AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

The effect of inhaled interferon-beta on worsening of asthma symptoms caused by viral infections: a randomised trial

Authors:

Ratko Djukanović, M.D.[1], Tim Harrison, M.D.[2], Sebastian L. Johnston, M.D.[3], Flic Gabbay, M.D.[4] Peter Wark, M.D.[5], Neil C Thomson, M.D.[6], Robert Niven, M.D.[7], Dave Singh, M.D.[8], Helen K Reddel, PhD[9], Donna E. Davies, PhD[1], Richard Marsden, B.A.[10], Christine Boxall, PhD[10], Sarah Dudley, PhD[10], Vincent Plagnol, PhD[11], Stephen T. Holgate, M.D.[1], Phillip Monk, PhD[10] and the **INTERCIA** study group*

Affiliations:

[1]NIHR Southampton Respiratory Biomedical Research Unit, Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, United Kingdom.

[2]Nottingham Respiratory Research Unit, University of Nottingham, Nottingham City Hospital, Nottingham, United Kingdom.

[3]Airway Disease Infection Section, National Heart & Lung Institute and Centre for Respiratory Infections, Imperial College, MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, Imperial College NHS Trust NIHR Comprehensive Biomedical Research Centre, London, United Kingdom.

[4]TranScrip Partners LLP, Reading, United Kingdom.

[5]Priority Research Centre for Asthma and Respiratory Diseases, Hunter Medical Research Institute and The University of Newcastle, Newcastle, New South Wales, Australia.

[6]Institute of Infection, Immunity and Inflammation, University of Glasgow and Gartnavel General Hospital, Glasgow, United Kingdom.

Copyright © 2014 by the American Thoracic Society

[7]Manchester Academic Health Sciences Centre, University of Manchester and University Hospital of South Manchester UHSM, Manchester, United Kingdom.

[8]Respiratory Translational Research Facility, North West Lung Research Centre, Wythenshawe Hospital, Manchester, United Kingdom.

[9]Woolcock Institute of Medical Research, University of Sydney, Glebe, Australia.

[10]Synairgen Research Ltd, Southampton University Hospital, Southampton, United Kingdom.

[11]Genetics Institute, University College London, London, United Kingdom

*INTERCIA: **INTERferon-beta** for the treatment of **C**old-**I**nduced **A**sthma exacerbations. Members of the INTERCIA study group are listed in the online supplement.

Name and address of corresponding author:

Ratko Djukanović, mailpoint 810, Level F, Sir Henry Wellcome Laboratories, South Block, Clinical and Experimental Sciences, University Hospital Southampton, Tremona Road, Southampton SO16 6YD, United Kingdom

Email: r.djukanovic@soton.ac.uk

Telephone : +44 2381 204195; Fax : +44 2380 511 761

Contributions of authors:

Ratko Djukanovic was the chief investigator of the study and, as such, was centrally involved in its design, conduct and analysis and led the writing of the manuscript. Tim Harrison, Sebastian Johnston and Helen Reddel were involved in the design, conduct (as PIs in their centres) and analysis of the trial and the writing of the manuscript. Peter Wark, Neil Thomson, Robert Niven and Dave Singh were PIs in their centres and were involved in the

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

writing of the manuscript. Flic Gabbay played an important role in the trial design and contributed to the writing of the manuscript. Christine Boxall and Sarah Dudley contributed to the laboratory analyses of the measured biomarkers and writing of the manuscript. Vincent Plagnol undertook the analysis of the whole genome expression analysis. Richard Marsden played a key role in the design and conduct of the study and also critically reviewed the manuscript. Stephen Holgate and Donna Davies provided strategic input to the trial design and analysis and contributed to the writing of the manuscript. Phillip Monk played a key role in the trial design, conduct of the laboratory analyses and analyses of all the data, and writing of the manuscript.

Support for the study:

This trial was funded by Synairgen Research Limited, a University of Southampton spinout company.

Running head:

Nebulised interferon-beta treatment of asthma

Keywords: Innate immunity, treatment, respiratory virus

Descriptor number: 1.27

Total word count: 3649

Abstract word count: 245

Copyright © 2014 by the American Thoracic Society

At a glance commentary:

Scientific Knowledge on the Subject - Asthmatics are more likely to develop lower respiratory tract symptoms due to a cold, i.e. an upper respiratory tract infection (URTI), and there is no specific anti-viral treatment that can prevent URTI-induced asthma exacerbations.

