg/mL and 1358 µg/mL for the 10 mg/kg and 50 mg/kg dose groups, respectively. Blood pressure and ECGs were also evaluated in the 4-week GLP toxicology study in cynomolgus monkeys and no effects were observed at IV dosages of CNTO 3157 up to 50 mg/kg/q3d.

Antibodies to CNTO 3157 were detected in the cynomolgus monkeys and mice that were treated with CNTO 3157. The frequency of antibodies to CNTO 3157 was lower in repeat dose toxicology studies in mice and cynomolgus monkeys.

There was no evidence of toxicity in the 1 month repeat-dose GLP toxicology studies following administration of CNTO 3157 at dose levels in CD-1 mice up to 200 mg/kg/week or cynomolgus monkeys up to 50 mg/kg every 3 days (q3d), both pharmacologically relevant species for toxicity testing. Switching infusion rate in cynomolgus monkeys from bolus IV administration to a 15 minute infusion eliminated the adverse effects noted with bolus administration of 100 mg/kg.

CNTO 3157 was well tolerated in mice following 3 months of either IV or SC administration at dosages up to 200 mg/kg/week. In cynomolgus monkeys, SC administration of CNTO 3157 was well tolerated at dosages up to 75 mg/kg q3d. However, q3d IV administration of CNTO 3157 for 3 months at 50 mg/kg resulted in minimal to slight multifocal glomerulopathy that appears to be the result of immune complex formation/deposition.

An in vitro tissue cross reactivity study demonstrated broad, predominately cytoplasmic binding of biotinylated CNTO 3157 with similar binding for human, cynomolgus monkey and CD-1 mice. An apparent low affinity, cytoplasmic binding to platelets was observed for all 3 species. There was no evidence of platelet effects (thrombus or thrombocytopenia) in 1 month repeat dose toxicology studies with CNTO 3157. In addition, flow cytometric analysis failed to demonstrate any specific binding of CNTO 3157 to human platelets.

Host resistance models in BALB/c mice were used to assess immunotoxicologic risk. A slight, transient delay in immune response (IgM) to influenza infection was observed, however there was not a toxicologically relevant impact on viral clearance or overall IgG response. No overt immunotoxicologic risk was identified for CNTO 3157 following high dosage administration (200 mg/kg/week) in these mouse models. No TLR3-specific overt immunotoxicologic risk was identified through testing in TLR3-knock out (KO) mice.

Hypersensitivity reactions were enhanced in influenza-infected, CNTO 3157 treated mice relative to non-infected, CNTO 3157-treated satellite TK mice. Influenza infection in BALB/c mice resulted in enhanced hypersensitivity reactions (increased mortality and clinical signs) following the third weekly dose administration of CNTO 3157 at 50 mg/kg/week; minimal hypersensitivity reactions, without lethality, were observed at 100 mg/kg/week and no reactions were observed at 200 mg/kg/week. Hypersensitivity reactions appear related to an apparent anti-CNTO 3157 antibody response; no effects were observed in influenza-infected TLR3-KO mice.

The inverse dose relationship and occurrence following repeat dose administration supports a role of antibodies to CNTO 3157 in the hypersensitivity reactions. CNTO 3157 is a human antibody that would be considered “foreign” in mice and therefore, an anti-CNTO 3157 antibody response would be expected. Influenza infection has been previously shown to enhance immunogenicity of a foreign antigen in rodents.[16,37] CNTO 3157 was evaluated for cytokine release potential in an in vitro human whole blood assay designed to detect an anti-CD28 superagonist mAb (CD28-SA)-type cytokine release activity. There was no evidence of CD28-SA type activity for CNTO 3157.

Pharmacokinetic and Immunogenicity Profile

In accordance with ICH guidance, traditional ADME studies were not performed as CNTO 3157 is an IgG-based therapeutic mAb (refer to ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals). However, the PK/TK studies presented here have adequately assessed the pharmacokinetics of CNTO 3157 and showed CNTO 3157 exposure in support of the toxicology programs.

CNTO 3157 exhibited dose proportional pharmacokinetics (PK) in single-dose PK and repeat dose toxicology studies in cynomolgus monkeys and in repeat dose toxicology studies in mice.

CNTO 3157 was well absorbed following SC dose administration with the bioavailability estimated to be from 69.27 to 79.65%. The T_{max} following SC administration was reached in a range of 0.5 to 2 days in the monkeys or 1 to 3 days in mice.

Elimination half-life (t1/2) following single or repeat dose IV or SC administration was approximately 6 to 16.5 days in mice and 7 to 14 days in cynomolgus monkeys. Some of the PK parameters, such as V_z and CL, were not estimated due to the impact of antibodies to CNTO 3157 that developed in the animal species.

The exposures of CNTO 3157 following IV or SC dose administrations in the toxicology studies were continuous and sufficient to support the safety evaluation programs. The incidence of antibodies to CNTO 3157 was high in cynomolgus monkeys following single dose administration. Eleven of 12 (91.7%) monkeys treated with CNTO 3157 developed antibodies to CNTO 3157. However, the incidence was moderate following repeat dose administrations in monkeys and mice (28.1% (SC) and 32.3% (IV) in monkeys, and 17.8% (SC) and 9.7% (IV) in mice).

Clinical Studies

Clinical study CNTO3157ASH1001 was a first-in-human (FIH), Phase 1, randomized, double-blind, placebo controlled study of CNTO 3157 that evaluated the safety, tolerability, PK, immunogenicity and pharmacodynamics (PD) of single ascending doses of CNTO 3157 in healthy subjects and of multiple doses in stable asthmatic subjects. In Part 1, 62 healthy subjects received a single 1-hour IV administration (30 minutes for the last 4 subjects in the 10 mg/kg dose cohort) of CNTO 3157 at 0.003, 0.01, 0.03, 0.1, 0.3, 1, 1.5, 3, or 10 mg/kg, or placebo. In Part 2, 17 stable asthmatic subjects were administered up to 4 30 minute IV infusions weekly of CNTO 3157 at 3 mg/kg, 10 mg/kg, or placebo. All dose cohorts were randomized in a 3:1 ratio of CNTO 3157 to placebo.

Results from the Phase 1, randomized, double-blind, placebo-controlled study, first-in-human (FIH) clinical study (CNTO3157ASH1001) evaluating single ascending doses of CNTO 3157 in healthy subjects and of multiple doses in stable asthmatic subjects indicate that CNTO 3157 was well tolerated without concerns for safety following single IV infusions up to 10 mg/kg in healthy subjects and up to 10 mg/kg x 4 weekly infusions in asthmatic subjects with no dose related pattern in adverse events or adverse drug reactions. There were no clinically significant

infusion reactions. One subject tested positive for antibodies to CNTO 3157 in Part 1 of the study. All samples tested up to Day 22 in Part 2 of the study were negative.

1.1. Overall Rationale for the Study

TLR3 is one of a family of membrane-bound pattern-recognition receptors that bridge the innate and adaptive arms of the immune system.[22,31] TLR3 binds dsRNA derived from viral, parasitic, bacterial and endogenous mammalian sources resulting in the expression of pro inflammatory cytokines/chemokines, interferons, and interferon response genes, as well the activation and maturation of immune cells.[2] In addition to constituting the genome of certain virus families, dsRNA is generated during the life cycle of many other viruses.[47] As such, it was initially hypothesized that TLR3 played a central role in host defense against viral infections. Although several studies have shown that TLR3 contributes to host immune responses upon infection with several viral respiratory pathogens such as human rhinoviruses (HRV),[15,38,43,51] influenza virus,[4,23] and human respiratory syncytial virus (RSV),[13,35,36] in vivo studies have shown that mice deficient in TLR3 are still capable of responding to viral infections.[8] Furthermore, the absence of TLR3 did not appear to increase viral replication/pathogenesis or impair the host's generation of adaptive antiviral responses, possibly due to the activity of other RNA-binding molecules such as TLR7, TLR8, MDA-5, RIG-I that can also initiate interferon-based anti-viral responses.[25] However, in a mouse model of coxsackie virus challenge, TLR3 deficiency has been linked to decreased survival.[33] A protective role for TLR3 against human coxsackie virus infections is suggested by a recent analysis of 57 viral myocarditis patients in which variant TLR3 alleles with reduced activity were found at a higher prevalence compared to control patients, although the prevalence of variant alleles in both groups in this study are much lower than have been reported in other studies.[11] A dominant-negative TLR3 allele, identified in 1 patient with myocarditis in the report of Gorbea et al, has also been associated with increased susceptibility to herpes simplex encephalitis upon primary infection with herpes simplex virus type 1 (HSV-1) in childhood; however, subjects with this allele do not have impaired responses to subsequent HSV-1 reactivations (eg, cold sores),[14,50] demonstrating that a lack of TLR3 activity does not inhibit development of protective immune responses to viruses. While these studies have looked at very small numbers of patients and do not demonstrate direct causality, these findings suggest that TLR3 may contribute to host defense during certain viral infections.

However, several studies have shown that excessive or prolonged TLR3 signaling contributes to morbidity and mortality in certain viral infection models including West Nile virus,[44] phlebovirus,[12] vaccinia virus,[17] and influenza A virus.[23] For example, during infection with influenza A virus, TLR3-deficient mice have increased survival and less virus-induced inflammatory pathology compared with wild-type mice.[23] Consistent with these animal studies, a role for TLR3 in driving detrimental inflammation during infection in humans has been proposed based upon increased TLR3 levels in lungs from fatal cases of influenza H1N1 2009 infections.[24] It is hypothesized that the continued activation of TLR3 by ligands released as a result of viral replication and/or cell killing or as by-products of the inflammatory process can trigger or enhance immune-mediated pathologies. This hypothesis is supported by the finding that intranasal administration of a TLR3 dsRNA agonist (polyinosinic:polycytidylic acid [poly(I:C)])

in mice induces increases in lung vascular permeability, leukocyte infiltration into the lung, and airway hyper-reactivity, a hallmark feature of asthma.[3,19,41]

Asthma is characterized by the presence of reversible bronchoconstriction, increased sensitivity to specific and non-specific bronchospasmic agents, and excessive mucus production accompanied by an underlying pathology of inflammation which may include airway tissue remodeling.[29] There were 1.75 million emergency department visits (1.11 million for adults and 0.64 million for children aged 0–17), 456,000 asthma hospitalizations (299,000 for adults and 157,000 for children aged 0–17), and 3,447 deaths due to asthma in 2007 (3,262 among adults and 185 among children aged 0–17).[1] In COPD, exacerbations related to viral infection constitute a similar unmet need to that seen in asthma. A therapy that could prevent or attenuate the severity of the host immune response in asthma and COPD exacerbations would thus address a significant unmet need. Although asthma and COPD can be exacerbated by infection with several types of respiratory tract viruses [6,10] depending upon the time of year, the majority of all asthma exacerbations (85% of all asthma exacerbations in children and 50% of all asthma exacerbations in adults) are caused by HRVs ("the common cold virus").[9,10,18,30,34,42]

While the underlying mechanisms driving viral-induced exacerbations of asthma and COPD are not fully understood, it is possible that the persistent activation of TLR3 by viral RNA could trigger or enhance immune-mediated pathologies associated with exacerbations of asthma and COPD. Detection of respiratory viral RNA after acute asthma exacerbations is common, and most often, persistence of non-infectious (defective) human rhinovirus viral RNA is found.[48] Since this viral RNA persistence is associated with elevated IL-10 mRNA and CXCL10/IP-10 gene expression, it has been suggested that increased CXCL10/IP-10 expression may be mediated by TLR3 activation.[40] Infection in tissue culture with human rhinovirus has been shown to increase cell-surface expression of TLR3.[15] Increased TLR3 mRNA levels have been reported in sputum from asthmatic subjects infected with HRV as compared with healthy subjects, and also in subjects with stable asthma or acute non-viral asthma.[49] Several preclinical studies have demonstrated that HRV infection of nasal and bronchial epithelial cells in culture specifically causes increased secretion of CXCL10/IP-10.[21,40,46] In addition, HRV-16 induced asthma exacerbations have been shown to correlate with CXCL10/IP-10 increases in serum[45] and nasal lavages,[40] and these increases in CXCL10/IP-10 correlated with symptom severity, viral titer, and lymphocytes in airway secretions. Similar findings have been observed in patients with COPD exacerbations.[32]

Collectively, these findings suggest that TLR3 plays a role in viral-induced exacerbations of asthma and COPD, and suggest that inhibition of TLR3 by CNTO 3157 may reduce the severity and/or frequency of viral-induced exacerbations by dampening the chronic inflammation that is observed in asthma and COPD, thus improving airway function. In support of this, preclinical studies have shown that an anti-murine TLR3 mAb can block poly(I:C)-induced inflammation in mice and in vitro,[5] and an anti-human TLR3 mAb can down-regulate the poly(I:C) induced production of inflammatory cytokines/chemokines (IL-6, CXCL8/IL-8, CCL2/MCP-1, CCL5/RANTES, and CXCL10/IP-10) in human lung epithelial cells.[7]

2. OBJECTIVES AND HYPOTHESIS

2.1. Objectives

2.1.1. Part 1

2.1.1.1. Primary

The primary objective of Part 1 of this study is to determine the safety and tolerability of a single IV administration of CNTO 3157 compared with placebo by examining the effects of pre-treatment on the respiratory manifestations of inoculation of healthy adult subjects with HRV-16.

2.1.1.2. Secondary

The secondary objectives are to assess the PK, PD, and immunogenicity of a single IV administration of CNTO 3157 in healthy adult subjects inoculated with HRV-16.

2.1.1.3. Exploratory

The exploratory objectives are to determine the impact of CNTO 3157 on host immune response biomarker profiles of healthy subjects inoculated with HRV-16 as assessed by proteomic profiling of serum and/or plasma, gene expression profiling in whole blood, nasal lavage samples, viral titers, and measurement of the fractional concentration of exhaled nitric oxide (FENO).

2.1.2. Part 2

2.1.2.1. Primary

The primary objective of Part 2 of this study is to determine the efficacy of pretreatment with CNTO 3157 compared with placebo in attenuating the respiratory manifestations of inoculation with HRV-16 in adult subjects with mild to moderate asthma.

2.1.2.2. Secondary

The secondary objectives are to assess the safety, tolerability, PK, PD, and immunogenicity of multiple IV administrations of CNTO 3157 compared with placebo in adult subjects with mild to moderate asthma inoculated with HRV-16.

2.1.2.3. Exploratory

The exploratory objectives are to determine the impact of CNTO 3157 on host immune response biomarker profiles of subjects as assessed by proteomic profiling of serum and/or plasma, gene expression profiling in whole blood, measurement of induced sputum biomarkers, nasal lavage samples, nasal brushings, viral titers, and measurement of FENO. In addition, the effect of four weekly doses of CNTO 3157 on asthma control prior to HRV-16 inoculation will be evaluated using standard assessments.

2.2. Hypothesis

2.2.1. Part 1

There is no hypothesis for Part 1 of the study.

2.2.2. Part 2

The hypothesis is that CNTO 3157 is superior to placebo in attenuating the respiratory manifestations of HRV-16 inoculation as measured by the maximum percent decrease relative to baseline in prebronchodilator FEV_1 assessed over 10 days following HRV-16 inoculation of asthmatic subjects.

3. STUDY DESIGN AND RATIONALE

3.1. Overview of Study Design

Safety results from the Phase 1 first-in-human study (CNTO3157ASH1001) indicate that CNTO 3157 was well tolerated without concerns for safety following single IV infusions up to 10 mg/kg in healthy subjects and up to 10 mg/kg x 4 weekly infusions in asthmatic subjects with no dose related pattern in adverse events or adverse drug reactions. Results from the NOCOMPOUNDASH1001 study evaluating the safety and clinical characteristics of a GMP-prepared challenge pool of HRV-16 in healthy subjects for use in viral challenge studies indicates that the challenge pool when administered at 100 or 1000 $TCID_{50}$ is safe and produces the expected clinical characteristics of a typical upper respiratory tract infection ("common cold"). The same challenge pool of HRV-16 used in the NOCOMPOUNDASH1001 study will be used in this study (CNTO3157ASH1002) administered at 1000 $TCID_{50}$. Additional details regarding both of these studies are available in the current versions of the respective Investigators' Brochures.

The purpose of this Phase 1, 2-part, randomized, multi-center, double-blind, parallel-design, placebo-controlled study is to evaluate the safety of CNTO 3157 in healthy adult subjects and safety and efficacy of CNTO 3157 in asthmatic adult subjects after intranasal inoculation with HRV-16.

In Part 1, following administration of study agent (CNTO 3157 or placebo), the severity of an upper respiratory tract infection due to inoculation with HRV-16 will be assessed for safety reasons. In Part 2, following administration of study agent (CNTO 3157 or placebo) and inoculation with HRV-16, efficacy and safety, will be assessed using standard assessments to evaluate asthma treatments (eg, FEV_1, PEFR, ACQ; see Section 9.4).

There is no primary efficacy analysis in Part 1. An Independent Data Monitoring Committee will be commissioned for this study (refer to Data Monitoring Committee, Section 11.12, for details). The study (Part 1 and Part 2) will be completed when the last subject completes the last visit (Week 11) in Part 2.

3.1.1. Part 1

3.1.1.1. Study Design Rationale – Part 1 (Healthy Adult Subjects)

Preclinical studies support the hypothesis that inhibition of TLR3 by CNTO 3157 may dampen the chronic inflammation that is observed in asthma, particularly the inflammation associated with viral-induced asthma exacerbations. However, as CNTO 3157 is an immunomodulatory agent, Part 1 is designed to assess the safety and tolerability of CNTO 3157 in healthy adult subjects inoculated with HRV-16 prior to testing the combination of CNTO 3157 and HRV-16 infection in asthmatic subjects. As detailed in Section 11.11, an external DMC will be commissioned to review unblinded safety data in both Part 1 and Part 2 of the study. If no safety concerns are identified in Part 1 of the study in healthy adults, Part 2 of the study in asthmatic subjects will be initiated.

A schematic diagram of Part 1 is presented in Figure 1.

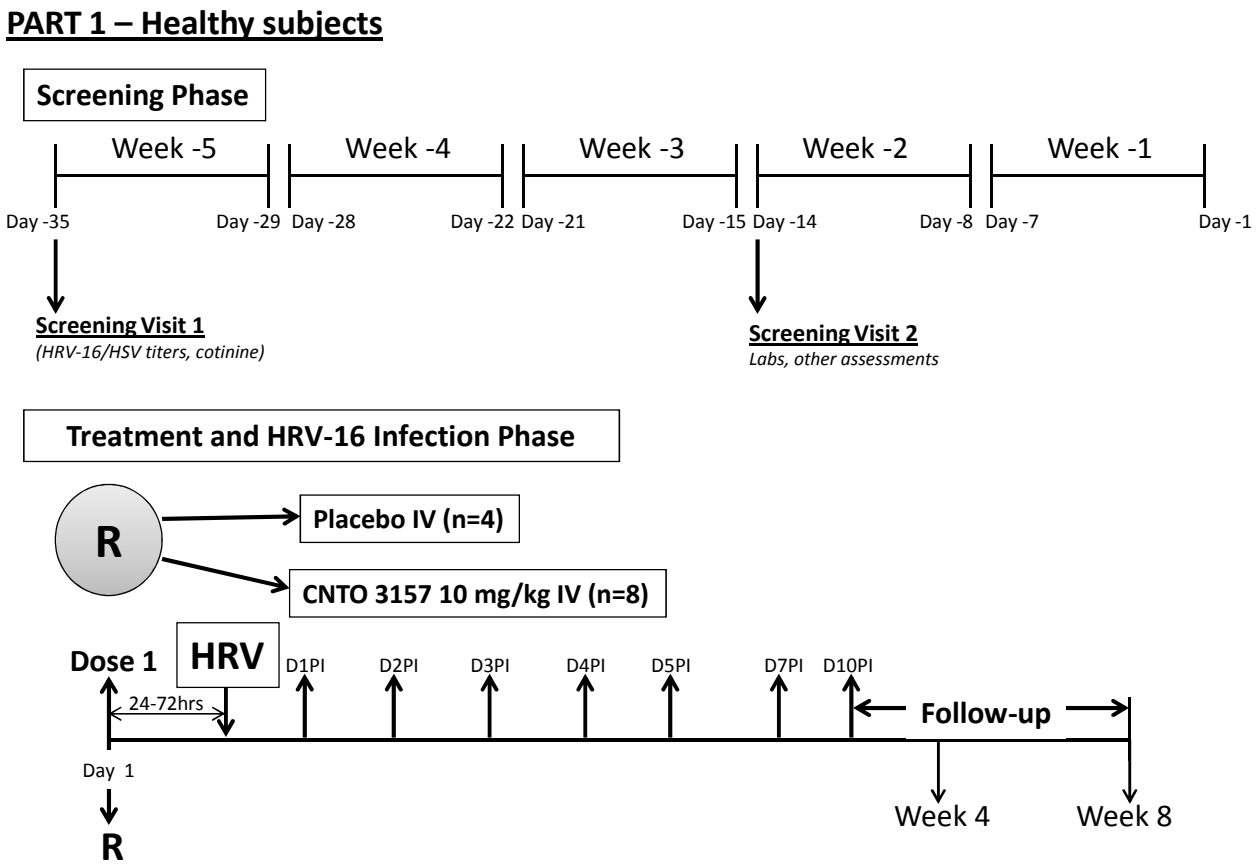


Figure 1: Schematic diagram of Part 1 of the CNTO3157ASH1002 study.