What This Study Adds to the Field – Although the trial did not meet its primary endpoint, exploratory sub-analysis of the difficult-to-treat-subgroup suggests that inhaled interferon-β (IFN-β) enhances innate immunity and may impact favourably on cold-induced asthma exacerbations in this patient population.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

ABSTRACT

Rationale

Ex vivo, bronchial epithelial cells from asthmatics are more susceptible to rhinovirus infection due to deficient induction of the anti-viral protein, interferon-beta. Exogenous interferon-beta restores anti-viral activity.

Objectives

To compare the efficacy and safety of inhaled interferon-beta to placebo administered to asthmatics after onset of cold symptoms to prevent or attenuate asthma symptoms caused by respiratory viruses.

Methods

147 asthmatics on inhaled corticosteroids (British Thoracic Society Steps 2-5), with a history of virus-associated exacerbations, were randomised to 14-day treatment with inhaled interferon-beta (n=72) or placebo (n=75) within 24 hours of developing cold symptoms and were assessed clinically, with relevant samples collected to assess virus infection and anti-viral responses.

Results

91% of randomised patients developed a defined cold. In this modified intention-to-treat population, asthma symptoms did not get clinically significantly worse (mean change in 6-item Asthma Control Questionnaire <0.5) and interferon-beta treatment had no significant effect on this primary endpoint, although it enhanced morning peak expiratory flow recovery (p=0.033), reduced the need for additional treatment, and boosted innate

Copyright © 2014 by the American Thoracic Society

immunity as assessed by blood and sputum biomarkers. In an exploratory analysis of the subset of more difficult-to-treat, Step 4/5 asthmatics (n=27 interferon-beta; n=31 Placebo), Asthma Control Questionnaire-6 increased significantly on placebo; this was prevented by interferon-beta (p=0.004).

Conclusions

While the trial did not meet its primary endpoint, it suggests that inhaled interferon-beta is a potential treatment for virus-induced deteriorations of asthma in difficult-to-treat asthmatics and supports the need for further, adequately powered, trials in this population.

Trial registration

ClinicalTrials.gov: NCT01126177

Funding

Synairgen Research Limited, U.K.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

INTRODUCTION

Exacerbations of asthma, the majority caused by respiratory viruses (1, 2), are a large unmet medical need, especially in more severe disease (3-5). Asthmatics are more likely to develop lower respiratory tract symptoms after an upper respiratory tract infection (URTI) even though the frequency and severity of colds in asthmatics is not higher than normal (2). Using culture of bronchial epithelial cells obtained by bronchoscopic brushings from corticosteroid-treated asthmatics, we have previously demonstrated that, when infected with rhinoviruses (RV), the asthmatic bronchial epithelium failed to mount an effective innate immune response involving interferon (IFN)-β (6) and IFN-λ (7). Suboptimal activation of anti-viral pathways resulted in greater viral replication and shedding, cytolysis of epithelial cells and mediator release (6, 7). A similar defect exists in asthmatic airway macrophages (8) and airway cells of asthmatic children (9, 10). Importantly, epithelial anti-viral activity could be corrected *ex vivo* by low concentrations of exogenous IFN-β (6, 11). Some other studies have been unable to replicate such differences between asthmatic and healthy individuals, but these were conducted in mild, corticosteroid-naive asthmatics (12) or in those with well controlled asthma(13).

In order to evaluate the clinical relevance of the IFN-β deficiency in asthma and explore the potential for IFN-β as a treatment for virus-induced asthma exacerbations, a series of clinical studies were conducted with inhaled IFN-β (SNG001) (see online data supplement). A dose-escalating study in asthmatic volunteers showed that nebulised IFN-β (6 million international units (MIU)) given once daily for 14 days is well tolerated and enhances innate immune responses in the airways, as assessed by several biomarkers of IFN-β-related anti-viral activity (neopterin, IFN-γ induced protein 10 (IP-10, CXCL10), myxoma resistance

Copyright © 2014 by the American Thoracic Society

protein 1 and 2'-5' oligoadenylate synthetase) measured in induced sputum (see Figure E1 in the online data supplement). This also provided evidence that CXCL10 may be a useful biomarker for clinical development of IFN-β.

A randomised placebo-controlled trial of IFN-β administered to asthmatics with a history of cold-induced exacerbations was, therefore, conducted to test the hypothesis that, when delivered by oral inhalation at the report of an URTI, IFN-β can prevent or substantially reduce the increase in asthma symptoms, thereby providing initial proof of concept for IFN-β as a potential treatment for virus-induced exacerbations.

Some of the results of these studies have been previously reported in the form of an abstract (14).

METHODS

STUDY DESIGN

This was a randomised, double-blind, parallel, placebo-controlled trial of IFN-β (SNG001) (see Figure 1 and 2) involving patients with a history of URTI-induced exacerbations, to test whether inhaled IFN-β can prevent or attenuate worsening asthma symptoms (defined as a rise in the 6-item Juniper Asthma Control Questionnaire [ACQ-6] (15)) caused by respiratory viruses if administered within 24 hours after reporting cold or influenza symptoms (ClinicalTrials.gov: NCT01126177). The primary endpoint was defined as the comparison of the mean change from Baseline to Day 8 in ACQ-6 in the modified intention to treat (mITT) population, i.e. those patients who fulfilled the Jackson (16) or Predy (17) criteria for a cold.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

Patients responded daily to text message questions about URTI symptoms. If they met the pre-set criteria for an URTI, they attended the research units within 24 hours (day 1) to begin treatment with inhaled IFN-β or placebo (randomised in a 1:1 ratio) given as single daily doses for 14 days. Patients were reviewed on days 4, 7, 10, 13 and 17 and recorded daily upper and lower respiratory symptoms and peak expiratory flow (PEF) measurements at home (see Table E1 in the online data supplement for the full study schedule).

STUDY TREATMENTS

SNG001 (Synairgen Research Ltd, Southampton, UK) consists of recombinant IFN-β1a formulated as an aqueous solution that, unlike some other commercial preparations, does not contain mannitol or human serum albumin and is pH neutral. Patients received 6 MIU of IFN-β or placebo (formulation buffer without IFN-β) from a portable mesh nebuliser delivered over 3 to 4 min (I-neb: Philips Respironics, Chichester, UK).

PATIENTS

The inclusion criteria for the pre-treatment phase included: a) age 18-65 years, b) asthma symptoms for ≥2 years confirmed by history and one of the following: Forced expiratory volume in 1 second ($FEV_{1)}$ reversibility to albuterol (≥12% and 200mL), bronchial hyperresponsiveness (at screening or historical), emergency admission or attendance at primary care or out-of-hours clinics for worsening asthma, c) history of cold-induced exacerbations and ≥1 exacerbation suspected to be caused by a respiratory virus in the past 24 months requiring oral corticosteroids and/or antibiotics, d) treatment with regular inhaled corticosteroids (ICS) (i.e. British Thoracic Society (BTS) Guidelines Step 2 and above) (18).

Copyright © 2014 by the American Thoracic Society

Patients entered the treatment phase if they fulfilled the following criteria: a) URTI symptoms within the last 24 hours presenting as cold symptoms (blocked or runny nose and a sore or scratchy throat) or influenza-like illness (temperature >37.8^{o}C, plus two of the following: headache, cough, sore throat, myalgia), b) patient's belief that they have a cold or flu, and c) continued regular ICS since screening and d) a post-bronchodilator FEV_1 ≥35% of predicted.

CLINICAL AND LABORATORY ASSESSMENTS

Patients were screened by history, physical examination, lung function testing and bronchial hyperresponsiveness (if asthma diagnosis required confirmation). To establish asthma control at screening baseline, patients completed the ACQ-6, and over 7 days, recorded twice daily PEF and responded to asthma symptom questions from the Asthma Index Questionnaire (AIQ) (19) (twice daily) and the Jackson Cold Score questionnaire (JCSQ) (16) (in the evening) by text message.

After establishing the screening baseline, patients responded to daily text messages asking them if they had symptoms of an URTI (see online data supplement for more details). If the response suggested that patients were experiencing cold or influenza symptoms, patients were telephoned to assess eligibility for the treatment phase using the JCSQ to confirm the onset of cold symptoms within the past 24 hours. For the next 28 days, beginning on treatment day 1, the text messages switched to questions from the JCSQ and four questions from the AIQ (symptoms of chest tightness, wheeze, cough and shortness of breath, scored 0-3). The ACQ-6 was completed by telephone interview on day 1 (as treatment baseline) and on treatment day 8.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

Nasal lavage was collected during the first week and screened for a panel of respiratory viruses by quantitative real-time polymerase chain reaction (qPCR). Sputum induction was attempted at selected sites at screening and on treatment days 4 and 7 to quantify rhinovirus virus load and anti-viral biomarkers by qPCR and pro-inflammatory biomarkers by Luminex. Blood samples were collected for measurement of serum CXCL10 by ELISA and genome-wide gene expression analysis.