The study procedures in Part 1 are described in Section 9.1. Subjects will participate in Part 1 for a total of approximately 13 weeks. Part 1 includes a Screening Phase of up to 5 weeks prior to randomization during which subjects will undergo 2 screening visits. After randomization, subjects will receive a single intravenous (IV) infusion of study agent (placebo or CNTO 3157 10 mg/kg). Twenty-four to 72 hours after dosing, subjects will receive a single intranasal inoculation with HRV-16 and will be followed intensely for 10 days to assess clinical symptoms and HRV-16 associated inflammation, PK, and biomarkers. Subjects will also return for follow-up assessments at Week 4 and Week 8

There is no formal interim database lock (DBL) planned for Part 1 of the study. If there are no safety concerns upon review of blinded data, Part 2 will proceed as planned. In addition, an independent DMC will review safety data after all subjects in Part 1 have received their single infusion of study agent (placebo or CNTO 3157), have been inoculated with HRV‑16, and have completed the D10PI visit assessments. No subject will be inoculated in Part 2 until this DMC review is complete and a recommendation to proceed is received.

3.1.1.2. Dose Rationale – Part 1

In Part 1, 12 healthy adult subjects will be randomized in a 2:1 ratio to receive a single, intravenous (IV) infusion of CNTO 3157 10 mg/kg (8 subjects) or placebo (4 subjects). The 10 mg/kg dose of CNTO 3157 was selected based on the safety, tolerability, and PK/PD profile from the Phase 1 study (CNTO3157ASH1001) in healthy adult subjects and adult subjects with mild asthma.

In CNTO3157ASH1001, ascending single doses of CNTO 3157 or placebo were administered to 8 sequential staggered parallel cohorts (0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, or 10 mg/kg) of healthy subjects as a 1 hour IV infusion for the majority of subjects, with the exception of the last 4 subjects in the 10 mg/kg cohort who received a 30 minute IV infusion. There were no dose-related safety or tolerability concerns at any dose or either infusion rate.

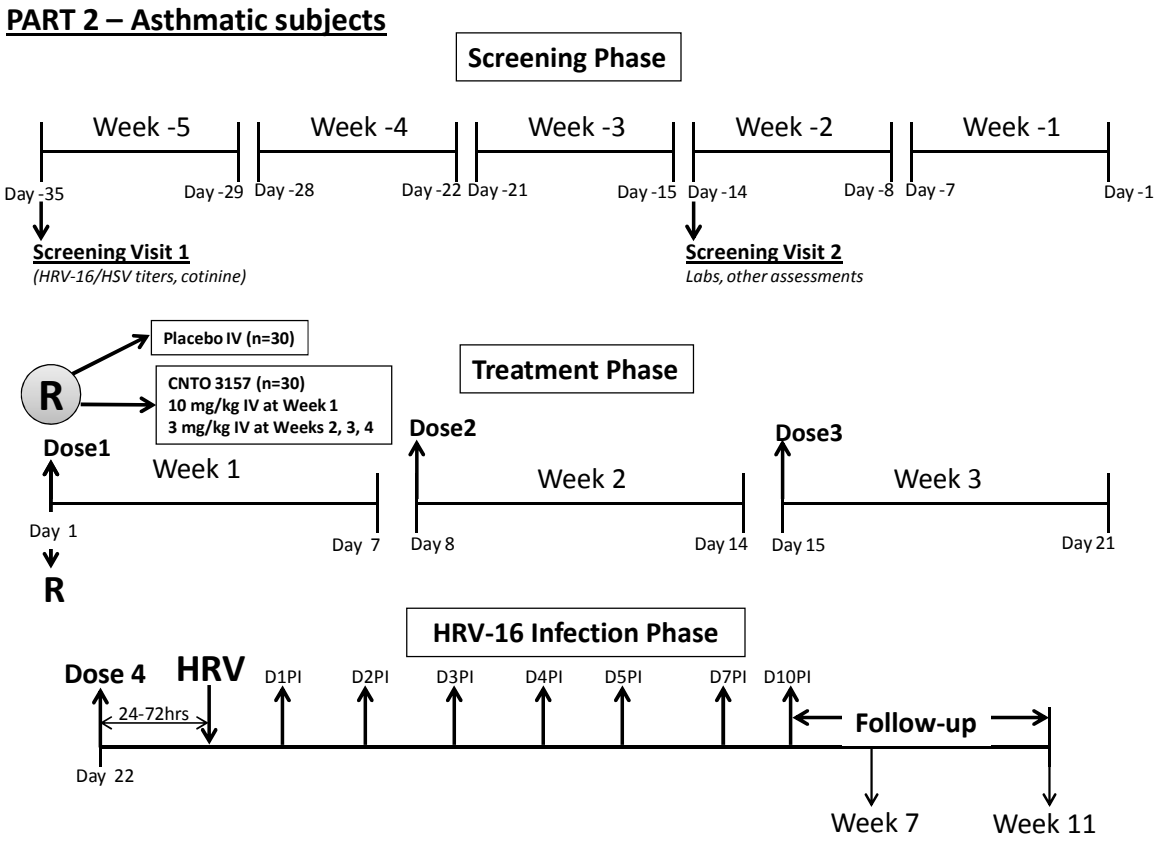
A preliminary PK analysis for Part 1 of the CNTO3157ASH1001 study has been conducted. The pharmacokinetics of CNTO 3157 exhibited target-mediated disposition in healthy subjects. The clearance decreased as the dose increased, and reached a plateau at higher dose levels (eg, 3 mg/kg and 10 mg/kg). This PK profile is consistent with saturation of the target (TLR3) by CNTO 3157 at higher doses (eg, 10 mg/kg). The proposed dose of 10 mg/kg in Part 1 of this study is expected to produce a similar PK profile as that observed in the CNTO3157ASH1001 study at 10 mg/kg. Based upon this assumption, a single IV infusion of 10 mg/kg should be sufficient to achieve saturation of the target at the time of inoculation with HRV-16. In addition, preliminary results of biomarker analysis from the CNTO3157ASH1001 study support biological inhibition of the target with this dosing.

3.1.2. Part 2

3.1.2.1. Study Design Rationale – Part 2 (Adult Asthmatic Subjects)

Part 2 is designed to determine if treatment with CNTO 3157 can attenuate the respiratory manifestations (nasal and chest consequences) of a viral infection induced by intranasal inoculation with HRV-16 in asthmatic subjects. A population of subjects with mild to moderate, controlled asthma was chosen for this study as subjects with uncontrolled or more severe asthma may be at greater risk for more serious respiratory complications of HRV-16 infection.

The study procedures in Part 2 are described in Section 9.2. Subjects will participate in Part 2 for a total of approximately 17 weeks. Part 2 includes a Screening Phase of up to 6 weeks prior to randomization during which subjects will undergo 2 screening visits. After randomization, subjects will receive 4 intravenous infusions of placebo at Week 1, Week 2, Week 3, and Week 4 or 1 infusion of 10 mg/kg of CNTO 3157 at Week 1 followed by 3 infusions of 3 mg/kg of CNTO 3157 at Week 2, Week 3, and Week 4. Twenty-four to 72 hours after the Week 4 dose, subjects will receive a single intranasal inoculation with HRV-16 and will be followed intensely for 10 days to assess clinical symptoms and HRV-16 associated inflammation, PK, and biomarkers. Subjects will return for follow-up assessments at Week 7 and Week 11. The database lock will occur after all subjects in Part 2 have completed their Week 11 visit. A schematic diagram of Part 2 is presented in Figure 2.



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

Figure 2: Schematic diagram of Part 2 of the CNTO3157ASH1002 study.

3.1.2.2. Dose Rationale – Part 2

In Part 2, 60 subjects with stable asthma will be randomized in a 1:1 ratio to receive either:

- 4 IV infusions of placebo at Week 1, Week 2, Week 3, and Week 4 (30 subjects), or
- 1 IV infusion of CNTO 3157 10 mg/kg at Week 1 followed by 3 infusions of 3 mg/kg of CNTO 3157 at Week 2, Week 3, and Week 4 (30 subjects).

In the second part of the ongoing CNTO3157ASH1001 study, CNTO 3157 or placebo was administered to mild asthmatic subjects in 2 cohorts. Eight asthmatic subjects in each cohort received up to 4 weekly IV infusions of 3 mg/kg or 10 mg/kg of CNTO 3157 or placebo. Preliminary PK data is available for the 3 mg/kg cohort up to Week 5. The trough concentrations of CNTO 3157 prior to each dose administration increased with repeat dosing suggesting that steady state had not been attained after the fourth dose.

In addition, a slower elimination rate was observed after the fourth dose when compared with that following the first dose (see Figure 3), suggesting an enhancement of target saturation by repeat dosing. This evidence suggests that multiple doses increase the concentration of CNTO 3157 and enhance target saturation in asthmatic patients.

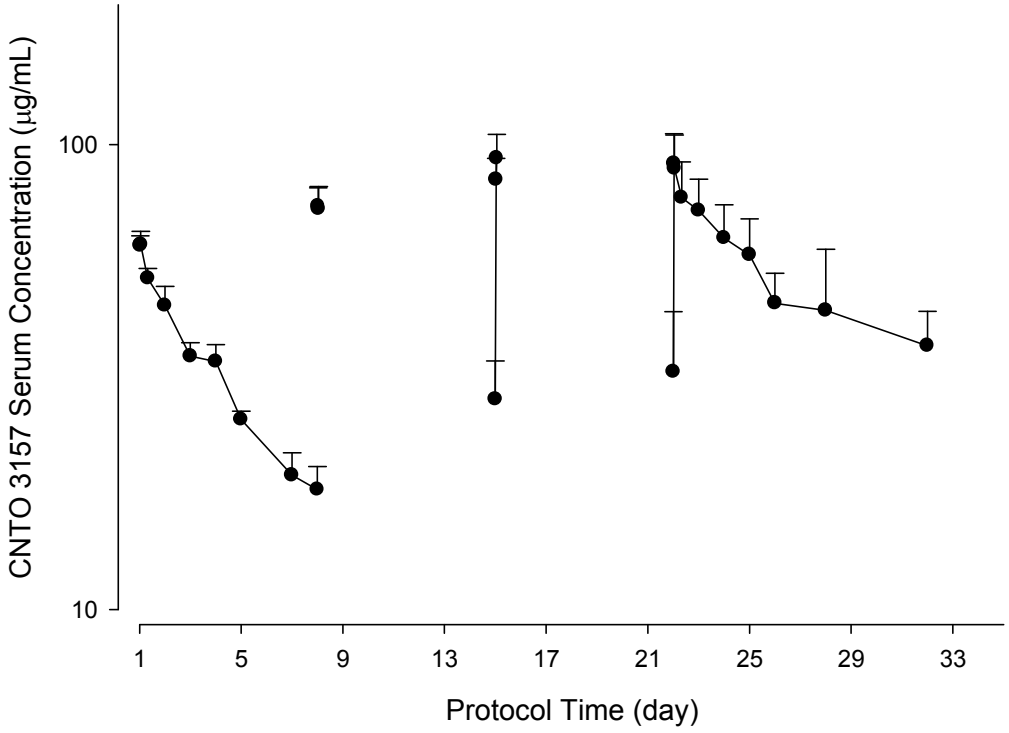


Figure 3: Mean (SD) concentration-time profiles following q1w IV infusion (30min) of 3 mg/kg of CNTO 3157 in asthmatic subjects (Part II, lowest quantifiable concentration in a sample = 0.008 µg/mL)

In order to maximize the probability of saturating of the target with CNTO 3157 prior to inoculation with HRV-16, asthmatic subjects in Part 2 who were randomized to the CNTO 3157 group will receive an initial infusion of 10 mg/kg followed by 3 subsequent infusions of 3 mg/kg at weekly intervals.

This proposed dose regimen in Part 2 of this study is expected to generate a slightly higher exposure than that observed in Part 2 of the CNTO3157ASH1001 study in the 3 mg/kg cohort; however, for safety considerations, it will not exceed the exposure in the 10 mg/kg cohort.

Repeated administration of CNTO 3157 at 3 mg/kg and 10 mg/kg in asthmatic subjects was well-tolerated in the first in human study. No serious infections, anaphylaxis, or other infusion related events were reported. No adverse events (AEs) related to study procedures were reported. The reported AEs do not exhibit a dose-dependent relationship. No AEs were reported at a frequency to cause any clinical concern. No clinically significant changes from the baseline were observed for vital signs, ECGs, telemetry (continuous cardiac monitoring), and/or laboratory parameters. There were no clinically significant infusion reactions.

Based on the safety information from the FIH CNTO3157ASH1001 study in healthy subjects and mild asthmatic subjects, the proposed dose regimen is expected to be in an appropriate range to ensure target engagement while still being safe.

3.2. Randomization and Blinding – Part 1 and Part 2

Subjects meeting study eligibility criteria will be randomly assigned using permuted block randomization into 1 of 2 treatment groups at a 2:1 ratio (CNTO 3157:placebo) in Part 1 or into 1 of 2 treatment groups in Part 2 at a 1:1 ratio (CNTO 3157:placebo).

A placebo control will be used to establish the frequency and magnitude of changes in clinical and other endpoints that may occur in the absence of active treatment in addition to background therapy. Randomization will be used to minimize bias in the assignment of subjects to treatment groups, to increase the likelihood that known and unknown subject attributes (eg, demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups. Blinded treatment will be used to reduce potential bias during data collection and evaluation of endpoints.

Further details on Treatment Allocation and Blinding are provided in Section 5.

DNA and Biomarker Collection

It is recognized that genetic variation can be an important contributory factor to interindividual differences in drug distribution and response and can also serve as a marker for disease susceptibility and prognosis. Pharmacogenomic research may help to explain interindividual variability in clinical outcomes and may help to identify population subgroups that respond differently to a drug. The goal of the pharmacogenomic component is to collect DNA to allow the identification of genetic factors that may influence the PK, PD, efficacy, safety, or tolerability of CNTO 3157 and to identify genetic factors associated with asthma and other chronic pulmonary diseases.

Biomarker samples will be collected to evaluate the mechanism of action of CNTO 3157 and potentially help to explain interindividual variability in clinical outcomes or may help to identify population subgroups that respond differently to a drug. The goal of the biomarker analyses is to evaluate the PD response of CNTO 3157 and aid in evaluating the drug-clinical response relationship.

DNA and biomarker samples may be used to help address emerging issues and to enable the development of safer, more effective, and ultimately individualized therapies.

4. SUBJECT SELECTION

Part 1 of the study will enroll healthy adult subjects. Part 2 of the study will enroll subjects with mild to moderate, controlled asthma who may be on no controller medications or are receiving low or medium dose ICS (≤ 500 µg/day fluticasone or equivalent) with or without additional permitted controllers (eg, long-acting beta-agonists [LABAs], leukotriene receptor antagonist [LTRAs] ,refer to Section 8.2).

The inclusion and exclusion criteria for enrolling subjects in Part 1 and Part 2 of this study are described in the following subsections. If there is a question about the inclusion or exclusion criteria below, the investigator should consult with the appropriate sponsor representative before enrolling a subject in the study.

For a discussion of the statistical considerations of subject selection, refer to Section 11.4, Sample Size Determination.

4.1. Part 1

4.1.1. Inclusion Criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study. Each subject must:

1. Demonstrate an understanding of the study and sign an informed consent form prior to any study related procedures.
2. Be willing and able to adhere to the prohibitions and restrictions specified in this protocol
3. Be 18 (or the legal age of consent in the jurisdiction in which the study is taking place) to 65 years of age, inclusive at the time of signing the informed consent
4. Have a body weight in the range of 40 to 125 kg inclusive. Have a body mass index (BMI) of 19 to 32 kg/m^2 inclusive.
5. Be healthy with no clinically significant abnormalities as determined by medical history, physical examination, blood chemistry assessments, hematologic assessments, coagulation and urinalysis, measurement of vital signs, and 12-lead ECG performed at Screening Visit 2.
6. The results of the following laboratory tests performed at the central laboratory must be within the limits specified below.

    - Serum ALT levels ≤2 x ULN
    - Serum AST levels ≤2 x ULN

    Only 1 repeat testing is allowed. Note: the investigator may consider the subject eligible if the previously abnormal laboratory test result is within normal range on a repeat testing in the central laboratory.

    Except for the tests specified above, if the results of the serum chemistry panel, hematology, or urinalysis are outside the normal reference ranges, the subject may be included only if the investigator and Sponsor's responsible study physician judge the abnormalities or deviations from normal to be not clinically significant. This determination must be recorded in the subject's source documents and initialed by the investigator.

7. Have been exposed to HSV-1 as documented by a positive serology test result for HSV-1 performed at Screening Visit 1, but have no signs or symptoms suggestive of an active HSV-1 infection and are not receiving prescription treatment for HSV-1.

8. If a woman, before entry she must be:

   - Postmenopausal, defined as:
     - >45 years of age with amenorrhea for at least 18 months, or
     - >45 years of age with amenorrhea for at least 6 months and a serum follicle stimulating hormone (FSH) level >40 U/L, or
   - Menstrual
     - Surgically sterile (have had a hysterectomy or bilateral oophorectomy, tubal ligation, or otherwise be incapable of pregnancy), or
     - If heterosexually active, practicing a highly effective method of birth control, including hormonal prescription oral contraceptives, contraceptive injections, contraceptive patch, intrauterine device, double-barrier method (eg, condoms, diaphragm, or cervical cap, with spermicidal foam, cream, or gel), or male partner sterilization, consistent with local regulations regarding use of birth control methods for subjects participating in clinical trials, for the duration of their participation in the study, or
     - Not heterosexually active

   Note: women who are not heterosexually active at screening must agree to utilize a highly effective method of birth control if they become heterosexually active during their participation in the study.

   Women must agree to continue using these methods of contraception throughout the study and for 6 months after receiving the last dose of study agent (placebo or CNTO 3157).

9. If a woman is of childbearing potential, she must have a negative serum β-human chorionic gonadotropin (β-hCG) pregnancy test at Screening Visit 2 and not be lactating.

10. If a man and heterosexually active with a woman of childbearing potential, he must agree to use a double barrier method of birth control and to not donate sperm during the study up to 6 months after last dose.

11. Agree to refrain from the use of alcohol 24 hours before all study visits.

12. Not be taking restricted medications at Screening Visit 1 for the proscribed time periods **AND** agree to refrain from taking any restricted medication as described in Section 8.1 unless medically necessary.

13. To participate in the optional pharmacogenomic component of this study, subjects must have signed the informed consent form for pharmacogenomic research indicating willingness to participate in the pharmacogenomic component of the study (where local regulations permit). Refusal to give consent for this component does not exclude a subject from participation in the clinical study.

4.1.2. Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study. The subject will be excluded if he or she:

1. Has any condition, including findings in the medical history or by any assessment, that in the opinion of the Investigator, in consultation with the Sponsor's medical monitor, constitute a risk or a contraindication for the participation of the subject in the study, prevent the subject from meeting or performing study requirements, or that could interfere with the study objectives, conduct or evaluation.

    - For chronic conditions that require medications and are considered mild (eg, hypercholesterolemia, mild hypertension), the investigator should consult with the Sponsor's medical monitor prior to randomizing the subject.

2. At Screening Visit 1 and throughout the study, works with (or lives with a family member who cares for) the elderly, (eg, nursing home), or lives with someone who may be at risk from transmission of the HRV-16 challenge agent, including but not limited to, individuals with chronic lung disease (including asthma), a premature infant, or an immunocompromised individual.

3. Has had any acute illness, including a common cold, within 4 weeks prior to Screening Visit 1, or has had a major illness or hospitalization within 6 months prior to Screening Visit 1.

4. Has active allergic rhinitis or perennial allergy symptoms (eg, due to ragweed) at Screening Visit 2 or expects to have active allergic rhinitis or perennial allergy symptoms during the study

5. Has a current infection (eg, sepsis, pneumonia or pyelonephritis), or has been hospitalized and/or received antimicrobials for a serious infection during the 6 months prior to Screening Visit 1.

6. Has chronic or recurrent infectious disease, including, but not limited to active herpes simplex virus (HSV) infection (including cold sores or genital herpes), chronic sinusitis, renal infection (eg, recurrent pyelonephritis); active tuberculosis (TB), chest infection (eg, bronchiectasis); urinary tract infection; skin wound, or ulcer.

7. Has a serum neutralizing antibody titer to HRV-16 >2-fold dilution (ie, can be diluted more than 2-fold and still retain HRV-16 neutralizing activity) in the blood sample collected at Screening Visit 1.

8. Has positive serology to human immunodeficiency virus 1 or 2 (HIV-1/2), hepatitis B virus (HBV), or hepatitis C virus (HCV) at Screening Visit 2.

9. Has any known malignancy or has a history of malignancy with the exceptions of:

   - basal cell carcinoma or squamous cell carcinoma in situ of the skin that has been treated with no evidence of recurrence within 6 months prior to Screening Visit 1.
   - squamous cell carcinoma of the skin (not in situ) or cervical carcinoma in situ that has been treated with no evidence of recurrence within 5 years prior to Screening Visit 1.

10. Has abnormal nasal anatomy or mucosa by visual inspection during the nasal examination at Screening Visit 2.

11. Has a history of bleeding disorders or frequent (1 or more times per month) nose bleeds.

12. Has had major surgery within 12 weeks prior to the Screening Visit 1, or will not have fully recovered from surgery, or has planned surgery through the end of the study. [Note: subjects with planned minor surgical procedures to be conducted under local anesthesia may participate.]

13. Is unable or unwilling to undergo multiple venipunctures because of poor tolerability or lack of easy access to veins.