STATISTICAL ANALYSES

Primary analysis was conducted on data only from those patients who fulfilled the Jackson (16) or Predy (17) criteria for a cold: these patients constituted the modified intention-to-treat (mITT) population. All statistical tests were two-sided with a 5% level of significance, with no adjustments for multiplicity. The primary hypothesis (that IFN-β is superior to placebo in respect of the change from treatment baseline ACQ-6 (Day 1) to day 8 in the mITT population) was tested by analysis of covariance, including terms for pooled site and baseline value. Sample size calculation, based on data from a prospective multicenter study of asthma control associated with a cold (20), determined that 56 patients per treatment-arm provided 80% power to detect a mean treatment difference of 0.5 in the change from baseline in ACQ-6 8 days after onset of a cold, a change accepted as clinically relevant (21), with a between-patient standard deviation of 0.93. A planned blinded sample size re-calculation (22) was performed mid-study, resulting in no required modification.

Additional secondary trial endpoints are provided in the online data supplement; the statistical analysis plan (SAP) also stated that exploratory analyses in patient subgroups

Copyright © 2014 by the American Thoracic Society

defined by asthma severity may be investigated, although the definition of severity was not pre-specified.

RESULTS

PATIENTS

From a total of 319 recruited patients, 147, considered by the investigators to be developing a cold, were randomised into the treatment phase (intention-to-treat (ITT) population). Subsequent analysis showed that the majority (134 patients) went on to develop a cold, as judged by fulfilling either the Jackson or Predy cold criteria (16); these were, therefore, treated as the mITT population (see Table 1).

RELATIONSHIP BETWEEN VIRUS INFECTIONS AND ASTHMA SYMPTOMS

Respiratory viruses were detected by qPCR in nasal lavage in 63% of patients; more than one virus was detected in some samples. The majority of viruses detected were rhinovirus (68%), the rest being adenovirus (3%), bocavirus (23%), coronavirus (9%), enterovirus (4%), human metapneumovirus (1%), parainfluenza virus (4%) and respiratory syncytial virus (3%). Analysis of the time-course, conducted in the placebo group to avoid active treatment effects, showed that both asthma and cold symptoms increased markedly from screening baseline, peaking on day 2 of the treatment phase and gradually returning to baseline (see Figure 3A). There was a similar trend in the IFN-β group (Figure 3B). For most patients, complete sets of asthma symptom scores were available only from day 2, i.e. one day after starting treatment. At presentation, the URTI (cold) and asthma symptoms were positively correlated (r_s=0.48, p<0.0001) (see Figure 3C).

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

ANALYSIS OF DATA FROM THE mITT POPULATION

Due to missing data, 11 subjects were excluded from analysis of the primary outcome. Analysis of the effect of treatment on ACQ-6 from treatment baseline (day 1) to day 8, the primary outcome, showed that IFN-β was not superior to placebo in the mITT population (see Figure 4A). However, IFN-β was superior to placebo when assessing morning PEF (mean between group difference 19.47 L/min, 95% CI 1.62, 37.31; p=0.033 for area under the curve (AUC)), with steady improvement during IFN-β treatment but an initial decline during the first week of placebo treatment (see Figure 4C). There was also a trend towards reduced sputum rhinovirus load in the active group on day 4 (mean between-group difference -1.20 $\log_{10}$ copies/g, (95% CI -2.46, 0.07; p=0.063) (see Figure 4E).

Assessment of blood and sputum biomarkers, using a combination of immunoassay and qPCR, showed changes consistent with enhanced anti-viral activity and associated attenuation of pro-inflammatory responses in patients on IFN-β treatment. Prior to treatment, serum levels of the anti-viral cytokine, CXCL10, increased in both the active and placebo treated groups relative to screening but remained elevated over several days only during IFN-β treatment (see Figure 5A), whilst it fell rapidly on placebo (differences were significant on days 4, 7, 10 and 13 (p=0.037, 0.001 <0.001, <0.001, respectively, analysed on the log scale via analysis of covariance, including day 1 as a covariate)). There was a significant reduction in the pro-inflammatory biomarker CCL4 concentration (p=0.035) in the fluid phase of sputum from patients receiving IFN-β on treatment day 7 (see Figure 5B) and a trend towards reduced CXCL8 concentration (p=0.109) (see Figure 5C), without any between-group differences in these biomarkers on day 4. Analysis of sputum cell pellets by qPCR showed significantly increased gene expression for anti-viral biomarkers OAS1 (see

Copyright © 2014 by the American Thoracic Society

Figure 5D), Mx1 (see Figure 5E) and CXCL10 (see Figure 5F) on day 7 (p=0.0003, 0.0001 and 0.0008 respectively) as a consequence of treatment, with trends towards increases on day 4 for OAS1 and Mx1 (p=0.07, 0.06 respectively) but not CXCL10.

Fewer patients who received IFN-β required additional treatment, i.e. oral corticosteroids or antibiotics, for worsening asthma symptoms during the treatment period. Including one subject on placebo who just failed to meet the mITT JCSQ criteria due to data missing on day 1, five patients receiving placebo required additional treatment (4 required oral corticosteroids and 1 required antibiotics), and one of these was hospitalized, while only one patient on IFN-β required oral corticosteroids. Of importance, all the placebo treated patients who required additional treatment were from the difficult-to-treat patient population categorised as Step 4 according to the BTS severity criteria (18), in keeping with these patients having more severe exacerbations with viral infections.

ANALYSIS OF PATIENT SUBSETS BASED ON DIFFICULTY TO TREAT

Analysis of the placebo treated patients classified using the BTS criteria, showed that only the difficult-to-treat asthmatics at Step 4/5 (i.e. not the Step 2 and 3) had developed a clinically significant increase in asthma symptoms (rise in ACQ-6 ≥0.5) after a cold (see Figure 4B), supporting the observation that this group was more likely to require additional corticosteroid or antibiotic treatment. Further analysis of treatment effects on the primary outcome in this group of 54 patients, showed a mean ACQ-6 increase of 0.53 in the placebo group (n=30) and a decrease of 0.10 in the IFN-β group (n=24), a between-group difference of -0.63 (p=0.004) (see Figure 4B and Table 2a). Correspondingly, the percentage of patients

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

in whom individual changes in ACQ-6 were ≥0.5 was significantly lower for IFN-β (17%) as compared with placebo (50%) (p=0.012). Similarly, analysis of PEF changes during the treatment period showed significant IFN-β-related effects in Step 4/5 patients (mean between-group difference 31.42 L/min, 95% CI 3.21, 59.63; p=0.029 for AUC) (see Figure 4D and Table 2b).

In the Step 2 and 3 subgroups, the active and placebo treatments were not statistically different in respect of either ACQ-6 or PEF. Although the mean rise in ACQ-6 in the Step 2 subgroup receiving IFN-β was >0.5, this did not correspond to the observed changes in PEF which were in favour of IFN-β (see Table 2b).

Analysis of the effect of IFN-β treatment on CXCL10 responses in the whole mITT group was also performed on the BTS Step 4/5 subgroup; this showed a treatment-related increase in serum levels of similar significance to those in the entire mITT population (see Figure E2 in the online data supplement).

Blood samples collected from patients in the mITT population provided a unique opportunity to assess in a post-hoc analysis the effect of treatment on genome-wide gene expression. Good quality data from the analysis were obtained for 50, 43, and 48 patients from the Step 4/5 mITT group on days 1, 4 and 7, respectively. Differentially expressed gene lists for IFN-β versus placebo treatment were generated for days 4 (88 genes) and 7 (94 genes) by selecting probes that were significant at p<0.05 and had a fold change of >1.25 following analysis of covariance, including day 1 as a covariate. Analysis of these data using Ingenuity Pathway Analysis software (Ingenuity® Systems [www.ingenuity.com], Mountain View, CA, USA) suggested enhanced type I interferon signalling on both days (see Figure 6). After correction for multiple testing using a Benjamini-Hochberg procedure, interferon

Copyright © 2014 by the American Thoracic Society

signalling was the only canonical pathway that was significantly associated with the dataset ($p=0.015$, $p=1.02x10^{-5}$ on days 4 and 7, respectively). An upstream regulator analysis showed predicted activation of transcriptional factors associated with type I interferon signalling e.g. interferon regulatory factors and STAT1/ STAT2 (23).