14. Has ever received CNTO 3157.

15. Has known allergies, hypersensitivity, or intolerance to any study agent (placebo, CNTO 3157, HRV-16) or its excipients (refer to Investigator's Brochures for CNTO 3157 and HRV-16).

16. Known or suspected intolerance or hypersensitivity to any biologic medication or known allergies or clinically significant reactions to murine, chimeric, or human proteins, to monoclonal antibodies or antibody fragments.

17. Has a history of recurrent adverse drug/food reactions, severe allergic reaction, angioedema or anaphylaxis.

18. Has received an investigational drug (including investigational vaccines) within 5 half-lives (or 12 weeks if the half life is unknown) or used an invasive investigational medical device before the planned first dose of study agent, plans to enroll, or is currently enrolled in an interventional investigational study.

19. Has received any live attenuated vaccination within 4 weeks prior to Screening Visit 1 or are expected to receive any live attenuated vaccinations during the trial or up to 3 months after the last administration of study agent. Inactivated, injectable influenza and pneumococcal vaccines are permissible.

20. Has taken any of the restricted medications as described in Section 8.1 for the indicated periods before Screening Visit 1.
21. Has received any systemic cytotoxic, immunosuppressant, or immunomodulatory agents for at least 12 weeks prior to Screening Visit 1.
22. Regularly used tobacco within 6 months of Screening Visit 1 or has a history of smoking ≥ 10 pack years (1 pack year = 20 cigarettes smoked per day for 1 year) or equivalent, or a positive urine cotinine test from the sample collected at Screening Visit 1.
23. Has, or has had, a substance abuse (drug or alcohol) problem within the previous 3 years.
24. At Screening Visit 2, has a positive urine toxicology screen for substances of abuse, including, but not limited to alcohol, cocaine, cannabinoids, amphetamines, benzodiazepines, barbiturates, opiates, tricyclic antidepressants, and methadone. Use of these substances need not be considered exclusionary with a valid prescription and following consultation with the Sponsor.
25. Has donated blood or has had a blood loss of more than 450 mL within 60 days prior to Screening Visit 1 or plans to donate blood during the study.
26. Is a woman who is pregnant, or breast-feeding, or planning to become pregnant or is a man who plans to father a child while enrolled in this study or within 6 months after study agent (placebo or CNTO 3157).
27. Plans to donate sperm or eggs while enrolled in this study or up to 6 months after last dose of study agent (placebo or CNTO 3157).
28. Has any condition that, in the opinion of the investigator, would make participation not be in the best interest (eg, compromise the well-being) of the subject or that could prevent, limit, or confound the protocol-specified assessments.
29. Lives in an institution on court or authority order.
30. Is an employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator.

NOTE: Investigators should ensure that all study enrollment criteria have been met at screening. If a subject's status changes (including laboratory results) after screening but before the first dose of study agent is given such that they now meet an exclusion criterion, they should be excluded from participation in the study.

4.1.3. Randomization Criteria

At the end of the screening period (at Day 1 visit), <u>in addition</u> to the preceding criteria, all of the following criteria must be met in order to be eligible <u>for randomization</u>:

1. Still meet all inclusion and exclusion criteria.

2. No upper respiratory illness in the screening period or signs or symptoms of other acute illnesses, moderate or severe rhinorrhea, a Cold Symptom Assessment Score ≥2 or any other major illness during the screening period in order to be eligible for randomization.

4.2. Part 2

4.2.1. Inclusion Criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study. Each subject must:

1. Demonstrate an understanding of the study and sign an informed consent form prior to any study related procedures.

2. Be willing and able to adhere to the prohibitions and restrictions specified in this protocol

3. Be 18 (or the legal age of consent in the jurisdiction in which the study is taking place) to 65 years of age, inclusive at the time of signing the informed consent

4. Have a body weight in the range of 40 to 125 kg inclusive. Have a body mass index (BMI) of 19 to 32 kg/m^2 inclusive.

5. Have a physician documented diagnosis of asthma for at least 6 months prior to Screening Visit 2.

6. Have objective evidence of asthma by fulfilling at least 1 of the following criteria:

   - bronchodilator reversibility of 12% or greater and at least a 200 mL improvement in FEV_1 postbronchodilator after administration of up to 8 puffs of a short-acting β2-agonist (SABA) at Screening Visit 2,

     <u>OR</u>

   - -documented reversibility of 12% or greater and at least a 200 mL improvement in FEV_1 postbronchodilator after administration of up to 8 puffs of a SABA within 12 months before Screening Visit 1

     <u>OR</u>

   - -documented airway reactivity within 12 months of Screening Visit 1 to histamine (PC20 histamine <8 mg/mL) or methacholine (PC20 methacholine <16 mg/mL)

7. Have stable asthma based on physician assessment at Screening Visit 2.

   - Permitted concomitant medications for asthma must have been at a stable dose for the 4 weeks prior to Screening Visit 1.

8. Have an ACQ symptom score <1.5 at Screening Visit 2.

9. Have a prebronchodilator forced expiratory volume in the first second (FEV_1) ≥65% of predicted normal value at Screening Visit 2.

10. Have worsening of asthma symptoms (eg, increased use of rescue medication, increased wheeze or shortness of breath, requirement for corticosteroid use) during naturally acquired upper respiratory tract infections ("a common cold") on questioning at Screening Visit 2.

    - The subject must answer "yes" to the question: *"Do you usually have worsening of your symptoms of asthma (eg, shortness of breath wheezing, cough phlegm) when you get a cold?"*

11. Be otherwise healthy with no clinically significant abnormalities other than stable asthma (intermittent or persistent) as determined by medical history, physical examination, blood chemistry assessments, hematologic assessments, coagulation and urinalysis, measurement of vital signs, and 12-lead ECG performed at Screening Visit 2.

12. The results of the following laboratory tests performed at the central laboratory must be within the limits specified below.

    - Serum ALT levels ≤2 x ULN
    - Serum AST levels ≤2 x ULN

    Only 1 repeat testing is allowed. Note: the investigator may consider the subject eligible if the previously abnormal laboratory test result is within normal range on a repeat testing in the central laboratory.

    Except for the tests specified above, if the results of the serum chemistry panel, hematology, or urinalysis are outside the normal reference ranges, the subject may be included only if the investigator and Sponsor's responsible study physician judge the abnormalities or deviations from normal to be not clinically significant. This determination must be recorded in the subject's source documents and initialed by the investigator.

13. Have been exposed to HSV-1 as documented by a positive serology test result for HSV-1 in a blood sample taken at Screening Visit 1, but have no signs or symptoms suggestive of an active HSV-1 infection and are not receiving prescription treatment for HSV-1.

14. If a woman, before entry she must be:

- Postmenopausal, defined as:
  - >45 years of age with amenorrhea for at least 18 months, or
  - >45 years of age with amenorrhea for at least 6 months and a serum follicle stimulating hormone (FSH) level >40 U/L, or
- Menstrual
  - Surgically sterile (have had a hysterectomy or bilateral oophorectomy, tubal ligation, or otherwise be incapable of pregnancy), or
  - If heterosexually active, practicing a highly effective method of birth control, including hormonal prescription oral contraceptives, contraceptive injections, contraceptive patch, intrauterine device, double-barrier method (eg, condoms, diaphragm, or cervical cap, with spermicidal foam, cream, or gel), or male partner sterilization, consistent with local regulations regarding use of birth control methods for subjects participating in clinical trials, for the duration of their participation in the study, or
  - Not heterosexually active

Note: women who are not heterosexually active at screening must agree to utilize a highly effective method of birth control if they become heterosexually active during their participation in the study.

Women must agree to continue using these methods of contraception throughout the study and for 6 months after receiving the last dose of study agent (placebo or CNTO 3157).

15. If a woman is of childbearing potential, she must have a negative serum β-human chorionic gonadotropin (β-hCG) pregnancy test at Screening Visit 2 and not be lactating.

16. If a man and heterosexually active with a woman of childbearing potential, he must agree to use a double barrier method of birth control and to not donate sperm during the study up to 6 months after last dose.

17. Agree to refrain from the use of alcohol 24 hours before all study visits.

18. Agree, if eligible, to attempt to provide induced sputum samples at all relevant visits.

19. Not be taking restricted medications at Screening Visit 1 for the proscribed time periods **AND** agree to refrain from taking any restricted medication as described in Section 8.2.

20. To participate in the optional pharmacogenomic component of this study, subjects must have signed the informed consent form for pharmacogenomic research indicating willingness to participate in the pharmacogenomic component of the study (where local regulations permit). Refusal to give consent for this component does not exclude a subject from participation in the clinical study.

4.2.2. Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study.

1. Has a history of any other chronic lung disease, including chronic obstructive pulmonary disease (COPD), bronchiolitis, bronchiectasis, allergic bronchopulmonary aspergillosis (mycosis), occupational asthma, sleep apnea, pulmonary hypertension, or any other obstructive pulmonary disease, liver or renal insufficiency; significant cardiac, vascular, pulmonary, gastrointestinal, endocrine, neurologic, hematologic, rheumatologic, psychiatric, or metabolic disturbances, or other body system disorders that are clinically significant in the opinion of the investigator.
2. Has ever had an episode of life-threatening asthma defined as respiratory arrest or requiring intubation for asthma.
3. Has been hospitalized (for greater than 24 hours) due to asthma in the 5 years prior to Screening Visit 1.
4. Has experienced an asthma exacerbation in the 12 weeks prior to Screening Visit 1 requiring management with systemic steroids.
5. Is receiving high dose ICS (>500 µg/day to fluticasone or equivalent). Use of low or medium dose ICS (≤500 µg/day fluticasone or equivalent) with or without permitted controller medications e.g LABA, LTRA is allowed (as indicated in Section 8.2).
6. Has active allergic rhinitis or perennial allergy symptoms (eg, due to ragweed) at Screening Visit 2 or expects to have active allergic rhinitis or perennial allergy symptoms during the study.
7. Has any condition, including findings in the medical history or by any assessment, that in the opinion of the Investigator, in consultation with the Sponsor's medical monitor, constitute a risk or a contraindication for the participation of the subject in the study, prevent the subject from meeting or performing study requirements, or that could interfere with the study objectives, conduct or evaluation.
    - For chronic conditions that require medications and are considered mild (eg, hypercholesterolemia), the investigator should consult with the Sponsor's medical monitor prior to randomizing the subject.
8. At Screening Visit 1 and throughout the study, works with (or lives with a family member who cares for) the elderly, (eg, nursing home), or lives with someone who may be at risk from transmission of the HRV-16 challenge agent, including but not limited to, individuals with chronic lung disease (including asthma), a premature infant, or an immunocompromised individual.
9. Has had any acute illness, including a common cold, within 4 weeks prior to Screening Visit 1, or has had a major illness or hospitalization within 6 months prior to Screening Visit 1.

10. Has a current infection (eg, sepsis, pneumonia or pyelonephritis), or has been hospitalized and/or received antimicrobials for a serious infection during the 6 months prior to Screening Visit 1.

11. Has chronic or recurrent infectious disease, including, but not limited to active herpes simplex virus (HSV) infection (including cold sores or genital herpes), chronic sinusitis, renal infection (eg, recurrent pyelonephritis); active tuberculosis (TB), chest infection (eg, bronchiectasis); urinary tract infection; skin wound, or ulcer.

12. Has a serum neutralizing antibody titer to HRV-16 >2-fold dilution (ie, can be diluted more than 2-fold and still retain HRV-16 neutralizing activity) in the blood sample collected at Screening Visit 1.

13. Has positive serology test result to human immunodeficiency virus 1 or 2 (HIV-1/2), hepatitis B virus (HBV), or hepatitis C virus (HCV) at Screening Visit 2.

14. Has any known malignancy or has a history of malignancy with the exceptions of:

    - basal cell carcinoma or squamous cell carcinoma in situ of the skin that has been treated with no evidence of recurrence within 6 months prior to Screening Visit 1.
    - squamous cell carcinoma of the skin (not in situ) or cervical carcinoma in situ that has been treated with no evidence of recurrence within 5 years prior to Screening Visit 1.

15. Has abnormal nasal anatomy or mucosa by visual inspection during the nasal examination at Screening Visit 2.

16. Has taken any of the restricted medications for the indicated time periods as described in Section 8.2 before Screening Visit 1.

17. Has a history of bleeding disorders or frequent (1 or more times per month) nose bleeds.

18. Has had major surgery within 12 weeks prior to the Screening Visit 1, or will not have fully recovered from surgery, or has planned surgery through the end of the study. [Note: subjects with planned minor surgical procedures to be conducted under local anesthesia may participate.]

19. Be unable or unwilling to undergo multiple venipunctures because of poor tolerability or lack of easy access to veins.

20. Has ever received CNTO 3157.

21. Has known allergies, hypersensitivity, or intolerance to any study agent (placebo, CNTO 3157, HRV-16) or its excipients (refer to Investigator's Brochures for CNTO 3157 and HRV-16).

22. Known or suspected intolerance or hypersensitivity to any biologic medication or known allergies or clinically significant reactions to murine, chimeric, or human proteins, to monoclonal antibodies or antibody fragments.

23. Has a history of recurrent adverse drug/food reactions, severe allergic reaction, angioedema or anaphylaxis.

24. Has received an investigational drug (including investigational vaccines) within 5 half-lives (or 12 weeks if the half life is unknown) or used an invasive investigational medical device before the planned first dose of study agent, plans to enroll, or is currently enrolled in an interventional investigational study.

25. Has received any live attenuated vaccination within 4 weeks prior to Screening Visit 1 or are expected to receive any live attenuated vaccinations during the trial or up to 3 months after the last administration of study agent. Inactivated, injectable influenza and pneumococcal vaccines are permissible.

26. Has received any systemic cytotoxic, immunosuppressant, or immunomodulatory agents for at least 12 weeks prior to Screening Visit 1.

27. Regularly used tobacco within 6 months of Screening Visit 1 or has a history of smoking ≥ 10 pack years (1 pack year = 20 cigarettes smoked per day for 1 year) or equivalent, or a positive urine cotinine test from the sample collected at Screening Visit 1.

28. Has, or has had, a substance abuse (drug or alcohol) problem within the previous 3 years.

29. At Screening Visit 2, has a positive urine toxicology screen for substances of abuse, including, but not limited to alcohol, cocaine, cannabinoids, amphetamines, benzodiazepines, barbiturates, opiates, tricyclic antidepressants, and methadone. Use of these substances need not be considered exclusionary with a valid prescription and following consultation with the Sponsor.

30. Has donated blood or has had a blood loss of more than 450 mL within 60 days prior to Screening Visit 1 or plans to donate blood during the study.

31. Is a woman who is pregnant, or breast-feeding, or planning to become pregnant or is a man who plans to father a child while enrolled in this study or within 6 months after the last dose of study agent (placebo or CNTO 3157).

32. Plans to donate sperm or eggs while enrolled in this study or up to 6 months after last dose of study agent (placebo or CNTO 3157).

33. Has any condition that, in the opinion of the investigator, would make participation not be in the best interest (eg, compromise the well-being) of the subject or that could prevent, limit, or confound the protocol-specified assessments.

34. Lives in an institution on court or authority order.

35. Is an employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator.

NOTE: Investigators should ensure that all study enrollment criteria have been met at screening. If a subject's status changes (including laboratory results) after screening but before first dose of study agent (placebo or CNTO 3157) is given such that they now meet an exclusion criterion, they should be excluded from participation in the study.

4.2.3. Randomization Criteria

At the end of the screening period (at Day 1 visit), in addition to the preceding criteria, all of the following criteria must be met in order to be eligible for randomization:

1. ACQ score <1.5.

2. Prebronchodilator FEV_1 ≥65% of the predicted value and the measured value of clinic prebronchodilator FEV_1 must not have increased or decreased by >20% relative to baseline (Day 1) between Screening Visit 2 and the Day 1 visit.

3. No worsening of asthma symptoms that required treatment with a change in asthma therapy between the first screening visit and randomization.

4. Still meet all inclusion and exclusion criteria.

5. No upper respiratory illness in the screening period or signs or symptoms of other acute illnesses, moderate or severe rhinorrhea, a Cold Symptom Assessment Score ≥2 or any other major illness during the screening period in order to be eligible for randomization.

6. Demonstrate acceptable compliance with the handheld electronic device (diary) usage in the 7 days prior to the randomization visit, which should be at least 70% (≥10 out of the 14 possible device entries [2 possible entries per day]).

   Lack of compliance due to mechanical malfunction of the device or other unusual, uncontrollable circumstances based upon the investigators' judgment will not be considered as non-compliance. In such cases, the screening period may be extended for 7 days for subjects to demonstrate acceptable compliance.

4.3. Eligibility Criteria for Sputum Induction

As stated in Section 4.2.3, all asthmatic subjects are required to have a prebronchodilator FEV_1 ≥65% at randomization. In recognition of intra-subject variability between visits, the following are provided as guidelines for investigators to consider for those subjects whose postbronchodilator FEV_1 may be below 65% at visits where sputum induction is to be performed.

- If the pre-induction postbronchodilator FEV_1 is ≥60%, then sputum induction may be performed starting with 3% and increasing to 4% and then 5% hypertonic saline if FEV_1 remains within the ranges as detailed in the Sputum Induction Manual.
- If the pre-induction postbronchodilator FEV_1 is ≥50% and <60%, then sputum induction may be performed starting with normal saline, and then increasing to 3% and 4% if FEV_1 remains within the ranges as detailed in the Sputum Induction Manual.
- If the pre-induction postbronchodilator FEV_1 is <50%, spontaneous sputum may be collected.
- If postbronchodilator FEV_1 at any scheduled sputum visit has fallen by ≥20% from postbronchodilator FEV_1 at Screening Visit 2 only normal saline should be used.

If a subject is eligible, but unable to produce a sputum sample at Screening Visit 2, the subject may make an additional attempt to produce a sputum sample after Screening Visit 2, but the next attempt must be at least 2 days after the first attempt, and at least 7 days prior to the Day 1 visit.

If a subject is unable to produce an acceptable sputum sample, they may still be enrolled in the study.

5. TREATMENT ALLOCATION AND BLINDING

Treatment Allocation

Procedures for Randomization and Stratification

Subjects in Part 1 will be randomly assigned to 1 of 2 treatment groups in a 2:1 ratio (CNTO 3157 versus placebo) based on a computer-generated randomization schedule prepared before the study by the interactive voice or web response system (IVRS or IWRS) provider under the supervision of the sponsor. Subjects in Part 2 will be randomly assigned to 1 of 2 treatment groups in a 1:1 ratio (CNTO 3157 versus placebo) based on a computer-generated randomization schedule prepared before the study by IVRS or IWRS provider under the supervision of the sponsor.

The IVRS/IWRS will assign a unique treatment code, which will dictate the treatment assignment and matching study agent (placebo or CNTO 3157) for the subject. The requestor must use his or her own user identification and personal identification number when contacting the IVRS, and will then give the relevant subject details to uniquely identify the subject.

Blinding

Since Part 1 and Part 2 include separate subjects, they can be considered as 2 separate experiments. The blinding related considerations are similar.

Blinding related considerations for both Part 1 and Part 2:

The investigator will not be provided with randomization codes. The codes will be maintained within the interactive voice response system (IVRS) and/or interactive web response system

(IWRS), which has the functionality to allow the investigator to break the blind for an individual subject.

Data that may potentially unblind the treatment assignment (ie, study agent serum concentrations, study agent preparation/accountability data, treatment allocation, biomarker or other specific laboratory data) will be handled with special care to ensure that the integrity of the blind is maintained and the potential for bias is minimized. This can include making special provisions, such as segregating the data in question from view by the investigators, clinical team, or others as appropriate until the time of database lock (DBL) and unblinding. If data from Part 1 of the study are reviewed prior to completing the study by a select group of internal or external individuals for planning purposes, provisions will be made to ensure maintenance of the blind.

Under normal circumstances and unless otherwise stated, the blind should not be broken until all subjects have completed the study and the database is locked. Otherwise, the blind should be broken only if specific emergency treatment/course of action would be dictated by knowing the treatment status of the subject. In such cases, the investigator may in an emergency determine the identity of the treatment by contacting the IVRS/IWRS. It is recommended that the investigator contact the Sponsor or its designee if possible to discuss the particular situation, before breaking the blind. Telephone contact with the Sponsor or its designee will be available 24 hours per day, 7 days per week. In the event the blind is broken, the Sponsor must be informed as soon as possible. The date and reason for the unblinding must be documented by the IVRS/IWRS, in the appropriate section of the CRF, and in the source document. The documentation received from the IVRS/IWRS indicating the code break must be retained with the subject's source documents in a secure manner so as to not unblind the treatment assignment to the subject, the study site, or Sponsor personnel. The investigator is also advised not to reveal the study treatment assignment to the subject, the study site, or Sponsor personnel.

Subjects who have had their treatment assignment unblinded will be discontinued from further administrations of study agent (placebo or CNTO 3157), but should continue to return for scheduled evaluations.

In general, randomization codes will be disclosed fully only if the study is completed and the clinical database is closed.

6. DOSAGE AND ADMINISTRATION

Subjects in Part 1 will receive a single IV infusion of either placebo or CNTO 3157 10 mg/kg at Day 1 24 to 72 hours prior to inoculation with HRV-16.

Subjects in Part 2 will receive 4 intravenous infusions of placebo at Week 1 (Day 1), Week 2 (Day 8), Week 3 (Day 15), and Week 4 (Day 22) or 1 infusion of 10 mg/kg of CNTO 3157 at Week 1 followed by 3 infusions of 3 mg/kg of CNTO 3157 at Week 2, Week 3, and Week 4. The final infusion (Week 4) will occur 24 to 72 hours prior to inoculation with HRV-16. Infusions will be administered over a period of not less than 30 minutes.

Only the unblinded pharmacist/designee will know which treatment the subject receives. The subject, medical monitor, sponsor study personnel (with the exception of the Independent Drug Monitors assigned to the site and the Sponsor Lead Independent Drug Monitor [IDM]), principal investigator, and all the investigator staff will be blinded; therefore, only the pharmacist/designee and an IDM will be unblinded to subject treatment assignments.

Information on adverse events during or following study agent infusion, infusion-site reactions, and delayed hypersensitivity (serum sickness-like) reactions is provided in Section 12.3.2, Section 12.3.3, and Section 12.3.4, respectively.

7. TREATMENT COMPLIANCE

Study agent (placebo or CNTO 3157) and HRV-16 will be administered at the investigational sites according to the Time and Events Schedule. The designated study personnel (unblinded pharmacist) will maintain a log of all study agent (placebo, CNTO 3157, and HRV-16) dispensed and returned. Study agent supplies (placebo, CNTO 3157, and HRV-16) will be inventoried and accounted for throughout the study (see Section 14.1.4).

8. PRESTUDY AND CONCOMITANT THERAPY

All prestudy treatments/therapies administered within 6 weeks before screening must be recorded at the first screening visit.

As certain conmeds (eg, systemic cytotoxic/immunosuppressant/immunomodulatory agents) may affect the clinical symptoms of HRV-16 infection, including biomarker or other clinical efficacy evaluations, for at least 3 months prior to and during the screening period, the use of any restricted medications as described in Section 8.1 and Section 8.2 is not allowed. The use of any of restricted medications as described in Section 8.1 and Section 8.2 at any time post randomization is strongly discouraged, and subjects who require any of the above types of agents should be withdrawn from receiving any additional administrations of study agent and inoculation HRV-16, but will remain in the study for all study evaluations.

All treatments (eg, prescription or over-the-counter medications, including vaccines, vitamins, herbal supplements) different from the study agent must be recorded in the CRF. Modification of an effective preexisting therapy should not be made for the explicit purpose of entering a subject into the study.

The sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which restricted therapies are administered.

8.1. Part 1 - Healthy Adult Subjects

Permitted and restricted concomitant medications for healthy adult subjects in Part 1 are listed in Table 1.

Table 1: Concomitant Medications in Part 1 (Healthy Adult Subjects)	
PERMITTED MEDICATIONS (PART 1)	
Medication Class	**Period**
PRN use of acetaminophen/paracetamol at recommended doses (≤1 gram/6 hours and ≤3 grams/day), low dose aspirin (75 to 81 mg/day), and continued pre-existing use of vitamins or multivitamins at recommended doses is allowed.	Allowed any time throughout the study.
Oral contraceptives	Allowed any time throughout the study.
Topical medications except drug patches	Allowed any time throughout the study.
RESTRICTED MEDICATIONS (PART 1)	
Medication Class	**Period**
Systemic steroids	12 weeks prior to Screening Visit 1 until end of Part 1 of the study.
Intranasal steroids	4 weeks prior to Screening Visit 1 until end of Part 1 of the study.
Allergy or cold medications	2 days prior to Screening Visit 1 until end of Part 1 of the study.
Allergy immunotherapy	12 weeks prior to Screening Visit 1 until end of Part 1 of the study.
Investigational medications of any nature	5 half-lives (or 12 weeks if the half life is unknown) prior to Screening Visit 1 until end of Part 1 of the study.
Prescription medications	4 weeks prior to Screening Visit 1 until end of Part 1 of the study unless for mild stable conditions and approved by sponsor's medical monitor.
The use of cytotoxic, immunosuppressant, immunomodulatory agents including, but not limited to, cyclosporine A, azathioprine, mycophenolate, mofetil, interferon gamma, anakinra, infliximab, adalimumab, certolizumab, etanercept, golimumab, or ustekinumab	For 12 weeks prior to Screening Visit 1 until end of Part 1 of the study.

Unless contraindicated (eg, allergy to components), inactivated, injected influenza and pneumococcal vaccines are permitted as recommended by guidelines.

8.2. Part 2 - Asthmatic Subjects

Permitted and restricted concomitant medications for asthmatic subjects in Part 2 are listed in Table 2.

Unless contraindicated (eg, allergy to components), inactivated, injected influenza and pneumococcal vaccines are permitted as recommended by guidelines.

No concomitant medications will be provided by the Sponsor.

Table 2: Concomitant Medications in Part 2 (Asthmatic Subjects)	
PERMITTED MEDICATIONS (PART 2)	
Medication Class	**Period**
All short acting β2 agonists taken as needed at recommended doses	Allowed any time throughout the study.
Leukotriene receptor antagonist (eg, montelukast)	Throughout study if already prescribed at least 4 weeks prior to Screening Visit 1.
PRN use of acetaminophen/paracetamol at recommended doses (≤1 gram/6 hours and ≤3 grams/day), low dose aspirin (75 to 81 mg/day), and continued pre-existing use of vitamins or multivitamins at recommended doses is allowed	Allowed any time throughout the study.
NSAIDS	Allowed any time throughout the study.
Allergy immunotherapy	If started 12 weeks prior to Screening Visit 1 and planned to be continued throughout study at a stable dose.
LABA (if prescribed with a medium or low dose ICS)	Throughout study if already prescribed at least 4 weeks prior to Screening Visit 1.
Oral contraceptives	Allowed any time throughout the study.
Topical medications except drug patches	Allowed any time throughout the study.
RESTRICTED MEDICATIONS (PART 2)	
Medication Class	**Period**
Systemic steroids	12 weeks prior to Screening Visit 1 to end of study unless prescribed during the study for acute asthma exacerbation or other significant illness.
Intranasal steroids	4 weeks prior to Screening Visit 1 until end of Part 2 of the study.
Allergy or cold medications	2 days prior to Screening Visit 1 until end of Part 2 of the study.
Investigational medications of any nature	5 half-lives (or 12 weeks if the half life is unknown) prior to Screening Visit 1 until end of Part 2 of the study.
Prescription medications	4 weeks prior to Screening Visit 1 until Week 7 unless for mild stable conditions and approved by sponsor's medical monitor.
High dose ICS (>500 µg/day fluticasone or equivalent).	4 weeks prior to Screening Visit 1 until end of Part 2 of the study (unless medically necessary).
Omalizumab	12 weeks prior to Screening Visit 1 until end of Part 2 of the study.
Cromolyns, theophylline, oral β2 agonists, short-acting or long-acting anticholinergics (eg, ipratropium or tiotropium)	2 weeks prior to Screening Visit 1 until end of Part 2 of the study.
The use of cytotoxic, immunosuppressant, immunomodulatory agents including, but not limited to, cyclosporine A, azathioprine, mycophenolate, mofetil, interferon gamma, anakinra, infliximab, adalimumab, certolizumab, etanercept, golimumab, or ustekinumab	For 12 weeks prior to Screening Visit 1 until end of Part 2 of the study.

8.2.1. Treatment of Asthma Exacerbations

Asthma may worsen to a moderate degree following HRV-16 infection; therefore, subjects must obtain and fill a prescription for a short-acting rescue medication (eg, salbutamol/albuterol) from the investigator for use as needed.

Rescue medication will not be provided by the Sponsor. Although it is not anticipated that many subjects will experience a clinically significant asthma exacerbation following experimental HRV-16 inoculation, guidelines for management should follow clinical practice recommendations based on the international guidelines on management of exacerbations.[28] In this study, subjects will be instructed to call the investigator or designee as their symptoms of worsening of asthma develop. The investigator or designee will assess the severity of the episode and initiate appropriate treatment if indicated. If, based on the assessment of symptoms, functional parameters, and the subjects' history, the episode of worsening of asthma is considered severe by the investigator, a clinic visit should be performed if possible (Note: This may be an additional, unscheduled visit). In the event of a medical emergency, subjects should seek urgent care immediately, and then inform the investigator.

The treatment of exacerbations depends on the subject's condition and the response to treatment. The investigator will assess the severity of the episode and initiate appropriate treatment. Administration of systemic corticosteroids will result in discontinuation from study agent administration and inoculation with HRV-16 (see Section 10.2).

Each subject should be provided the site contact information and emergency clinic phone number.

9. STUDY EVALUATIONS

The Time and Events Schedules for Part 1 and Part 2 summarize the frequency and timing of efficacy, pharmacokinetic, pharmacodynamic, biomarker, pharmacogenomic, medical resource utilization, and safety assessments applicable to Part 1 and Part 2 of this study.

Additional serum or urine pregnancy tests may be performed for female subjects of child-bearing potential, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the subject's participation in the study.

The total blood volume for Part 1 of the study is approximately 190 mL. For each subject, the maximum amount of blood drawn from each subject in Part 1 in this study will not exceed 200 mL. Repeat or unscheduled samples may be taken for safety reasons.

The total blood volume for Part 2 of the study is approximately 235 mL. For each subject, the maximum amount of blood drawn from each subject in Part 2 of this study will not exceed 250 mL. Repeat or unscheduled samples may be taken for safety reasons.

Blood samples will be collected only from subjects who have consented to participate in the optional pharmacogenomic (DNA) component of the study. In the event of DNA extraction failure, a replacement pharmacogenomic blood sample may be requested from the subject.

Adverse events and concomitant medications will be collected at each visit throughout the study.

9.1. Study Procedures in Part 1

When multiple procedures are performed at a given visit, the following guideline for order of procedures is recommended (however, any deviations from this recommended order of events will not be considered a protocol violation). All visit-specific patient-reported outcomes (PRO) assessments during a visit should be conducted before any tests, procedures, or other consultations for that visit to prevent influencing subject perceptions. FENO must precede spirometry. The recommended order of procedures is:

- Cold question
- Cold Symptom Assessment Score
- Cold and Chest Symptom Scale
- Physical exam
- Vital signs
- ECG
- FENO sampling
- Spirometry (on D3PI)
- Blood samples
- Urine samples
- Nasal lavage
- Administer study agent (infusion)
- Post-infusion PK samples
- Post-infusion vital signs and post-infusion adverse events collection

On the study day when HRV-16 inoculation is performed, inoculation with HRV-16 should be done after all other procedures for that visit are performed.

9.1.1. Screening Phase (Part 1)

In Part 1, healthy adult subjects will undergo 2 screening visits approximately 35 days prior to study agent administration.

9.1.1.1. Screening Visit 1

At Screening Visit 1, subjects will sign an informed consent, and samples to determine the serum neutralizing antibody titer levels to HRV-16 and the presence of antibodies to HSV-1 will be collected from potential subjects. In addition, subjects will be queried about their smoking history and a urine cotinine test will be done. Eligibility criteria that can be reviewed at this visit will be evaluated. Since approximately 70% of the population will have sufficient neutralizing antibody titers to HRV-16 to be resistant to infection when challenged with virus, all subjects

will need to be screened for the presence of neutralizing antibodies to HRV-16. To allow successful challenge with HRV-16, those subjects with a neutralizing serum antibody titer to HRV-16 (serum can be diluted more than 1:2 and still retain HRV-16 neutralizing activity) will be excluded. The period between Screening Visit 1 and inoculation with HRV-16 should be no longer than 45 days to minimize the chance of seroconverting to HRV-16 or having their anti-HRV-16 titers increase prior to HRV-16 inoculation due to natural exposure. In addition, a dominant negative TLR3 allele has been associated with increased susceptibility to herpes simplex encephalitis upon primary infection with HSV-1 in childhood[50]; however, subjects with this allele do not have impaired responses to subsequent HSV-1 reactivations (eg, cold sores). As an added precaution, subjects without detectable antibodies to HSV-1 (indicating previous primary infection) will be excluded.

A urine sample for a cotinine test will be collected at Screening Visit 1.

All concomitant and prestudy treatments/therapies administered within 6 weeks before screening must be recorded at Screening Visit 1.

9.1.1.2. Screening Visit 2

At Screening Visit 2, potential subjects (those without HRV-16 neutralizing antibody titers, with antibodies to HSV-1 and a negative urine cotinine test result from Screening Visit 1) will be evaluated for each of the inclusion/exclusion criteria, including: a review of medical history and prior and current concomitant medication use, physical and nasal exam, collection of vital signs, a urine drug screen, and a laboratory analyses including hematology, chemistry, urinalysis, serum pregnancy testing (female subjects of child-bearing potential only), and other study procedures as detailed in the Time and Events Schedule. Nasal lavage will be done for the measurement of nasal inflammation. Serum samples for biomarker assessments will be collected. In addition, spirometry and a 12-lead ECG will be performed.

9.1.1.3. Screen Failure/Rescreening

If a subject is a screen failure but at some point in the future meets all of the subject eligibility criteria, the subject may be rescreened on 1 occasion only after consultation with the Sponsor. Subjects who are rescreened will be assigned a new subject number, undergo the informed consent process, and then restart a new screening phase.

9.1.2. Double-Blind Treatment Phase (Part 1)

Day 1/Day of Randomization

On the day of randomization prior to administration of study agent, study staff will perform a brief physical exam including weight, measure vital signs including body temperature (oral), perform a 12-lead ECG (prior to infusion), record each subject's Cold Symptom Assessment Score and cold and chest symptom scales, and perform spirometry, and confirm inclusion/exclusion criteria. Predose whole blood and serum samples for biomarker assessments, and FENO will be collected. Predose (baseline) PK samples for the measurement of serum

concentration of CNTO 3157 and antibodies to CNTO 3157 will be collected. Nasal lavage will be done immediately prior to dosing for the measurement of nasal inflammation.

Subjects will be randomized in a 2:1 ratio to receive a single IV infusion of 10 mg/kg of CNTO 3157 (8 subjects) or placebo (4 subjects), respectively, and a serum PK sample will be collected at 5 minutes post-infusion. Vital signs will be measured and any adverse events will be collected at 5 minutes, 2 hours, and 4 hours post-infusion, and a 12-lead ECG will be performed 4 hours post-infusion.

At Study Day 1 (predose), a pharmacogenomic blood sample will be collected from subjects who consent separately to the pharmacogenomic component of the study (where local regulations permit). Participation in pharmacogenomic research is optional.

Day 2/Intranasal Inoculation with HRV-16

Subjects will return the following day (to occur 24 to 72 hours after dosing on Study Day 1) for intranasal inoculation with HRV-16. A brief physical exam will be performed, and vital signs will be measured. A blood sample will be collected from subjects prior to inoculation with HRV-16 to determine serum neutralizing antibody titer levels to HRV-16 on the day of inoculation. Chest and cold symptom scale and cold symptoms scores will be recorded, and a PK sample to measure serum concentrations of CNTO 3157 will be collected. Prior to inoculation with HRV-16, a throat swab will be collected for multiplex PCR screening for the presence of other respiratory viruses.

Per design of this study, subjects will receive a single inoculation of HRV-16 administered in a total volume of approximately 1.0 mL administered via 4 intranasal inoculations (2 instillations per naris). If at any time during the instillation of HRV-16 into a subject's nostrils, the subject experiences significant discomfort or any adverse event, further administration of the remaining portion of the HRV-16 inoculum to that subject will be discontinued, but the subject will continue to return for all subsequent study visits. Refer to Section 10.2.2 regarding stopping criteria for inoculation with HRV-16.

Day 3 to Day 12/Post-HRV-16 Inoculation Phase

Subjects will return daily for 5 days post inoculation (D1PI through D5PI) with HRV-16 to assess the presence and severity of chest and cold symptoms using symptom scoring systems in the context of the previous 24 hours via an interactive interview with the study staff. In addition, FENO, spirometry, pharmacokinetic, biomarker and other assessments will be performed at the timepoints as specified in the Time and Events Schedule. In addition, subjects' responses to the Subject Cold Question (see Section 9.4.1) will be collected. In addition, nasal lavage samples for assessing HRV-16 titers and biomarkers will be collected daily during this period.

Subjects will return to the study site 7 days (D7PI) following inoculation with HRV-16 and will have nasal lavage, FENO, spirometry, pharmacokinetic, and biomarker assessments. The presence and severity of chest and cold symptoms using symptom scoring systems will also be

determined. Serum samples for determining antibody titers to HRV-16 will be collected. Other assessments as noted in the Time and Events Schedule will be performed.

Ten days post inoculation with HRV-16 (D10PI), subjects will return for FENO, nasal lavage, assessment of the severity of their chest and cold symptoms using symptom scoring systems, and other assessments as noted in the Time and Events Schedule.

9.1.3. Follow-Up Phase (Part 1)

Subjects will return for follow-up visits at Week 4 and Week 8 at which time safety and other assessments (eg, PK sampling, antibodies to HRV-16, assessments of the presence and severity of chest and cold symptoms) will be performed as indicated in the Time and Events Schedule.

For those subjects who have provided PG consent, a second pharmacogenomic (DNA) sample will be collected at Week 8 for possible epigenetic analysis.

Subjects will be followed for safety through Week 8 in Part 1. Any clinically significant abnormalities persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

Investigators may recontact the subject to obtain long-term follow-up information to determine the subject's safety (see Section 16.2.3, Informed Consent).

9.2. Study Procedures in Part 2

When multiple procedures are performed at a given visit, the following guideline for order of procedures is recommended (however, any deviations from this recommended order of events will not be considered a protocol violation). All visit-specific patient-reported outcomes (PRO) assessments during a visit should be conducted before any tests, procedures, or other consultations for that visit to prevent influencing subject perceptions. FENO must precede spirometry, and postbronchodilator spirometry must precede sputum induction. The recommended order of procedures is:

- Review of data from electronic peak flow meter and handheld electronic device
- ACQ
- TNOSS
- Cold question
- Cold Symptom Assessment Score
- Cold and Chest Symptom Scale
- Physical exam
- Vital signs
- ECG
- FENO sampling

- Spirometry (prebronchodilator and postbronchodilator when indicated)
- Induced sputum (in eligible subjects)
- Blood samples
- Urine samples
- Nasal lavage
- Nasal brushing
- Administer study agent (infusion)
- Post-infusion PK samples
- Post-infusion vital signs and post-infusion adverse events collection

On the study day when HRV-16 inoculation is performed, inoculation with HRV-16 should be done after all other procedures for that visit are performed.

Refer to the Time and Events Schedule for Part 2 for a complete listing of assessments.

9.2.1. Screening Phase (Part 2)

Asthmatic subjects will undergo 2 screening visits in Part 2 approximately 35 days prior to the first dose of study agent administration.

9.2.1.1. Screening Visit 1

As in Part 1, at Screening Visit 1, subjects in Part 2 will sign an informed consent, and samples to determine the serum neutralizing antibody titer levels to HRV-16 and the presence of antibodies to HSV-1 will be collected from potential subjects (see Section 9.1.1). In addition, subjects will be queried about their smoking history and a urine sample for a cotinine test will be collected. The period between Screening Visit 1 and Study Day 1 (randomization) should not exceed 35 days (42 days in the event an extension is required for diary compliance issues). All subjects without a neutralizing serum antibody titer to HRV-16 (serum can be diluted more than 1:2 and still retain HRV-16 neutralizing activity), detectable serum antibodies to HSV-1, and a negative cotinine test at Screening Visit 1 may proceed to Screening Visit 2.

All concomitant and prestudy treatments/therapies administered up to 6 weeks before screening must be recorded at Screening Visit 1.

A urine sample for a cotinine test will be collected at Screening Visit 1.

9.2.1.2. Screening Visit 2

At Screening Visit 2 in Part 2, potential subjects (those without HRV-16 neutralizing antibody titers, with antibodies to HSV-1 and a negative urine cotinine test result from Screening Visit 1) will be evaluated for each of the inclusion/exclusion criteria, including: a review of medical history and prior and current concomitant medication use, physical and nasal exam, a 12-lead ECG, collection of vital signs, a urine drug screen, and a laboratory analyses including hematology, chemistry, urinalysis, serum pregnancy testing (female subjects of child-bearing

potential only), and other study procedures as detailed in the Time and Events Schedule. Nasal lavage will be done for the measurement of nasal inflammation. Serum samples for biomarker assessments will be collected.

At Screening Visit 2, electronic diary devices will be issued to each subject to collect data for determining eligibility requirements and to collect subject reported data (eg, rescue medication use,). Site staff will review the use of the electronic diary with the subject at this visit. Spirometry measurements will be collected. Once all screening procedures are complete, subjects must complete at least 7 days of their electronic diary before the Day 1 visit as indicated in Section 4.2.3.

Eligible subjects (as determined by FEV_1 criteria) will undergo attempts at sputum induction at Screening Visit 2. An acceptable induced sputum sample is not required for inclusion in the study; however, all subjects in Part 2 must agree to attempt to provide induced sputum samples.

9.2.1.3. Repeat Testing During Screening (Part 2 only)

Repeat testing in Part 2 of only those laboratory tests specified in the inclusion criterion (Section 4.2.1, criterion 12) is permitted.

9.2.1.4. Screen Failure/Rescreening

If a subject is a screen failure but at some point in the future meets all of the subject eligibility criteria, the subject may be rescreened on 1 occasion only after consultation with the Sponsor. Subjects who are rescreened will be assigned a new subject number, undergo the informed consent process, and then restart a new screening phase.