MULTIPLE REGRESSION ANALYSIS

A multiple regression analysis was undertaken to explore whether any of the baseline assessments appeared to be related to subsequent changes in ACQ-6 (a score which reflects asthma symptoms over the previous 7 days) and to any potential treatment effects. The numbers of exacerbations in the last 24 months, screening lung function, BTS Step, use of long acting β-agonists, baseline cold and asthma symptom scores were all identified as potentially related to the change in ACQ-6 from treatment baseline to day 8. Based on a subsequent stepwise regression model, the daily asthma symptom score on treatment day 2 (positive slope; the higher the score the greater the increase in ACQ-6) and the interaction between study treatment and BTS Step appeared to have the most influence on the ACQ-6 change. Interestingly, daily cold and asthma symptom scores were worse in the active- compared to the placebo-treated BTS Step 2 patients immediately before the start of treatment. Taking daily asthma symptom scores at the start of treatment (day 2) into account, the estimated difference in change in ACQ-6 between IFN-β and placebo reduced in both BTS Steps 2 (from 0.41 to 0.3, $p=0.29$) and 3 (from 0.19 to 0.09, $p=0.73$) but increased in favour of IFN-β in the BTS Step 4/5 group (from -0.63 to -0.76, $p<0.001$), see Table E3 in the online data supplement.

SAFETY

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

Overall, inhaled IFN-β was well tolerated; no patient had to stop treatment and there was little difference between placebo and IFN-β in the frequency of treatment emergent adverse events with the exception of palpitations experienced by 5 subjects on IFN-β and none on placebo. All were mild and not considered clinically significant and no other cardiac events were recorded (see Table E4 and E5 in the online data supplement).

DISCUSSION

This study is the first demonstration of the ability of a biologic to improve anti-viral responses in patients with asthma that is also associated with a beneficial clinical effect. It is, in our view, a good example of translation of observations of enhanced innate immune responses from *ex vivo* studies (6) into proof of concept clinical studies. The study suggests that, when given at the time of reporting an URTI, inhaled IFN-β can ameliorate the way the airways of asthmatic patients respond to viral infection. While the trial did not show a positive effect on ACQ-6, the primary outcome, treatment with IFN-β had a positive effect on morning lung function (PEF) and, in parallel, enhanced innate immunity both systemically and in the lungs as assessed by serum CXCL10 concentration and induction of genes for anti-viral biomarkers CXCL10, Mx1 and OAS1 in induced sputum. This was associated with reduced pro-inflammatory cytokines in sputum and a trend towards reduced sputum rhinovirus load. Analysis of the moderate to severe asthmatics (Step 4 and 5 according to BTS guidelines) suggested a beneficial clinical effect of treatment, as shown by a mean 0.63 reduction in ACQ-6 score in patients treated with IFN-β (p=-0.004 as compared with those on placebo) that is widely viewed as being clinically significant. Genome-wide gene

Copyright © 2014 by the American Thoracic Society

expression analysis using circulating blood cells from this subset of patients also showed that treatment with nebulised IFN-β enhanced systemic innate immunity.

The lack of effect on the primary outcome is not surprising because the whole mITT population, which also included mild asthmatics, did not sustain an increased ACQ-6 ≥0.5 after cold onset during placebo treatment, suggesting that, overall, the exacerbations were not clinically significant in the whole population studied. Further, pre-specified analysis (as stated in the SAP) of patient subgroups, with asthma severity classified by BTS treatment step criteria (18, 21), showed a significant protective effect of IFN-β in patients from BTS Steps 4/5, i.e. those patients who require more intensive asthma maintenance treatment. The study, therefore, suggests that IFN-β treatment may be most appropriate for the more difficult to control asthmatics in whom the underlying disease process is likely to be more severe, thus requiring more intensive treatment, and in whom the risks of exacerbations, health impact and treatment costs are greatest (24).