9.2.2. Double-Blind Treatment Phase (Part 2)

9.2.2.1. Day 1/Day of Randomization

On the day of randomization in Part 2 prior to administration of study agent, study staff will review daily diary data, confirm inclusion/exclusion criteria, perform a brief physical exam, measure vital signs including body temperature (oral), weight measurement for dose determination, perform a 12-lead ECG (prior to infusion), record subject's Cold Symptom Assessment Score and Chest and Cold Symptom Scale, review concomitant medications, review any adverse events, collect FENO, spirometry, ACQ, and Total Nasal and Ocular Symptom Score (TNOSS) measurements. Nasal lavage and nasal brushings will be collected immediately prior to dosing for the measurement of nasal and lung inflammation. Predose (baseline) PK samples for the measurement of serum concentration of CNTO 3157 and antibodies to CNTO 3157 will be collected. Predose whole blood and serum samples for biomarker assessments will be collected.

Subjects with stable asthma will be randomized in a 1:1 ratio to receive 4 IV infusions of either placebo (30 subjects) or 1 infusion of 10 mg/kg of CNTO 3157 at Day 1 (Week 1) followed by 3 infusions of 3 mg/kg of CNTO 3157 (30 subjects) at weekly intervals through Week 4.

On Day 1 (Week 1), subjects will receive the first infusion of study agent (placebo or CNTO 3157), and a serum PK sample will be collected at 5 minutes post-infusion. Vital signs will be measured, a 12-lead ECG will be performed 4-hours post infusion, and any adverse events will be collected at 5 minutes, 2 hours, and 4 hours post-infusion.

At Study Day 1 (pre-dose), a pharmacogenomic blood sample will be collected from subjects who consent separately to the pharmacogenomic component of the study (where local regulations permit). Participation in pharmacogenomic research is optional.

9.2.2.2. Week 2 to Week 4

Subjects will return weekly for 3 additional infusions of study agent (placebo or CNTO 3157) at Week 2, Week 3, and Week 4. Assessments will be performed as detailed in the Time and Events Schedule. The study staff will review daily diary data, current medication use, and perform a brief physical exam, record subject's cold symptom self-assessments and cold and chest scales, measure vital signs including body temperature (oral), and review any adverse events. FENO, spirometry, ACQ, and nasal lavage and serum biomarkers will be assessed and should be collected just prior to the infusion. Samples to determine serum CNTO 3157 concentrations will be collected. On the day of the fourth infusion (Study Day 22 visit), the following additional assessments will be performed: TNOSS, sputum induction, whole blood biomarkers, sample for antibodies to CNTO 3157. In addition, a 12-lead ECG (triplicate tracing) will be performed 4 hours post-infusion for the fourth infusion only.

9.2.2.3. Intranasal Inoculation with HRV-16

Subjects will return 24 to 72 hours after the fourth (Week 4) infusion of study agent for intranasal inoculation with HRV-16. A brief physical exam will be performed, vital signs will be measured, and concomitant medications and any adverse events will be reviewed. A blood sample will be collected from subjects prior to inoculation with HRV-16 to determine serum neutralizing antibody titer levels to HRV-16 on the day of inoculation. Cold and Chest Symptom Scale and Cold Symptom Assessment Scores will be recorded, and a PK sample to measure serum concentrations of CNTO 3157 will be collected. Other assessments, including daily diary review, spirometry, FENO, nasal lavage, and serum biomarkers, will be performed as indicated in the Time and Events Schedule. Prior to inoculation with HRV-16, a throat swab will be collected for multiplex PCR screening for the presence of other respiratory viruses.

Per design of this study, subjects will receive a single inoculation of HRV-16 administered in a total volume of approximately 1.0 mL administered via 4 bilateral intranasal inoculations (2 instillations per naris). If at any time during the instillation of HRV-16 into a subject's nostrils, the subject experiences significant discomfort or any adverse event, further administration of the remaining portion of the HRV-16 inoculum to that subject will be discontinued, but the subject will continue to return for all subsequent study visits. Refer to Section 10.2.2 regarding stopping criteria for inoculation with HRV-16.

9.2.2.4. Post-HRV-16 Inoculation Assessment Phase

Subjects will return daily for 5 days post-inoculation with HRV-16 (D1PI through D5PI) to assess the presence and severity of chest and cold symptoms using symptom scoring systems in the context of the previous 24 hours via an interactive interview with the study staff. In addition, FENO, spirometry, pharmacokinetic, biomarker and other assessments will be performed at the timepoints as specified in the Time and Events Schedule. Subjects' responses to the Subject Cold Question (see Section 9.4.1) will be collected as specified in the Time and Events Schedule. A nasal brushing will be collected 48 hours post inoculation with HRV-16. In addition, nasal lavage samples for assessing HRV-16 titers and biomarkers (protein) will be collected daily during this period, as well as other assessments as noted in the Time and Events Schedule.

Subjects will return to the study site 7 days following inoculation with HRV-16 (D7PI) and will have nasal lavage, FENO, spirometry, daily diary review, pharmacokinetic, sputum induction, and biomarker assessments collected. The presence and severity of chest and cold symptoms using symptom scoring systems will also be determined. Serum samples for determining CNTO 3157 concentration and antibody titers to HRV-16 will be collected. Other assessments as indicated in the Time and Events Schedule will be performed.

Ten days post inoculation with HRV-16 (D10PI), subjects will return for FENO, spirometry, ACQ, nasal lavage, assessment of the severity of their chest and cold symptoms using symptom scoring systems, and other assessments as noted in the Time and Events Schedule.

9.2.3. Follow-Up Phase (Part 2)

Subjects will return for follow-up visits at Week 7 and Week 11 at which time safety and other assessments (eg, whole blood and serum biomarkers, PK samples, FENO, spirometry, daily diary data review, cold and chest symptom assessments, and ACQ) will be performed as indicated in the Time and Events Schedule.

For those subjects who have provided PG consent, a second pharmacogenomic (DNA) sample will be collected at Week 11 for possible epigenetic analysis.

Subjects will be followed for safety through Week 11 in Part 2. Any clinically significant abnormalities persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

Investigators may recontact the subject to obtain long-term follow-up information to determine the subject's safety (see Section 16.2.3, Informed Consent).

9.3. Early Termination

If a subject terminates from the study early with no plans to continue follow-up visits, an early termination visit should be scheduled as soon as possible at the time of the subject's discontinuation. At the early termination visit, all assessments scheduled for the final visit (Week 8 for the healthy subjects and Week 11 for asthmatic subjects) visit should be performed.

9.4. Efficacy

9.4.1. Evaluations

Efficacy in Part 2 will be assessed using standard assessments to evaluate asthma treatments (eg, FEV_1, ACQ).

Efficacy evaluations will include pre- and postbronchodilator spirometry (including FEV_1, etc.), collection of ACQ, daily asthma symptom diaries collected on a handheld electronic device (including the number of nocturnal awakenings, rescue medication use, impact on activities, and PEF), and assessment of nasal and ocular symptoms using TNOSS. These evaluations are widely accepted as standard endpoints for demonstrating therapeutic efficacy in terms of reduction of signs and symptoms of asthma. TNOSS will be used to evaluate the potential effect of CNTO 3157 on nasal and ocular symptoms which often co-exist in subjects with asthma.

9.4.1.1. Pulmonary Function Testing at Clinic Visits

Throughout the study, it is preferable that for any individual subject at each study visit, pulmonary function tests are administered by the same pulmonologist or technician in order to ensure consistency of technique. The equipment for respiratory assessments provided by the Sponsor must be used for this study. All personnel administering pulmonary function tests must be trained before administering the tests to any subjects in the study. All pulmonary function tests will be performed as specified in the Time and Events schedule and in accordance with the spirometry manual provided to each site.

FEV_1, forced vital capacity (FVC), peak expiratory flow (PEF), and forced expiratory flow at 25-75% of vital capacity (FEF_{25-75}) will be measured according to the Body Temperature, Pressure, Saturated standard (BTPS) convention using the spirometer provided by the Sponsor, which is suitable for research purposes, calibrated and maintained to acceptable respiratory function laboratory standards.[26] Pulmonary function tests may be repeated up to 8 times to obtain 3 acceptable, repeatable readings according to American Thoracic Society (ATS) guidelines. Acceptable repeatability is achieved when the difference between the largest and the next largest FVC is ≤0.150 L and the difference between the largest and next largest FEV1 is ≤0.150 L.

Subjects are required to have refrained from using short-acting bronchodilators (SABA) for at least a 6-hour period or long-acting bronchodilators (LABA) for at least a 12-hour period preceding each spirometry session.

To determine bronchodilator reversibility at screening, the following steps will be undertaken:

1. The subject has 3 acceptable tests of FEV_1, FVC, PEF, and FEF_{25-75}, recorded as described previously.

2. Four separate actuations (total dose 400 mg) of albuterol/salbutamol by metered dose inhaler (MDI) are delivered at approximately 30-second intervals. Use of a nonelectrostatic spacer is recommended. The breath is then held for 5 to 10 seconds before the subject exhales.

3. Three additional acceptable spirometry efforts are recorded between 10 to 15 minutes later.

4. If the patient does not achieve ≥12% and ≥200 mL improvement from the prebronchodilator FEV_1 value, then the patient will be given 2 additional actuations of albuterol/salbutamol by MDI and spirometry will be repeated 10 to 15 minutes later.

5. Step 4 may be repeated 1 time (for a total of 8 actuations of albuterol/salbutamol) if the acute bronchodilator reversibility inclusion criterion (>12% and >200 mL) was not achieved after 6 actuations.[39]

Bronchodilator reversibility will be calculated at screening with the formula below:

[(highest post - albuterol FEV_1 - highest pre - albuterol FEV_1)/highest pre - albuterol FEV_1]*100

For all subsequent determinations of bronchodilator reversibility, the subject will be given the same number of actuations of β2 agonist that were given at the Screening Visit 2.

9.4.1.2. Fractional Exhaled Nitric Oxide (FENO)

Fractional exhaled nitric oxide will be measured for all asthmatic subjects at an exhalation flow rate of 50 mL/s according to ATS guidelines using an exhaled nitric oxide (NO) meter that is approved for asthma measurements. FENO testing must precede any other pulmonary procedures. Two replicate FENO measurements will be obtained that agree at the 10% level and up to a total of 8 measurements will be performed to achieve this level of agreement.

9.4.1.3. Asthma Control Questionnaire (ACQ)

Asthmatic subjects will be asked to complete the Asthma Control Questionnaire, an instrument designed to evaluate asthma control defined as "the full range of clinical impairment that patients with asthma may experience as a result of the disease".[20] The control options cover a continuum from 'well controlled' to 'life threatening'. In addition, the subject's percent predicted FEV_1 value is scored. All 7 items are scored on a 7-point scale (0=good control, 6=poor control) with the mean score as an overall summary score. Higher scores reflect poorer control. The recall period is 7 days. The questionnaire will be provided in an electronic format and completed during the site visits as noted in the Time and Events Schedule.

9.4.1.4. Electronic Data Collection Devices

An electronic peak flow meter and a handheld electronic device (eDiary) will be issued to each subject at Screening Visit 2 and will be used to record the following data twice daily for the duration of the study:

- Night-time awakenings and symptom severity of coughing, wheezing, breathlessness, chest tightness, and activity limitation.
- Use of inhaled rescue medication (expressed as the number of occasions, not puffs, of rescue medication use for control of symptoms and not for prophylaxis [e.g. exercise]).
- Morning and evening PEF measurements.

At each clinic visit, study staff, investigator, or designee must review the electronic device data with the subject for completeness and consistency with subject-reported AEs. Subject- and investigator-completed scores and assessments designated by the sponsor that will be recorded directly into an electronic device will be considered source data.

Peak expiratory flow rates are to be measured twice daily. Subjects should attempt to collect these readings before any use of inhaled rescue medication in the morning and at least 4 hours following any use of inhaled rescue medication in the evening. In addition, subjects should attempt to collect these morning and evening readings before any use of LABA. It is important that the subject measure PEF twice daily even if he or she does not refrain from the use of inhaled SABA or LABA as suggested above. The subject will receive appropriate training on the use of the handheld electronic device and electronic peak flow meter at Screening Visit 2.

9.4.1.5. Total Nasal and Ocular Symptom Score (TNOSS)

Total Nasal and Ocular Symptom Score (TNOSS) is the assessment of 4 individual nasal symptom (nasal congestion, nasal itching, rhinorrhea, and sneezing) and 3 individual ocular symptoms (eye itching/burning, eye tearing/watering, and eye redness) and will be used in Part 2 only (asthmatic subjects). Each symptom severity is rated by the subject using a 5-point categorical response scale from 0 to 4 where 0=absent/no symptom, 1=mild symptoms 2=moderate symptoms, 3=severe symptoms, and 4=extremely severe symptoms, with a 24-hour recall period. The TNOSS will be presented to the asthma subjects in an electronic format on the handheld electronic device and completed on Day 1 and Day 22 of the study.

9.4.1.6. Subject Cold Question

On Study Days 3-7 and 9 (Part 1) and Study Days 24-28 and 30 (Part 2), subjects will be asked *"Did you develop a cold after you were inoculated and/or do you currently have a cold?"*

9.4.1.7. Cold Symptom Assessment Score

Clinical symptoms to HRV-16 will be assessed by using the Cold Symptom Assessment Score form (Attachment 4). Subjects report the presence and severity of their cold symptoms in the context of the previous 24 hours via an interactive interview with the study staff. The symptoms consist of nasal congestion (stuffy nose), rhinorrhea (runny nose), sore throat, sneezing, cough, headache, malaise (feeling run down, tired) and chilliness. Severity is rated using a 5 point scale (0=none, 1=mild, 2=moderate, 3=severe, and 4=very severe). Scores can range from 0-32, with higher scores indicating more symptoms.

9.4.1.8. Cold and Chest Symptom Scale

Clinical (chest) symptoms of HRV-16 will be assessed by using the Cold and Chest Symptom Scale form (Attachment 5). Subjects report the presence and severity of their chest symptoms in the context of the previous 24 hours via an interactive interview with the study staff.

The Cold and Chest Symptom Scale contains 15 items composing 2 domains, a total cold score and a total chest score. Together, these 2 domains comprise the chest symptom assessment score.

- **Total Cold Score domain:** sneezing, runny nose, blocked or stuff nose, sore throat or hoarse voice, headache or face pain, generally unwell, chills, fever or shivery and cough **and**
- **Total Chest Score domain:** cough on waking, wheeze on waking, daytime cough, daytime wheeze, daytime chest tightness, daytime breathlessness and nocturnal cough, wheeze and breathlessness.

Severity is rated using a 5 point scale: 0 using a 5 point scale (0=none, 1=mild, 2=moderate, 3=severe, and 4=very severe). Scores can range from 0-60, with higher scores indicating more symptoms.

9.4.2. Endpoints

9.4.2.1. Part 1

The endpoints for Part 1 are:

- AUC of change from baseline in Cold Symptom Assessment Score, Cold and Chest Symptom Scale, and log-transformed FENO from the day of HRV-16 inoculation through 10 days following HRV-16 inoculation.
- Change from baseline in Cold Symptom Assessment Score, Cold and Chest Symptom Scale, and log-transformed FENO over time.
- Change from baseline over time in FEV_1, percent-predicted FEV_1, FVC, FEV_{25-75}, and FEV_1/FVC

9.4.2.2. Part 2

Primary Endpoint

The primary endpoint is the maximum percent decrease relative to baseline in the pre bronchodilator FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16. The baseline is defined as the average of all prebronchodilator FEV_1 prior to study agent administration.

Major Secondary Endpoints

The major secondary endpoints in Part 2 of the study are:

- AUC of the percent change from baseline in clinic assessed prebronchodilator FEV_1 through 10 days following HRV-16 inoculation
- Change from baseline in ACQ reported approximately 10 days following inoculation with HRV-16 (D10PI)
- AUC of the change from baseline in Cold Symptom Assessment Scores from the day of HRV-16 inoculation through 10 days following HRV-16 inoculation
- AUC of the change from baseline in Cold and Chest Symptom Scale from the day of HRV-16 inoculation through 10 days following HRV-16 inoculation
- AUC of change from baseline in morning (AM) PEFR through 10 days following inoculation with HRV-16

Other Secondary Endpoints

To further evaluate the treatment effect of CNTO 3157 in the asthmatic population, analyses are planned for the following other secondary efficacy endpoints:

Pulmonary function tests:

- The maximum decrease from baseline in the prebronchodilator FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16
- AUC of the change from baseline in clinic assessed prebronchodilator FEV_1, percent-predicted FEV_1, FVC, FEV_{25-75}, and FEV_1/FVC through 10 days following inoculation with HRV-16
- Change from baseline over time in prebronchodilator and postbronchodilator parameters: percent-predicted FEV_1, FEV_1, FVC, FEV_{25-75}, and FEV_1/FVC
- Change from baseline in morning/evening PEFR over time

Symptoms and other measures:

- AUC of change from baseline in average total asthma symptom diary score through 10 days following inoculation with HRV-16
- Change from baseline in TNOSS
- Change from baseline in Cold Symptom Assessment Score, Cold and Chest Symptom Scale, average total asthma symptom diary score, average number of nocturnal awaking, and average rescue medication use over time
- Number of symptom-free days through 10 days following inoculation with HRV-16
- Time to the maximum decrease relative to baseline in prebronchodilator FEV_1 assessed 10 days following HRV-16 inoculation
- Change from baseline in log-transformed FENO over time

Exploratory Endpoints

To account for the potential separate effects of study agent and viral inoculation upon efficacy assessments (eg, pre-bronchodilator FEV_1), exploratory endpoints defined as changes and/or percent changes relative to the assessment immediately prior to the HRV-16 inoculation will be explored.

9.5. Pharmacokinetics and Immunogenicity

9.5.1. Evaluations

Venous blood samples of 5 mL will be collected for determination of serum concentrations of CNTO 3157 and the immunogenicity of CNTO 3157 as specified in the Time and Events Schedule.

Samples will be used to evaluate the pharmacokinetics, as well as the immunogenicity of CNTO 3157 (antibodies to CNTO 3157). Samples collected for analyses of CNTO 3157 serum

concentration and antibody to CNTO 3157 may additionally be used to evaluate safety or efficacy aspects that address concerns arising during or after the study period, for further characterization of immunogenicity or for the evaluation of relevant biomarkers. Genetic analyses will not be performed on these serum samples. Subject confidentiality will be maintained. At visits where serum concentration and antibodies to CNTO 3157 will be evaluated, 1 blood draw of sufficient volume can be used. Venous blood samples will be collected and each serum sample will be divided into 3 aliquots (1 each for pharmacokinetics, antibodies to study agent, and a back-up).

9.5.2. Analytical Procedures

Pharmacokinetics

Serum samples will be analyzed to determine concentrations of CNTO 3157 using a validated, specific, and sensitive [eg, ELISA, MSD] method by or under the supervision of the sponsor.

Immunogenicity

The detection and characterization of antibodies to CNTO 3157 will be performed using a validated assay method by or under the supervision of the sponsor. All samples collected for detection of antibodies to CNTO 3157 will also be evaluated for CNTO 3157 serum concentration to enable interpretation of the antibody data.

9.5.3. Pharmacokinetic Parameters

Serum concentration of CNTO 3157 will be analyzed for all subjects with all available samples. The serum concentration-time data of CNTO 3157 will be analyzed for all evaluable subjects for PK parameters. All calculations will be based on actual sampling times. PK parameters and concentrations will be summarized by treatment group among PK evaluable subjects.

9.5.3.1. Part 1

Individual subject serum concentration-time data of CNTO 3157 will be analyzed for all subjects in Part 1 with all available samples. PK parameters to be calculated will include, but are not limited to AUC_t, AUC_{inf}, C_{max}, V_z, CL, and $T_{1/2}$ following the single dose administration.

9.5.3.2. Part 2

Individual subject serum concentration-time data of CNTO 3157 will be analyzed for all subjects in Part 2 with all available samples. PK parameters to be calculated will include, but are not limited to $AUC_{(t1-t2)}$, $C_{max,n}$, $C_{min,n}$, and $T_{1/2}$ following multiple dose administration.

9.5.4. Antibodies to CNTO 3157

Serum samples will be screened for antibodies binding to CNTO 3157 and the titer of confirmed positive samples will be reported. Other analyses may be performed to verify the stability of antibodies to CNTO 3157 and/or further characterize the immunogenicity of CNTO 3157. Antibodies to CNTO 3157 will be evaluated on blood drawn from all subjects according to the Time and Events schedule. Additionally, samples should also be collected at the final visit for

subjects who withdraw from the study and from subjects who experience an adverse event suspected to be related to immunogenicity (eg, infusion reactions, injection site reactions or hypersensitivity). These samples will be tested by the sponsor or sponsor's designee.

9.6. Pharmacokinetic/Pharmacodynamic Evaluations

PK/PD analysis may be conducted to evaluate the relationship between exposure to CNTO 3157 and appropriate efficacy/safety outcomes (eg, change in FEV_1, ACQ).

9.7. Biomarkers

Whole blood, serum, nasal lavage and nasal brushing (Part 2 only) samples for biomarker analyses will be collected according to the Time and Events Schedule. All nasal lavage samples will be evaluated for the presence/absence of HRV-16, HRV-16 titers, and other biomarkers which may include, but are not limited to, IL-6, CXCL8/IL-8, and CXCL10/IP-10. RNA from whole blood will be analyzed for transcriptome profiling. In addition, FENO will be measured as detailed in Section 9.4.1.2 at times indicated in the Time and Events Schedule.

Biomarker results will be presented in a separate report.

9.7.1. Nasal Lavage and Brushings

Nasal lavage samples will be collected by instillation of 5 mL of 0.9% saline into each nostril. The wash will be immediately expelled into a waxed paper cup and kept chilled or frozen at -70°C until processed. All nasal lavage samples will be evaluated for the presence/absence of HRV-16, HRV-16 titers, and other exploratory biomarkers.

In Part 2, RNA will be extracted from nasal epithelial cells collected by brushing the inferior turbinate with a standard cytology brush after administration of a local anesthetic.

9.8. Pharmacogenomic (DNA) Evaluations

Complete or selected genomic testing will be done to search for links of specific genes to disease or response to drug. Only DNA research related to CNTO 3157 or to the diseases for which this drug is developed will be performed. A list of candidate genes that are potentially relevant to CNTO 3157 or asthma is provided in Attachment 3. This list may be updated at any time as hypotheses evolve. Genome wide pharmacogenomics testing may be undertaken in this study in consenting subjects. Further, a subject may withdraw such consent at any time without affecting their participation in other aspects of the study, or their future participation in the study.

Pharmacogenomic research allows for the storage of DNA samples for future genetic research related to CNTO 3157 or the indication(s) for which it is developed. Details of long-term storage of samples are provided in Section 16.2.5. Stored DNA samples and relevant clinical data will be de-identified after the Clinical Study Report has been issued. This involves removing personal identifiers and replacing the study subject identifier with a new number to limit the possibility of linking genetic data to a subject's identity.

9.9. Safety Evaluations

Details regarding the Independent Data Monitoring Committee are provided in Section 11.11.

Any clinically significant abnormalities persisting at the end of the study/early discontinuation will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

The study will include the following evaluations of safety and tolerability according to the timepoints provided in the Time and Events Schedule:

Adverse Events

Adverse events will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally-acceptable representative) for the duration of the study. Adverse events will be followed by the investigator as specified in Section 12, Adverse Event Reporting.

Clinical Laboratory Tests

Blood samples for serum chemistry and hematology will be collected. The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the adverse event section of the CRF. The following tests will be performed by the central laboratory or at the investigational site as specified:

- Hematology Panel

  -hemoglobin
  -hematocrit
  -red blood cell (RBC) count
  -white blood cell (WBC) count with differential
  -platelet count

- Serum Chemistry Panel

  -sodium
  -potassium
  -chloride
  -bicarbonate
  -blood urea nitrogen (BUN)
  -creatinine
  -glucose
  -aspartate aminotransferase (AST)
  -alanine aminotransferase (ALT)
  -gamma-glutamyltransferase (GGT)
  -total and direct bilirubin
  -alkaline phosphatase
  -creatine phosphokinase (CPK)
  -lactic acid dehydrogenase (LDH)
  -uric acid
  -calcium
  - phosphate
  -albumin
  -total protein

- Urinalysis

  Dipstick (at site)
  -specific gravity
  -pH
  [Sediment (if dipstick result is abnormal)]
  -red blood cells

-glucose
-protein
-blood
-ketones
-bilirubin
-urobilinogen
-nitrite
-leukocyte esterase
-white blood cells
-epithelial cells
-crystals
-casts
-bacteria

If dipstick result is abnormal, flow cytometry will be used to measure sediment. In case of discordance between the dipstick results and the flow cytometric results, the sediment will be examined microscopically at a central laboratory.

Additional Laboratory Tests

See Time and Events Schedules for details on timing of these tests:

- Serum β-human chorionic gonadotropin (β-hCG) pregnancy and Urine Pregnancy Testing for women of childbearing potential only
- Serology (hepatitis B surface antigen [HBsAg], hepatitis B surface antibody [HBsAb], hepatitis B core antibody [HBcAb], human immunodeficiency virus [HIV], and hepatitis C virus antibody)
- Urine albumin, protein, and creatinine
- Urine cotinine
- Urine toxicology screen for substances of abuse, including, but not limited to cocaine, cannabinoids, amphetamines, benzodiazepines, barbiturates, opiates, tricyclic antidepressants, and methadone.
- -prothrombin time (PT)
- -partial thromboplastin time (PTT)

Electrocardiogram (ECG)

During the collection of ECGs, subjects should be in a quiet setting without distractions (eg, television, cell phones). Subjects should rest in a supine position for at least 5 minutes before ECG collection and should refrain from talking or moving arms or legs. If blood sampling or vital sign measurement is scheduled for the same timepoint as ECG recording, the procedures should be performed in the following order: ECG(s), vital signs, and blood draw.

In Part 1, an ECG will be performed at Screening Visit 2, Study Day 1, and 4 hours after administration of study agent. In Part 2, an ECG will be performed at Screening Visit 2, Study Day 1, and 4 hours after the first administration of study agent (Week 1), and in triplicate 4 hours after the last administration of study agent (Week 4). Where triplicate ECGs are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart. The full set of triplicates should be completed in less than 4 minutes.

Twelve-lead ECGs will be recorded at a paper speed of 25 mm per second for at least 10 seconds until 4 regular consecutive complexes are available. Computer-generated interpretations of ECGs should be reviewed for data integrity and reasonableness by the investigator.

Vital Signs (body temperature (oral), pulse/heart rate, respiratory rate, blood pressure)

Blood pressure and heart rate measurements should be preceded by at least 5 minutes of rest in a supine position in a quiet setting without distractions (eg, television, cell phones).

Respiratory rate will be measured over at least 30 seconds. Oral temperature will be measured using a method in accordance with the site's standard operating procedure.

Physical and Nasal Examination

A complete physical examination including body weight and height will be conducted at Screening Visit 2. A brief physical examination (cardiac and pulmonary assessment) will be conducted at times indicated in the Time and Events Schedule. A nasal examination for abnormal nasal anatomy or mucosa will be performed by visual inspection at Screening Visit 2.

Any clinically significant abnormalities identified during the study and persisting at the end of the study will be followed by the investigator until resolution or until reaching a clinically stable endpoint.

9.10. Sample Collection and Handling

The actual dates and times of sample collection must be recorded in the CRF or laboratory requisition form.

Refer to the Time and Events Schedule for the timing and frequency of all sample collections.

Instructions for the collection, handling, and shipment of samples are found in the laboratory manual that will be provided for sample collection and handling.

10. SUBJECT COMPLETION/WITHDRAWAL

10.1. Completion

10.1.1. Part 1

A subject will be considered to have completed Part 1 of the study if he or she has completed the Week 8 visit in Part 1. Subjects who prematurely discontinue study treatment for any reason before completion will not be considered to have completed the study.

10.1.2. Part 2

A subject will be considered to have completed Part 2 of the study if he or she has completed the Week 11 visit in Part 2. Subjects who prematurely discontinue study treatment for any reason before completion will not be considered to have completed the study.

10.2. Discontinuation of Study Agent and/or HRV-16 Inoculation

10.2.1. Subject Stopping Criteria – Study Agent

Administration of placebo or CNTO 3157 to a subject should be discontinued if:

- A subject in Part 2 has an asthma exacerbation requiring treatment with systemic steroids or institution of a prohibited medication
- A severe infusion reaction with CNTO 3157 occurs
- A subject takes any restricted medications as per Section 8.1 or Section 8.2
- A female subject becomes pregnant
- A significant other medical illness emerges that places the subject at risk from further participation
- A subject withdraws consent
- The Sponsor decides that further participation may jeopardize the subject's safety or the conduct of the study

10.2.2. Subject Stopping Criteria - HRV-16 Inoculation

Per design of this study, subjects in Part 1 and Part 2 will receive a single administration of HRV-16. If during the instillation of HRV-16 into a subject's nostrils, a subject experiences significant discomfort or any adverse event, further administration of the remaining portion of the HRV-16 inoculum to that subject will be discontinued.

Subjects will be ineligible for the scheduled inoculation with HRV-16 if any of the following occur:

- A complete dose of study agent was not given in Part 1 for any reason
- The first and fourth dose of study agent was not given in Part 2 for any reason. If a subject misses the second or third scheduled dose of study agent, the case must be discussed with the sponsor's medical monitor to determine if inoculation should occur.
- A significant other medical illness emerges that places the subject at risk from further participation
- For Part 2 of the study, prebronchodilator FEV_1 performed before inoculation has fallen by ≥20% absolute compared to baseline FEV_1 (average of FEV_1 at Screening Visit 2 and baseline)
- A subject in Part 2 has an asthma exacerbation requiring treatment with systemic steroids or institution of a prohibited medication
- The Sponsor decides that further participation may jeopardize the subject's safety or the conduct of the study
- A female subject becomes pregnant
- The subject withdraws consent

All subjects discontinuing from administration of study agent and/or HRV-16 inoculation will be encouraged to return for all scheduled visits for follow-up.

- If a subject in Part 1 has received the single infusion of study agent (placebo or CNTO 3157), but discontinues prior to being inoculated with HRV-16, they should be encouraged to return for the Week 4 and Week 8 follow-up visits. If the subject refuses, they should be encouraged to return for a final follow-up visit (performing the Week 8 assessments).
- If a subject in Part 2 has received any study agent (placebo or CNTO 3157), but discontinues prior to being inoculated with HRV-16, they should be encouraged to return for the Week 7 and Week 11 follow-up visits. If the subject refuses, they should be encouraged to return for a final follow-up visit (performing the Week 11 assessments).

10.3. Withdrawal From the Study

A subject will be withdrawn from the study for any of the following reasons:

- Lost to follow-up
- Withdrawal of consent
- Death

If a subject is lost to follow-up, every reasonable effort must be made by the study site personnel to contact the subject and determine the reason for discontinuation/withdrawal. The measures taken to follow up must be documented.

When a subject withdraws before completing the study, the reason for withdrawal is to be documented in the CRF and in the source document. Study agent assigned to the withdrawn subject may not be assigned to another subject.

Subjects who withdraw at or prior to D10PI may be replaced. Subjects who withdraw after D10PI will not be replaced.

A subject who withdraws from the study will have the following options regarding pharmacogenomic research:

- The DNA extracted from the subject's blood will be retained and used in accordance with the subject's original pharmacogenomic informed consent.
- The subject may withdraw consent for pharmacogenomic research, in which case the DNA sample will be destroyed and no further testing will take place. To initiate the sample destruction process, the investigator must notify the Sponsor site contact to request sample destruction. The Sponsor site contact will, in turn, contact the biomarker/pharmacogenomics representatives to execute sample destruction. If requested, the investigator will receive written confirmation from the Sponsor that the sample has been destroyed.

Withdrawal From Pharmacogenomic Research Only

The subject may withdraw consent for pharmacogenomic research while remaining in the clinical study. In such a case, any DNA extracted from the subject's blood will be destroyed. The

sample destruction process will proceed as described above. However, all samples will be made nonidentifiable after the Clinical Study Report is issued and thereafter cannot be identified for destruction. If the sample has already undergone conversion to the nonidentifiable format, the Sponsor will notify the investigator in writing.

Withdrawal From PK or Immunogenicity Research

Consent for future use of PK or immunogenicity samples is specified separately in the main informed consent form. These samples may be used in method development or to better understand analytical processes in the underlying disease of a specific subject population. No pharmacogenomic testing will be conducted on these samples.

The PK or immunogenicity samples may be stored long-term (see Section 16.2.5, Long-Term Storage of Samples for Future Research). The long-term storage allows use of these samples for research to improve or better understand bioanalytical methods and processes. Subjects who refuse to give consent for this component will not be excluded from participation in the clinical study. These samples will remain coded without subject-specific identification throughout the sample storage and analysis process.

10.4. Suspension/Termination of Study Treatments

10.4.1. Part 1

If upon review of the blinded safety data in Part 1, a significant safety signal is detected, the Sponsor may suspend dosing of study agent and intranasal inoculation with HRV-16 of any other subjects in Part 1 and will not proceed to Part 2 until such signal is reviewed by the DMC and a recommendation is received. Subjects who have received study agent will continue to be followed through Week 8.

If no safety concerns are identified out to 10 days post dose in Part 1 of the study in healthy adults, Part 2 of the study in asthmatic subjects will be initiated.

10.4.2. Part 2

If upon review of the blinded safety data in Part 2, a significant safety signal is detected, the Sponsor may suspend dosing of study agent and intranasal inoculation with HRV-16 of any other subjects in Part 2 until such signal is reviewed by the DMC and a recommendation is received. Subjects who have received study agent will continue to be followed through Week 11.

10.5. Study Interruption/Stopping Criteria

Details on study termination are provided in Section 17.9.

11. STATISTICAL METHODS

11.1. Statistical Design

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the efficacy and safety data is outlined below. Specific details will be provided in the Statistical Analysis Plan (SAP).

This is a 2-part, randomized, multi-center, double-blind, parallel-design, placebo-controlled, HRV-16 challenge study. Part 1 is designed to assess the safety and tolerability of CNTO 3157 compared with placebo in healthy adult subjects inoculated with HRV-16, and Part 2 is designed to determine if treatment with CNTO 3157 can attenuate the respiratory manifestations (nasal and chest consequences) of a viral infection induced by intranasal inoculation with HRV-16 in asthmatic subjects.

In Part 1 using permuted block randomization, approximately 12 subjects will be randomized in a 1:2 ratio to 1 of the following 2 treatment groups:

- Group 1 (n=4): Placebo IV infusion at Week 1
- Group 2 (n=8): CNTO 3157 10 mg/kg IV infusion at Week 1

Within 24 ±8 hours after the infusion of study agent, subjects will receive a single intranasal inoculation with HRV-16, and be followed for 10 days to assess clinical symptoms and HRV-16 associated inflammation, PK, and biomarkers.

In Part 2 using permuted block randomization, approximately 60 subjects will be randomized in a 1:1 ratio to 1 of the following 2 treatment groups:

- Group 1 (n=30): Placebo IV infusion at Week 1, Week 2, Week 3, Week 4
- Group 2 (n=30): CNTO 3157 10 mg/kg IV infusion at Week 1
  CNTO 3157 3 mg/kg IV infusion at Week 2, Week 3, Week 4

Within 24 to 72 hours after the fourth (last) infusion of study agent, subjects will receive a single intranasal inoculation with HRV-16, and be followed for 10 days to assess clinical symptoms and HRV-16 associated inflammation, PK, and biomarkers.

Enrolled subjects will be followed through Week 8 in Part 1 and Week 11 in Part 2 for safety unless study participation is discontinued early and the subject withdraws consent for further participation in the study. Otherwise, subjects should be followed for the duration of the study irrespective of the study agent received.

An interim data lock for Part 1 of this study may be conducted when Part 1 is complete for decision-making purposes. The data from Part 1 and Part 2 of this study will be cleaned and locked for analysis at the Week 11 DBL in Part 2. Details for this are presented in the Statistical Analysis Plan (SAP).

The primary endpoint is the maximum percent decrease relative to baseline in the prebronchodilator FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16. The baseline is defined as the average of all prebronchodilator FEV_1 prior to study agent administration. The analyses of the primary endpoint will be conducted following Week 11 DBL. Details of the analyses after the DBL will be described in the SAP.

At the Week 11 DBL, subject level unblinded data will be made available to the Sponsor. Subject level data will be unblinded to the Sponsor's data management, programming, pharmacology, clinical and biostatistics teams for analysis and reporting, while the subjects are still being followed in the study. Investigators, study site personnel, and the subjects will remain blinded until data through Week 11 in Part 2 have been locked.

In both Part 1 and Part 2, randomization will be performed by IVRS/IWRS using a permuted block method. There is no stratification in the randomization.

An independent DMC will monitor the safety of the study in unblinded fashion on a regular basis and whenever deemed necessary. More details are provided in Section 11.11.

11.2. Hypothesis

11.2.1. Part 1

There is no hypothesis for Part 1 of the study.

11.2.2. Part 2

The hypothesis is that CNTO 3157 is superior to placebo in attenuating the respiratory manifestations of HRV-16 inoculation as measured by the maximum percent decrease relative to baseline in prebronchodilator FEV_1 assessed over 10 days following HRV-16 inoculation of asthmatic subjects.

11.3. Primary Analysis

11.3.1. Part 1

There is no primary efficacy analysis in Part 1.

11.3.2. Part 2

The primary endpoint is the maximum percent decrease relative to baseline in prebronchodilator FEV_1 assessed at each treatment visit through Day 10 post-inoculation [D10PI] with HRV-16). The primary analysis will be based on the primary endpoint and will be conducted on the modified intent-to-treat (mITT) population, which includes all randomized subjects who receive at least 1 dose of study agent, have at least 1 measurement prior to study agent infusion, are inoculated with HRV-16, and have at least one post-inoculation prebronchodilator FEV_1 measurement.

Since all subjects to be included in the primary analysis have at least one post-inoculation measurement; therefore, no subject will have a missing primary endpoint assessment. Subjects

who have 1 or more of the following events through 10 days after inoculation with HRV-16 will be defined as treatment failures:

- Exacerbation (see Statistical Analysis Plan [SAP])
- Use of prohibited meds (refer to Section 8)

The measurement of the primary endpoint for subjects who meet any treatment failure rules will be replaced with the highest maximum percent decrease from baseline in prebronchodilator percent predicted FEV_1 of those who don't meet any treatment failure rules, ie, the worst value will be used for treatment failures in primary efficacy analysis.

An analysis of covariance (ANCOVA) model with treatment group as a fixed factor and baseline prebronchodilator FEV_1 as a covariate will be used in the primary efficacy analysis. The baseline value is defined as the average of all prebronchodilator FEV_1 prior to study agent administration. If significant non-normality is observed, appropriate non-parametric tests will be used to evaluate the differences between treatments.

Part 2 and hence the study will be considered positive if the primary analysis achieves statistical significance at a significance level of 0.1 (2 sided) and CNTO 3157 shows a better treatment effect than placebo.

In addition to the primary analysis, the following sensitivity analyses will also be performed:

- Sensitivity analysis 1: this analysis is similar to the primary analysis, but only includes subjects with all scheduled pre bronchodilator FEV_1 measurements through 10 days following inoculation with HRV-16.
- Sensitivity analysis 2: to directly compare the treatment effect among those who are infected after inoculation with HRV-16, a similar analysis will be conducted on the subgroup of the infected population. Subjects will be considered to have been infected with HRV-16 if HRV-16 is determined to be present in at least 1 of their nasal lavage samples collected on D1PI through D5PI **OR** if there is at least a 4-fold increase in the titer of serum neutralizing antibody to HRV-16 in their blood samples from the day prior to inoculation with HRV-16 to the Week 8 visit in Part 1 or the Week 11 visit in Part 2.

If deemed necessary, the primary endpoint will be analyzed on the per-protocol population which includes more compliant subjects. Details of the inclusion/exclusion rules will be provided in the SAP. In the per-protocol analysis, subjects will be analyzed based on their actual treatment group.

11.4. Sample Size Determination

11.4.1. Part 1

The sample size of 12 subjects in Part 1 is not based on statistical considerations.

11.4.2. Part 2

The sample size calculation is based on the primary endpoint, the maximum percent decrease relative to baseline in the prebronchodilator FEV_1 measurements assessed at each visit through 10 days following inoculation with HRV-16. To have 80% power to detect a clinically significant relative reduction of 50% (from 13% 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) are required. The assumption of a 13% reduction for placebo and the 10% SD are based upon a previous study in which a similar asthmatic population was inoculated with HRV-16 (Johnston et al, personal communication). In that study, the mean maximum percent decrease relative to baseline in prebronchodilator percent predicted FEV1 was 12.83% (n=9, SD=9.71%). Due to the limited data from the reference study, the power to detect a significant treatment difference at α=0.1 (2-sided) is calculated under various assumptions with n=30 per arm (see Table 3). The significance level of 0.1 was chosen as this is an early phase, proof of concept study.

Table 3: Power to detect a significant treatment difference in maximum percent decrease from baseline in prebronchodilator FEV_1

Mean[1] of Placebo Group (%)	Relative Reduction[2] of CNTO3157	Absolute Reduction[3] (Delta, %)	Mean[1] of CNTO3157 Group (%)	Power
15	50%	7.5	7.5	89%
	60%	9	6	96%
13	50%	6.5	6.5	80%
	60%	7.8	5.2	91%
12	50%	6	6	74%
	60%	7.2	4.8	87%
11	50%	5.5	5.5	68%
	60%	6.6	4.4	81%
10	50%	5	5	61%
	60%	6	4	74%

1. Mean maximum percent decrease from baseline in prebronchodilator FEV_1.
2. Relative reduction (% relative to the mean of placebo) in maximum percent decrease from baseline in prebronchodilator FEV_1.
3. Absolute reduction = treatment difference

11.5. General Statistical Methods

Demographic and baseline disease characteristic data will be summarized by treatment group in each study part. Descriptive statistics (eg, mean, median, standard deviation, interquartile range, minimum, and maximum) will be used to summarize continuous variables. Counts and percentages will be used to summarize categorical variables. Graphic data displays (eg, box plots) may also be used to present data.

Categorical data will be analyzed using chi-square test, CMH chi-square test, or logistic regression. Continuous responses will be analyzed using the same statistical method as in the primary efficacy analysis. Nonparametric methods will be adopted when the normality assumption is violated. Survival analysis techniques will be used for endpoints defined by time to an event. Log-rank test will be used to compare endpoints defined by time to an event.

For efficacy analysis, data will be analyzed according to the assigned treatment group. Unless otherwise stated, efficacy analyses in Part 1 and Part 2 will be based on a mITT population.

Safety, PK, and PD analyses in Part 1 and Part 2 will include all subjects treated with study agent and will be summarized based on the actual treatment received. Some safety, PK, and PD analyses may also be performed on the population inoculated with HRV-16.

11.6. Pharmacokinetic and Immunogenicity of CNTO 3157 Analysis

Individual subject serum concentration-time data of CNTO 3157 will be analyzed for all subjects with all available samples. All calculations will be based on actual sampling times.

In Part 1, PK parameters to be calculated will include, but are not limited to AUCt, AUCinf, Cmax, Vz, CL and T1/2 following the single dose administration.

In Part 2, PK parameters to be calculated will include, but are not limited to AUC(t1-t2), Cmax,n, Cmin,n, and T1/2 following multiple dose administration.

11.7. Biomarkers Analysis

Changes in the concentration of individual biomarkers from baseline to the selected posttreatment timepoints will be summarized. Associations between baseline levels and changes from baseline in select biomarkers and clinical response will be explored. The biomarker analysis will characterize the response of subjects to CNTO 3157 to determine if response to CNTO 3157 can be predicted and to further our understanding of asthma. Although biomarker analyses will be summarized in separate technical reports, for certain analyses, biomarker data (eg, FENO, sputum cell counts) may be used for the analysis of certain endpoints.

11.8. Pharmacogenomic Analyses

Results of pharmacogenomic analyses will be presented in a separate report.

11.9. Medical Resource Utilization

Medical resource utilization will be descriptively summarized by treatment group.

11.10. Safety Analyses

Safety data, including but not limited to, AEs, SAEs, spirometry data, infections, serious infections, mortality, changes in laboratory assessments, changes in vital signs, and incidence of formation of antibodies to CNTO 3157 will be summarized. Treatment-emergent AEs will be summarized by treatment group and MedDRA system organ class and preferred terms. Details will be specified in the SAP.

Adverse Events

The verbatim terms used in the CRF by investigators to identify adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported adverse events with onset during the treatment phase (ie, treatment-emergent adverse events, and adverse events that have worsened since baseline) will be included in the analysis. For each adverse event, the

percentage of subjects who experience at least 1 occurrence of the given event will be summarized by treatment group.

Summaries, listings, datasets, or subject narratives may be provided, as appropriate, for those subjects who die, who discontinue treatment due to an adverse event, or who experience a severe or a serious adverse event.

Clinical Laboratory Tests

Laboratory data will be summarized by type of laboratory test. Reference ranges and markedly abnormal results (specified in the Statistical Analysis Plan) will be used in the summary of laboratory data. Descriptive statistics will be calculated for each laboratory analyte at baseline and at each scheduled time point. Changes from baseline results will be presented in pre- versus posttreatment cross-tabulations (with classes for below, within, and above normal ranges). A listing of subjects with any laboratory results outside the reference ranges will be provided. A listing of subjects with any markedly abnormal laboratory results will also be provided.

Electrocardiogram (ECG)

The effects on cardiovascular variables will be evaluated by means of descriptive statistics and frequency tabulations. These tables will include observed values and changes from baseline values (the predose [Day 1] ECG will be used as baseline) to allow detection of clinically relevant changes in individuals.

Descriptive statistics of QTc intervals and changes from baseline will be summarized. The ECG variables that will be analyzed are heart rate, PR interval, QRS interval, QT interval, and corrected QT (QTc) interval using the following correction methods: QT corrected according to Bazett's formula (QTcB), QT corrected according to Fridericia's formula (QTcF), and QT correction derived by linear regression (QTcL).

Descriptive statistics of QTc intervals and changes from baseline will be summarized at each scheduled timepoint. The percentage of subjects with QTc interval >450 milliseconds, >480 milliseconds, or >500 milliseconds will be summarized, as will the percentage of subjects with QTc interval increases from baseline >30 milliseconds or >60 milliseconds.

All important abnormalities in ECG waveform that are changes from the baseline readings will be reported (eg, changes in T-wave morphology or the occurrence of U-waves).

Vital Signs

Vital signs (eg, systolic blood pressure, diastolic blood pressure) will be summarized by treatment group. Markedly abnormal criteria (to be specified in the Statistical Analysis Plan) will be used to identify markedly abnormal vital signs, which will be summarized by treatment group. A listing of subjects with any markedly abnormal vital signs will also be provided.

11.11. Data Monitoring Committee

An independent DMC will be established to monitor data on an ongoing basis to ensure the continuing safety of the subjects enrolled in this study. The DMC will consist of 3 to 6 members, including at least one medical expert in asthma and at least one statistician. None of the members will be participating as investigators in the current study. The members of the committee will be specified prior to study initiation. A DMC charter will be completed at an organizational meeting of the DMC prior to the initial review of safety data.

The DMC will review unblinded safety data after all 12 subjects have completed their Day 10 post-inoculation visit in Part 1 and periodically thereafter to review interim unblinded data. After the review, the DMC will make recommendations regarding the continuation of the study. The DMC responsibilities, authorities, and procedures will be documented in a separate DMC charter.

The safety reviews will focus on particular AEs, SAEs, and mortality. The DMC will have access to unblinded data and review tabulated safety summaries (if appropriate) and any additional data that the DMC may request during the conduct of the study. No formal statistical hypothesis testing will be performed. The content of the safety summaries, the DMC role and responsibilities, and the general procedures (including communications) will be defined and documented in the DMC charter prior to any DMC review.

During the study, the Sponsor's study responsible physician (or designee) will regularly review blinded safety data from the sites and notify the DMC and appropriate Sponsor personnel of any issues.

12. ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established Standard Operating Procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of safety information; all clinical studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

12.1. Definitions

12.1.1. Adverse Event Definitions and Classifications

Adverse Event

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not

related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH])

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

For the purpose of this study, any symptoms attributed to rhinovirus infection (eg, sneezing, rhinorrhea, nasal congestion, sore throat, cough, headache, malaise, chilliness, post-nasal drip, stuffy ears, pain in ears, sinus pain/pressure) will not be considered AEs unless the investigator deems that these symptoms are exceptional and should be recorded as an AE.

Note: The sponsor collects adverse events starting with the signing of the informed consent form (refer to Section 12.3.1, All Adverse Events, for time of last adverse event recording).

Serious Adverse Event

A serious adverse event based on ICH is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is Medically Important*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An adverse event is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For an investigational product, the expectedness of an adverse event will be determined by whether or not it is listed in the Investigator's Brochure.

Adverse Event Associated With the Use of the Agent

An adverse event is considered associated with the use of the agent if the attribution is possible, probable, or very likely by the definitions listed in Section 12.1.2.

12.1.2. Attribution Definitions

Not Related

An adverse event that is not related to the use of the agent.

Doubtful

An adverse event for which an alternative explanation is more likely, eg, concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

Possible

An adverse event that might be due to the use of the study agent. An alternative explanation, eg, concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Probable

An adverse event that might be due to the use of the study agent. The relationship in time is suggestive (eg, confirmed by dechallenge). An alternative explanation is less likely, eg, concomitant drug(s), concomitant disease(s).

Very Likely

An adverse event that is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, eg, concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (eg, it is confirmed by dechallenge and rechallenge).

12.1.3. Severity Criteria

Grading of AEs will be performed as described in Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

The investigator should use clinical judgment in assessing the severity of events not directly experienced by the subject (eg, laboratory abnormalities).

12.2. Special Reporting Situations

Safety events of interest on a sponsor medicinal product that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Overdose of a sponsor medicinal product
- Suspected abuse/misuse of a sponsor medicinal product
- Inadvertent or accidental exposure to a sponsor medicinal product
- Medication error involving a sponsor product (with or without subject/patient exposure to the sponsor medicinal product, eg, name confusion)

Special reporting situations should be recorded in the CRF. Any special reporting situation that meets the criteria of a serious adverse event should be recorded on the serious adverse event page of the CRF.

12.3. Procedures

12.3.1. All Adverse Events

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated informed consent form is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety). Serious adverse events, including those spontaneously reported to the investigator within 30 days after the last dose of study agent, must be reported using the Serious Adverse Event Form. The Sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

All events that meet the definition of a serious adverse event will be reported as serious adverse events, regardless of whether they are protocol-specific assessments.

All adverse events, regardless of seriousness, severity, or presumed relationship to study therapy, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record in the CRF their opinion concerning the relationship of the adverse event to study therapy. All measures required for adverse event management must be recorded in the source document and reported according to Sponsor instructions.

The Sponsor assumes responsibility for appropriate reporting of adverse events to the regulatory authorities. The Sponsor will also report to the investigator all serious adverse events that are unlisted (unexpected) and associated with the use of the agent. The investigator (or Sponsor where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol unless otherwise required and documented by the IEC/IRB.

Subjects (or their designees, if appropriate) must be provided with a "study card" indicating the following:

- A place for the subject's name
- Subject number
- A place for the investigator's name and 24-hour contact information
- Statement that the subject is participating in a clinical trial.

12.3.2. Adverse Events During or Following Study Agent Infusion

Before any infusion is started, the appropriate personnel, medication (epinephrine, inhaled beta agonists, antihistamines, and corticosteroids) and other requirements for the treatment of anaphylaxis must be available.

All prior infusion sites must be evaluated carefully at each study visit. Any adverse reaction (eg, erythema and/or induration) should be noted in the AE page of the eCRF.

A physician must be available immediately at the site at all times during the administration of study agent. The Sponsor's medical monitor or designee must be notified within 24 hours of any infusion reaction requiring interruption of study agent. Subjects experiencing a reaction during the study agent administration should be treated according to institutional guidelines.

The risks of an acute infusion reaction with CNTO 3157 are currently unknown. Infusion reactions (eg, fever, chills, chest pain/tightness, hypo- or hypertension, dyspnea, pruritus, urticaria and cardiopulmonary reactions) have been associated with IV mAb therapy. All subjects must be observed carefully for symptoms of an allergic reaction/hypersensitivity during the infusion and for at least 30 minutes after the infusion of study agent is finished. The investigator should use clinical judgment in assessing the intensity of infusion reactions. The following examples are for guidance only, are based upon previous examples of infusion reactions with mAb therapy in general, and may be considered either severe or nonsevere based upon the clinical circumstances: hypotension, shortness of breath, rash, urticaria, flushing, chest pain/tightness, tachyarrhythmias, fever, peripheral edema of extremities, chills/rigor, nausea/emesis, headache, diaphoresis, light headedness, somnolence, erythema, abdominal discomfort, shivers, polyarthralgias, sore throat, increased temperature, flu-like symptoms, joint stiffness and pain, myalgia, dyspnea, dysphagia, dizziness, bronchospasm, wheezing, cyanosis, hypoxia, oropharyngeal or laryngeal edema, cardiac arrest/respiratory arrest, angioedema/urticaria, ischemia or infarction.

Subjects with reactions during an infusion or during the 24-hour period following the infusion, resulting in bronchospasm with wheezing and/or dyspnea requiring ventilatory support, or symptomatic hypotension with a decrease in systolic blood pressure greater than 40 mmHg, will permanently stop study agent (if enrolled in Part 2, and scheduled to receive subsequent infusions).

If an infusion reaction is observed, treatment such as oral paracetamol/acetaminophen and/or oral antihistamine may be administered and the infusion rate slowed until the infusion reaction diminishes or resolves.

If a prophylactic medication is considered for subsequent administrations, the Sponsor's medical monitor or designee must be contacted prior to the start of the next scheduled study agent administration to discuss the use of such medications.

Subjects who experience a reaction during the administration of CNTO 3157 resulting in discontinuation of study agent (such as a severe infusion reaction) will have blood drawn for

determination of antibodies to CNTO 3157. The sample should be obtained within 4 hours of the onset of the reaction. Subjects discontinuing study agent will be asked to return for required assessments at all scheduled visits (see Time and Event Schedules) through Week 8 (Part 1) or Week 11 (Part 2).

12.3.3. Study Agent Infusion Site Reactions

An infusion-site reaction is any adverse reaction at an infusion site. All subjects must be observed carefully for symptoms of an infusion-site reaction. Subjects will be observed during the infusion and for at least 30 minutes after the infusion. If an infusion site reaction is observed, the subject should be treated at the investigator's discretion.

For subjects who have mild or moderate infusion-site reactions and are scheduled to receive additional infusions, subsequent infusions may be administered at the discretion of the investigator. If a subject has a severe infusion site reaction, discontinuation of further study agent infusions (if enrolled in Part 2) must be considered. Subjects discontinuing study agent will be asked to return for required assessments at all scheduled visits (see Time and Event Schedules) through Week 8 (Part 1) or Week 11 (Part 2).

12.3.4. Delayed Hypersensitivity (Serum Sickness-Like) Reactions

A delayed hypersensitivity (serum sickness-like) reaction is defined as a reaction following study agent re-administration consisting of myalgia and/or arthralgia with fever and/or rash (that does not represent signs and symptoms of other recognized clinical syndromes) occurring 1 to 14 days after an infusion of study agent. These may be accompanied by other events including pruritus, facial, hand, or lip edema, dysphagia, urticaria, sore throat and/or headache. If this reaction occurs, the subject should contact the investigator for evaluation and appropriate treatment and the subject must be permanently discontinued from study medication.

12.3.5. Serious Adverse Events

All SAEs occurring during clinical studies must be reported to the appropriate sponsor contact person by investigational staff within 24 hours of their knowledge of the event.

Information regarding SAEs will be transmitted to the sponsor using the Serious Adverse Event Form, which must be completed and signed by a member of the investigational staff, and transmitted to the sponsor within 24 hours. The initial and follow-up reports of a SAE should be made by facsimile (fax).

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available

- The event can be attributed to agents other than the study agent or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Suspected transmission of an infectious agent by the investigational agent (CNTO 3157 or placebo) will be reported as a SAE. Suspected transmission of an infectious agent (other than HRV-16) by the challenge inoculum will be reported as a SAE.

Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject's participation in a clinical study must be reported as a SAE, except hospitalizations for the following:

- Reasons other than an AE (eg, routine in-patient testing)
- Surgery or procedure planned before entry into the study (must be documented in the eCRF)

The cause of death of a subject in a clinical study 30 days after administration of study agent (CNTO 3157 or placebo), whether or not the event is expected or associated with the investigational agent, is considered a SAE.

12.3.6. Pregnancy

All initial reports of pregnancy must be reported to the Sponsor by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (eg, spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported using the Serious Adverse Event Form. Any subject who becomes pregnant during the study must be promptly withdrawn from study treatment. Subjects will be encouraged to complete an end-of-treatment visit (performing the Week 8 assessments in Part 1 and Week 11 assessments in Part 2).

Because the effect of the study agent on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

12.4. Contacting Sponsor Regarding Safety

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding safety issues or questions regarding the study are listed on the Contact Information page(s), which will be provided as a separate document.

13. PRODUCT QUALITY COMPLAINT HANDLING

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, ie, any dissatisfaction relative to the identity, quality,

durability, or reliability of a product, including its labeling or package integrity. PQCs may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from clinical studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information; all clinical studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

13.1. Procedures

All initial PQCs must be reported to the sponsor by the investigational staff as soon as possible after being made aware of the event.

If the defect is combined with a serious adverse event, the investigational staff must report the PQC to the sponsor according to the serious adverse event reporting timelines (refer to Section 12.3.2, Serious Adverse Events). A sample of the suspected product should be maintained for further investigation if requested by the sponsor.

13.2. Contacting Sponsor Regarding Product Quality

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding product quality issues are listed on the Contact Information page(s), which will be provided as a separate document.

14. STUDY AGENT INFORMATION

14.1. Study Agents

14.1.1. Physical Description of Study Agent(s)

CNTO 3157 is supplied as a sterile, preservative-free, lyophilized white solid cake in a single use, 8R Type 1 glass vial. Each vial contains a nominal target of 100 mg of lyophilized CNTO 3157 for reconstitution with 2 mL sterile water (without bacteriostat) for injection to yield a 50 mg/mL solution of CNTO 3157.

Refer to the Investigator's Brochure for CNTO 3157 for a list of excipients.

Placebo will be 5% dextrose and will be supplied from a commercial source.

14.1.2. Labeling of Study Agent(s)

Study agent labels will contain information to meet the applicable regulatory requirements.

14.1.3. Preparation, Handling, and Storage of Study Agent(s)

Aseptic procedures must be used during the preparation of study agents. CNTO 3157 will be reconstituted with sterile water for injection. The diluent for IV infusion is sterile, 5% dextrose, European Pharmacopoeia (EP).CNTO 3157 should be stored at 2°C to 8°C (36°F to 46°F) and

protected from exposure to light. Protection from light is not required during dose preparation or administration.

The study agent will be prepared according to the subject's weight and treatment assignment by the pharmacist or other appropriately licensed and authorized health professional who is not blinded to the treatment assignment. All study administrations must be calculated based on the subject's weight at the pre-dose timepoint on the initial day of dosing, and for Part 2, subsequent days of dosing thereafter.

The reconstituted CNTO 3157 should be clear solution and essentially free of visible particulate matter. In addition, the reconstituted Placebo and CNTO 3157 are to be administered by IV infusion with an in-line 0.22 μm filter.

Refer to the pharmacy manual/site investigational product manual for additional guidance on study agent preparation and handling.

14.1.4. Accountability for Study Agents(s)

The investigator is responsible for ensuring that all study agent received at the site is inventoried and accounted for throughout the study. The study agent administered to the subject must be documented on the drug accountability form. All study agent will be stored and disposed of according to the sponsor's instructions. Site staff must not combine contents of the study agent containers.

Study agent must be handled in strict accordance with the protocol and the container label, and must be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental conditions. Unused study agent must be available for verification by the sponsor's site monitor during on-site monitoring visits. The return to the sponsor of unused study agent will be documented on the drug return form. When the site is an authorized destruction unit and study agent supplies are destroyed on site, this must also be documented on the drug return form.

Potentially hazardous materials such as used ampules, needles, syringes and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for drug accountability purposes.

Study agent should be dispensed under the supervision of the investigator or a qualified member of the investigational staff, or by a hospital/clinic pharmacist. Study agent may not be relabeled or reassigned for use by other subjects. The investigator agrees neither to dispense the study agent from, nor store it at, any site other than the study sites agreed upon with the sponsor.

14.2. HRV-16 Challenge Agent

14.2.1. Physical Description of HRV-16

The HRV-16 challenge stock will be supplied as a frozen liquid.

14.2.2. Packaging of HRV-16

HRV-16 will be supplied in gamma-irradiated polypropylene cryovials containing approximately 1 mL of clarified tissue culture lysate (final pH 6.5 to 8.5) which has been passed through a 0.2 μm filter and snap frozen.

14.2.3. Labeling of HRV-16

HRV-16 study agent labels will contain information to meet the applicable regulatory requirements.

14.2.4. Preparation, Handling, and Storage of HRV-16

HRV-16 must be stored at -60°C or below. Vials will be thawed once and not refrozen or reused. A single thawed vial may be used to inoculate multiple subjects.

Refer to the Site Investigational Product Procedures Manual for additional guidance on handling HRV-16.

15. STUDY-SPECIFIC MATERIALS

The investigator will be provided with the following supplies (note: this is not an inclusive list):

- Investigator Brochures for CNTO 3157 and HRV-16
- Site Investigational Product Procedures Manual
- IVRS/IWRS Manual
- Spirometer and user manual
- FENO device (if applicable) and user manual
- Electronic handheld device (for PRO questionnaires) with peak flow meter and user manual

16. ETHICAL ASPECTS

16.1. Study-Specific Design Considerations

Potential subjects will be fully informed of the risks and requirements of the study and, during the study, subjects will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential adverse events of the study, and provide their consent voluntarily will be enrolled.

The total blood volume to be collected from every completed subject in Part 1 of the study will be approximately 190 mL, and total blood volume to be collected from every completed subject in Part 2 of the study will be approximately 235 mL. All female subjects of childbearing potential will have an additional blood sample collected at screening for serum pregnancy testing. An additional blood volume of approximately 10 mL will be collected from each subject who consents to participate in the pharmacogenomic study. This is considered to be an acceptable amount of

blood to be collected over this time period from the population in this study based upon the standard of the American Red Cross (1 pint/473 mL of blood for donation). Additional blood samples may be collected if indicated.

16.2. Regulatory Ethics Compliance

16.2.1. Investigator Responsibilities

The investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical study data are credible.

16.2.2. Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator (or sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents:

- Final protocol and, if applicable, amendments
- Sponsor-approved informed consent form (and any other written materials to be provided to the subjects)
- Investigator's Brochure (or equivalent information) and amendments/addenda
- Sponsor-approved subject recruiting materials
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any), the informed consent form, applicable recruiting materials, and subject compensation programs, and the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

Approval for the pharmacogenomic research component of the clinical study and for the pharmacogenomic informed consent form must be obtained from the IEC/IRB. Approval for the protocol can be obtained independent of approval for pharmacogenomic research.

During the study the investigator (or sponsor where required) will send the following documents and updates to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments
- Revision(s) to informed consent form and any other written materials to be provided to subjects
- If applicable, new or revised subject recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually)
- Reports of adverse events that are serious, unlisted/unexpected, and associated with the investigational study agent
- New information that may adversely affect the safety of the subjects or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects
- Report of deaths of subjects under the investigator's care
- Notification if a new investigator is responsible for the study at the site
- Annual Safety Report and Line Listings, where applicable
- Any other requirements of the IEC/IRB

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or trial conduct), the amendment and applicable informed consent form revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).

At least once a year, the IEC/IRB will be asked to review and reapprove this clinical study. The reapproval should be documented in writing (excluding the ones that are purely administrative, with no consequences for subjects, data, or study conduct).

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

16.2.3. Informed Consent

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the Sponsor and by the reviewing IEC/IRB and be in a language that the subject can read and understand. The informed consent should be in accordance with principles that originated in the Declaration of

Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Before enrollment in the study, the investigator or an authorized member of the investigational staff must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his or her disease. Subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. Finally, they will be told that the investigator will maintain a subject identification register for the purposes of long-term follow up if needed and that their records may be accessed by health authorities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the informed consent form the subject is authorizing such access, and agrees to allow his or her study physician to recontact the subject for the purpose of obtaining consent for additional safety evaluations, if needed, and subsequent disease-related treatments , or to obtain information about his or her survival status.

The subject will be given sufficient time to read the informed consent form and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained the consent, a copy of the informed consent form must be given to the subject.

Subjects will be asked to consent to participate in a pharmacogenomic research component of the study (where local regulations permit). After informed consent for the clinical study is appropriately obtained, the subject will be asked to sign and personally date a separate pharmacogenomic informed consent form indicating agreement to participate in optional pharmacogenomic research. A copy of the signed pharmacogenomic informed consent form will be given to the subject. Refusal to participate in the pharmacogenomics research component of the study will not result in ineligibility for the clinical study.

After informed consent for the clinical study is appropriately obtained, subjects meeting eligibility criteria for sputum induction will be asked to provide informed consent indicating agreement to provide induced sputum samples.

16.2.4. Privacy of Personal Data

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be

put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of study subjects confidential.

The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator to allow direct access to his or her original medical records for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The subject has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

Exploratory DNA, pharmacodynamic, biomarker, PK, and immunogenicity research is not conducted under standards appropriate for the return of data to subjects. In addition, the sponsor cannot make decisions as to the significance of any findings resulting from exploratory research. Therefore, exploratory research data will not be returned to subjects or investigators, unless required by law. Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.

16.2.5. Long-Term Storage of Samples for Future Research

Samples will be stored in accordance with country-specific regulatory requirements and company SOPs.

16.2.6. Country Selection

This study will only be conducted in those countries where the intent is to launch or otherwise help ensure access to the developed product, unless explicitly addressed as a specific ethical consideration in Section 16.1, Study-Specific Design Considerations.

17. ADMINISTRATIVE REQUIREMENTS

17.1. Protocol Amendments

Neither the investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor, and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the investigator and IEC/IRB must be provided to the sponsor or its designee. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

During the course of the study, in situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor

representative (see Contact Information page(s) provided separately). Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

17.2. Regulatory Documentation

17.2.1. Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

17.2.2. Required Prestudy Documentation

The following documents must be provided to the sponsor before shipment of study agent to the investigational site:

- Protocol and amendment(s), if any, signed and dated by the principal investigator
- A copy of the dated and signed, written IEC/IRB approval of the protocol, amendments, informed consent form, any recruiting materials, and if applicable, subject compensation programs. This approval must clearly identify the specific protocol by title and number and must be signed by the chairman or authorized designee.
- Name and address of the IEC/IRB, including a current list of the IEC/IRB members and their function, with a statement that it is organized and operates according to GCP and the applicable laws and regulations. If accompanied by a letter of explanation, or equivalent, from the IEC/IRB, a general statement may be substituted for this list. If an investigator or a member of the investigational staff is a member of the IEC/IRB, documentation must be obtained to state that this person did not participate in the deliberations or in the vote/opinion of the study.
- Regulatory authority approval or notification, if applicable
- Signed and dated statement of investigator (eg, Form FDA 1572), if applicable
- Documentation of investigator qualifications (eg, curriculum vitae)
- Completed investigator financial disclosure form from the principal investigator, where required
- Signed and dated clinical trial agreement, which includes the financial agreement
- Any other documentation required by local regulations

The following documents must be provided to the sponsor before enrollment of the first subject:

- Completed investigator financial disclosure forms from all clinical subinvestigators
- Documentation of subinvestigator qualifications (eg, curriculum vitae)

- Name and address of any local laboratory conducting tests for the study, and a dated copy of current laboratory normal ranges for these tests, if applicable
- Local laboratory documentation demonstrating competence and test reliability (eg, accreditation/license), if applicable

17.3. Subject Identification, Enrollment, and Screening Logs

The investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the sponsor site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure subject confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by assigned number.

The investigator must also complete a subject screening log, which reports on all subjects who were seen to determine eligibility for inclusion in the study.

17.4. Source Documentation

At a minimum, source documentation must be available for the following to confirm data collected in the CRF: subject identification, eligibility, and study identification; study discussion and date of informed consent; dates of visits; results of safety and efficacy parameters as required by the protocol; record of all adverse events and follow-up of adverse events; concomitant medication; drug receipt/dispensing/return records; study agent administration information; and date of study completion and reason for early discontinuation of study agent or withdrawal from the study, if applicable.

In addition, the author of an entry in the source documents should be identifiable.

At a minimum, the type and level of detail of source data available for a study subject should be consistent with that commonly recorded at the site as a basis for standard medical care. Specific details required as source data for the study will be reviewed with the investigator before the study and will be described in the monitoring guidelines (or other equivalent document).

17.5. Case Report Form Completion

Case report forms are provided for each subject in printed or electronic format.

Electronic Data Capture (eDC) will be used for this study. The study data will be transcribed by study personnel from the source documents onto an electronic CRF, and transmitted in a secure manner to the Sponsor within the timeframe agreed upon between the Sponsor and the site. The electronic file will be considered to be the CRF.

Worksheets may be used for the capture of some data to facilitate completion of the CRF. Any such worksheets will become part of the subject's source documentation. All data relating to the study must be recorded in CRFs prepared by the Sponsor. Data must be entered into CRFs in

English. Designated site personnel must complete the CRF as soon as possible after a subject visit, and the forms should be available for review at the next scheduled monitoring visit.

Every effort should be made to ensure that all subjective measurements (eg, questionnaires) to be recorded in the CRF are completed by the same individual who made the initial baseline determinations. The investigator must verify that all data entries in the CRFs are accurate and correct.

All CRF entries, corrections, and alterations must be made by the investigator or other authorized study-site personnel. If necessary, queries will be generated in the eDC tool.

If corrections to a CRF are needed after the initial entry into the CRF, this can be done in 3 different ways:

- Site personnel can make corrections in the eDC tool at their own initiative or as a response to an auto query (generated by the eDC tool)
- Site manager can generate a query for resolution by the investigational staff
- Clinical data manager can generate a query for resolution by the investigational staff

17.6. Data Quality Assurance/Quality Control

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and associated personnel before the study, and periodic monitoring visits by the Sponsor, and direct transmission of clinical laboratory data from a central laboratory. Written instructions will be provided for collection, preparation, and shipment of samples.

Guidelines for CRF completion will be provided and reviewed with study personnel before the start of the study.

The Sponsor will review CRFs for accuracy and completeness during on-site monitoring visits and after transmission to the Sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. After upload of the data into the clinical study database they will be verified for accuracy and consistency with the data sources.

17.7. Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all CRFs and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing

applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator must permit access to such reports.

17.8. Monitoring

The sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study site visit log that will be kept at the site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the CRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the sponsor and investigational staff and are accessible for verification by the sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the investigational staff.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. Findings from this review of CRFs and source documents will be discussed with the investigational staff. The sponsor expects that, during monitoring visits, the relevant investigational staff will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

17.9. Study Completion/Termination

17.9.1. Study Completion

The study is considered completed with the last visit for the last subject participating in the study. The final data from the investigational site will be sent to the Sponsor (or designee) after completion of the final subject visit at that site, in the time frame specified in the Clinical Trial Agreement.

17.9.2. Study Termination

The sponsor reserves the right to close the investigational site or terminate the study at any time for any reason at the sole discretion of the sponsor. Investigational sites will be closed upon study completion. An investigational site is considered closed when all required documents and study supplies have been collected and a site closure visit has been performed.

The investigator may initiate site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of an investigational site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study agent development

In addition, the study will be stopped for any of the following:

- If the Data Monitoring Committee recommends and/or sponsor agrees that the number and/or severity of AEs justify discontinuation of the study
- If the sponsor decides to discontinue the study
- If data becomes available that raises concern about the safety of the study agent so that continuation would pose new risks to the subject

Premature termination of the study must be mutually agreed upon by the investigator and the sponsor and must be documented. However, study results will be reported according to Section 17.11.

The recommendation to resume dosing, to terminate the trial, or to amend the protocol may be made by an internal safety review group. A decision to modify or discontinue dosing will be communicated to the IRBs/ECs and appropriate Health Authorities.

Study termination requires that no additional subjects will be treated with study agent. Subjects who have already received study agent will continue to be followed for safety through the remainder of the planned follow up period.

17.10. On-Site Audits

Representatives of the sponsor's clinical quality assurance department may visit the site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Subject privacy must, however, be respected. The investigator and staff are responsible for being present and available for consultation during routinely scheduled site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

17.11. Use of Information and Publication

All information, including but not limited to information regarding CNTO 3157 or the sponsor's operations (eg, patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the sponsor to the investigator and not previously published, and any data, including pharmacogenomic research data, generated as a result of this study, are considered confidential and remain the sole property of the sponsor. The investigator agrees to maintain this information in confidence and use this information only to accomplish this study, and will not use it for other purposes without the sponsor's prior written consent.

The investigator understands that the information developed in the clinical study will be used by the sponsor in connection with the continued development of CNTO 3157, and thus may be disclosed as required to other clinical investigators or regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated by the Sponsor and will contain CRF data from all investigational sites that participated in the study, and direct transmission of necessary auxiliary data (eg, clinical laboratory data from a central laboratory) into the Sponsor’s data base. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating investigator. Results of pharmacogenomic or exploratory biomarker analyses performed after the Clinical Study Report has been issued will be reported in a separate report and will not require a revision of the Clinical Study Report. Study subject identifiers will not be used in publication of pharmacogenomic or exploratory biomarker results. Any work created in connection with performance of the study and contained in the data that can benefit from copyright protection (except any publication by the investigator as provided for below) shall be the property of the Sponsor as author and owner of copyright in such work.

The Sponsor shall have the right to publish such data and information without approval from the investigator. If an investigator wishes to publish information from the study, a copy of the manuscript must be provided to the Sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the Sponsor in writing, the investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the Sponsor will review these issues with the investigator. The Sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multicenter study designs and substudy approaches, secondary results generally should not be

published before the primary endpoints of a study have been published. Similarly, investigators will recognize the integrity of a multicenter study by not submitting for publication data derived from the individual site until the combined results from the completed study have been submitted for publication, within 12 months of the availability of the final data (tables, listings, graphs), or the Sponsor confirms there will be no multicenter study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

Registration of Clinical Studies and Disclosure of Results

The sponsor will register and/or disclose the existence of and the results of clinical studies as required by law.

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Attachment 1: Blood Volume Sampling (Part 1)

Volume of Blood to be Collected From Each Subject in Part 1

Type of Sample	Volume per Sample (mL)	No. of Samples per Subject	Total Volume of Blood (mL)[a]
Hematology	2.0	2	4.0
Serum chemistry[b]	5.0	2	10.0
Serology (HRV-16)	10.0	5	50.0
Serology (HSV-1)	10.0	1	10.0
Serology (HIV, HBV, HCV)	10.0	1	10.0
Pharmacokinetic and Immunogenicity samples[d]	5.0	10	50.0
Biomarkers			
Serum protein biomarkers	5.0	6	30.0
Whole blood RNA biomarkers	2.5	5	12.5
Pharmacogenomic sample[c]	5.0	2	10.0
Loss by use of indwelling intravenous cannula			
Total			186.5

a. Calculated as number of samples multiplied by amount of blood per sample.
b. Serum chemistry includes serum β-hCG pregnancy tests (female subjects of child bearing potential only).
c. In the event of DNA extraction failure, a replacement pharmacogenomic blood sample may be requested from the subject.
d. Serum samples from pharmacokinetic, immunogenicity, and biomarker time points will be split into 3 aliquots (1 aliquot for PK, 1 aliquot for immunogenicity, and 1 aliquot as a backup).

Note: An indwelling intravenous cannula may be used for blood sample collection.

For each subject, the maximum amount of blood drawn from each subject in this study will not exceed 200.0 mL. Repeat or unscheduled samples may be taken for safety reasons.

Attachment 2: Blood Volume Sampling (Part 2)

Volume of Blood to be Collected From Each Subject in Part 2

Type of Sample	Volume per Sample (mL)	No. of Samples per Subject	Total Volume of Blood (mL)[a]
Hematology	2.0	3	6.0
Serum chemistry[b]	5.0	3	15.0
Serology (HRV-16)	10.0	3	30.0
Serology (HSV-1)	10.0	1	10.0
Serology (HIV, HBV, HCV)	10.0	1	10.0
Pharmacokinetic and Immunogenicity samples[d]	5.0	16	80.0
Biomarkers			
Serum protein biomarkers	5.0	11	55.0
Whole blood RNA biomarkers	2.5	7	17.5
Pharmacogenomic sample[c]	5.0	2	10.0
Loss by use of indwelling intravenous cannula			
Total			233.5

a. Calculated as number of samples multiplied by amount of blood per sample.
b. Serum chemistry includes serum β-hCG pregnancy tests (female subjects of child bearing potential only).
c. In the event of DNA extraction failure, a replacement pharmacogenomic blood sample may be requested from the subject.
d. Serum samples from pharmacokinetic, immunogenicity, and biomarker time points will be split into 3 aliquots (1 aliquot for PK, 1 aliquot for immunogenicity, and 1 aliquot as a backup).

Note: An indwelling intravenous cannula may be used for blood sample collection.

For each subject, the maximum amount of blood drawn from each subject in this study will not exceed 250 mL in Part 2.

Attachment 3: Candidate Gene List for Part 1 of Pharmacogenomics

Absorption, Distribution, Metabolism, and Excretion Genes and hepatoxicity-related: *ABC* family, *ACSL* family, *ACSM* family, *ACSS2, ACSS3, ADH* family, *AHR, ALDH* family, *AOX1, APAF1, ARNT, ARSA, ATM, ATP7A, ATP7B, BAD, BAK1, BAX, BCL* family, *BDH2, BFAR, BIK, BIRC* family, *BLK, BMF, BNIP3, BOK, CARD10, CASP* family, *CAT, CBR1, CBR3, CCL2, CCL5, CCND1, CCR2, CCR5, CD27, CD40, CD40LG, CD70, CD74, CDA, CES1, CES2, CFLAR, CHEK1, CHEK2, CHEK2, CHST* family, *CHUK, CIDEA, CIDEB, COMT, CRADD, CXC* family, *CYP11A1, CYP11B1, CYP11B2, CYP17A1, CYP19A1, CYP1A1, CYP1A2, CYP1B1, CYP20A1, CYP21A2, CYP24A1, CYP26A1, CYP27A1, CYP2A13, CYP2A6, CYP2A7, CYP2B6, CYP2C18, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP39A1, CYP3A4, CYP3A43, CYP3A5, CYP3A7, CYP46A1, CYP4B1, CYP4F11, CYP4F12, CYP4F2, CYP4F3, CYP4F8, CYP4Z1, CYP51A1, CYP7A1, CYP7B1, CYP8B1, DAPK2, DDIT3, DFFA, DFFB, DHRS* family, *DPYD, EPHX1, EPHX2, FADD, FAS, FAS, FASLG, FASLG, FMO* family, *GADD45A, GPR177, GPX* family, *GSR, GSS, GSTA* family, *GSTCD, GSTK1, GSTM* family, *GSTO1, GSTO2, GSTP1, GSTT1, GSTT2, GSTZ1, GZMA, GZMB, GZMH, GZMK, GZMM, HAGH, HGF, HLA-A, HLA-B, HLA-C, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-E, HLA-F, HLA-G, HLA-K, HLA-L, HNMT, HRK, HSD17B11, HSD17B14, IFNG, IFNGR1, IFNGR2, IFNR, IKBKB, IKBKG, IL10, IL10RA, IL10RB, IL12A, IL12B, IL12RB1, IL12RB2, IL13, IL13RA1, IL13RA2, IL18, IL18BP, IL18R1, IL18RAP, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL1R1, IL1R2, IL1RAP, IL1RN, IL4, IL4R, IL6, IL6R, IL8RA, IL8RB, IRF1, KRT18, KRT8, LEFTY2, LRRFIP1, LTA, LTB, LTBR, MAOA, MAOB, MAP3K7IP3, MAPK3, MAPK8, MAPK9, MCL1, MDM2, MET, MGST1, MGST2, MGST3, MPO, MST1, MYD88, NAIP, NAT1, NAT2, NFE2L2, NFKB* family, *NKRF, NNMT, NOD1, NOL3, NOS2A, NOS2C, NOS3, NOSIP, NOSTRIN, NQO1, NR1I2, NR3C1, PLEKHG5, POR, PPARA, PPARD, PPARG, PTGIS, PTGS1, PTGS2, PYCARD, RALBP1, RELA, RIPK1, RIPK2, RPA3, RPL17, SLC* family, *SLCO* family, *SOD2, SOD3, SPG7, SPINT1, SULT* family, *TANK, TBK1, TBXAS1, TERT, TGFB1, TGFB1I1, TGFB2, TGFB3, TGFBR1, TGFBR2, TGFBR3, TGFBRAP1, TGFBRE, TMED4, TNF, TNFRSF* family, *TP53, TPMT, TPP63, TRAF* family, *UGT* family, *UGT2B7, UGT8, VDAC1,* and *XDH.*

Target/Disease related genes:

TLR family, Myd88, TRIF, IPS-1, IRF-3

Attachment 4: Cold Symptom Assessment Score

Cold Symptom Assessment Score

For **each** symptom listed below, please **read** the question in the second column "Did you have a xx (symptom) during the past 24 hours?"

- For example "Did you have a **runny nose** during the past 24 hours?"
  - Check 'yes' or 'no' to indicate whether or not the subject had the symptom during the past 24 hours.

If the subject experienced the symptom and responds "yes", please ask the question in the 3rd column "Please rate the severity of your xx (symptom)" and indicate how severe it was for them.

- For example, if the subject answered 'yes' that they have had a **runny nose**, please ask "please rate the severity of your runny nose as either mild, moderate severe or very severe."

If the subject asks for the definitions of the severity scale, please explain that

- **Mild** means the symptom is barely noticeable
- **Moderate** means the symptom is clearly noticeable but does not interfere with daily activity
- **Severe** means the symptom interferes with daily activity
- **Very severe** means the symptom prevents participation in daily activity

If the subject did not experience the symptom, and responds "no" please check "NO" ONLY in the box and move onto the next symptom question.

- For example, if the subject did not have a runny nose, move on to the next symptom and ask "Did you have a blocked or stuffy nose during the past 24 hours?"

Cold Symptom Assessment Score Form

Symptom	Did you have a xx (symptom) during the past 24 hours?	Please rate the severity of your xx (symptom) as either: mild, moderate, severe or very severe			
		Mild = 1	Moderate = 2	Severe = 3	Very Severe = 4
Nasal congestion (stuffy nose)	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Rhinorrhea (runny nose)	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Sore throat	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Sneezing	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				

Cough	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Headache	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Malaise (feeling run down)	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Chilliness	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Total Cold Symptom Score					

Attachment 5: Cold and Chest Symptom Scale

Cold and Chest Symptom Scale

The Cold and Chest Symptom Scale contains 15 items composing 2 domains, a total cold score and a total chest score. Together, these 2 domains comprise the Cold and Chest Symptom Scale.

- **Total Cold Score domain:** sneezing, runny nose, blocked or stuff nose, sore throat or hoarse voice, headache or face pain, generally unwell, chills, fever or shivery and cough **and**

- **Total Chest Score domain:** cough on waking, wheeze on waking, daytime cough, daytime wheeze, daytime chest tightness, daytime breathlessness and nocturnal cough, wheeze and breathlessness.

For each symptom listed below, please read the question in the second column "Did you have a xx (symptom) during the past 24 hours?"

- For example "Did you have a runny nose during the past 24 hours?"
    - Check 'yes' or 'no' to indicate whether or not the subject had the symptom during the past 24 hours.

If the subject experienced the symptom and responds "yes", please ask the question in the third column "Please rate the severity of your xx (symptom)" and indicate how severe it was for them.

- For example, if the subject answered 'yes' that they have had a runny nose, please ask "please rate the severity of your runny nose as either mild, moderate severe or very severe."

If the subject asks for the definitions of the severity scale, please explain that

- Mild means the symptom is barely noticeable
- Moderate means symptom is clearly noticeable but does not interfere with normal daytime or night time activities
- Severe means the symptom mildly interferes with your ability to successfully perform normal daytime or night time activities
- Very severe means the symptom significantly interferes with your ability to adequately perform normal daytime or night time activities

If the subject did not experience the symptom, and responds "no" please check "NO" ONLY in the box and move onto the next symptom question.

- For example, if the subject did not have a runny nose, move on to the next symptom and ask "Did you have a blocked or stuffy nose during the past 24 hours?"

Cold and Chest Symptom Scale Form

Symptom	Did you have a xx (symptom) during the past 24 hours?	Please rate the severity of your xx (symptom) as either: mild, moderate, severe or very severe			
		Mild = 1	Moderate = 2	Severe = 3	Very Severe = 4
Sneezing	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Runny nose	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Blocked or stuffy nose	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Sore throat or hoarse voice	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Headache or face pain	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Generally unwell	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Chill, fever or shivery	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Cough	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Total Cold Score					

Symptom	Did you have a xx (symptom) during the past 24 hours?	Please rate the severity of your xx (symptom) as either: mild, moderate, severe or very severe			
		Mild = 1	Moderate = 2	Severe = 3	Very Severe = 4
Cough on waking	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Wheeze on waking	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Daytime Cough	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Daytime Wheeze	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Daytime chest tightness	Yes ☐ →	☐	☐	☐	☐

	No ☐ = 0				
Daytime breathlessness	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Nocturnal cough, wheeze, breathlessness	Yes ☐ →	☐	☐	☐	☐
	No ☐ = 0				
Total Chest Score					

LAST PAGE