Asthma exacerbations are a major target for novel therapeutic agents, including such biologics as omalizumab (25) and mepolizumab (26). Although effective at reducing exacerbations, these drugs are given long-term and systemically, and are pharmacodynamically unsuitable for acute administration. Inhaled corticosteroids, the mainstay of asthma treatment, also reduce the rate of exacerbations, but doubling their dose at the onset of an exacerbation does not reduce the severity or rate of recovery of the exacerbation (27). Thus, nebulised IFN-β treatment is the first therapy acting on the causal viral pathway that seems able to prevent worsening of asthma symptoms if administered shortly after patients become aware of a cold developing.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

IFN-β was selected for clinical development based on abundant evidence of its safety in multiple sclerosis (28-31) and efficacy in pre-clinical *in vitro* asthma studies (6, 11). Accordingly, measurement of the biomarker, CXCL10 in serum and anti-viral biomarkers in sputum cells (OAS1, Mx1 and CXCL10) demonstrated that inhaled IFN-β activated local anti-viral defence mechanisms effectively at the dose and frequency selected for this trial whilst, at the same time, attenuating pro-inflammatory mediators, as judged by concentrations of CCL4 and CXCL8 measured in airway secretions (sputum). Additionally, IFN-β treatment resulted in a modest, but significant, induction of systemic innate immune responses as judged by the genome-wide gene expression analysis of blood cells which showed significant differences in genes involved in type I interferon signalling. Together with the sustained and marked elevation of CXCL10 protein levels in blood, this suggests that treatment with IFN-β enhanced innate immunity which, in turn, improved the clinical outcome of the cold. The trend towards reduced load of rhinovirus in sputum observed with treatment suggests that better anti-viral defences may result in faster clearance of rhinoviruses. Further studies, including analysis of other viruses, are required for more definitive proof.

It was noted that BTS Step 2 patients (on low dose inhaled corticosteroids (ICS) and short-acting β-agonists) receiving IFN-β had a mean increase in ACQ-6 of 0.52. However, this was not significantly different to placebo and analysis of PEF data in this subgroup did not support a deleterious effect of IFN-β, mirroring the lack of IFN-β deficiency observed *in* vitro in mild patients(12), whilst this treatment had a positive effect on both ACQ-6 and PEF in patients at BTS Step 4/5. Although the number of patients in the BTS Step 2 group was

Copyright © 2014 by the American Thoracic Society

relatively small (15 placebo; 17 active), we cannot exclude the possibility that IFN-β treatment could have increased asthma symptoms in a small number of patients.

In summary, this study suggests for the first time that administration of IFN-β by inhalation can enhance innate immunity both locally within the lungs and, to a lesser extent, systemically, thereby compensating for the IFN-β deficiency that we have previously demonstrated *ex vivo* in the epithelium of patients with moderate-severe asthma(6). The possible beneficial clinical effect of treatment seen in patients with moderate-severe asthma that was associated with this enhancement suggests that this treatment may impact favourably on cold-induced asthma exacerbations. The trial was designed and powered on the basis of the entire mITT patient population; therefore, further, adequately powered studies, focusing on more difficult-to-treat asthmatics, are now needed to test the hypothesis that IFN-β is effective in this high risk patient population.

REFERENCES

1. Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, Cunningham A, Robinson BS, Myint SH, Ward ME, Tyrrell DA, Holgate ST. The relationship between upper respiratory infections and hospital admissions for asthma: A time-trend analysis. *Am J Respir Crit Care Med* 1996;154:654-660.
2. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, Johnston SL. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: A longitudinal cohort study. *Lancet* 2002;359:831-834.
3. Papadopoulos NG, Christodoulou I, Rohde G, Agache I, Almqvist C, Bruno A, Bonini S, Bont L, Bossios A, Bousquet J, Braido F, Brusselle G, Canonica GW, Carlsen KH, Chanez P, Fokkens WJ, Garcia-Garcia M, Gjomarkaj M, Haahtela T, Holgate ST, Johnston SL, Konstantinou G, Kowalski M, Lewandowska-Polak A, Lodrup-Carlsen K, Makela M, Malkusova I, Mullol J, Nieto A, Eller E, Ozdemir C, Panzner P, Popov T, Psarras S, Roumpedaki E, Rukhadze M, Stipic-Markovic A, Todo Bom A, Toskala E, van Cauwenberge P, van Drunen C, Watelet JB, Xatzipsalti M, Xepapadaki P, Zuberbier T. Viruses and bacteria in acute asthma exacerbations--a ga(2) len-dare systematic review. *Allergy* 2011;66:458-468.
4. Jackson DJ, Johnston SL. The role of viruses in acute exacerbations of asthma. *J Allergy Clin Immunol* 2010;125:1178-1187.
5. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: Gina executive summary. *Eur Respir J* 2008;31:143-178.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

6. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005;201:937-947.
7. Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, Kebadze T, Mallia P, Stanciu LA, Parker HL, Slater L, Lewis-Antes A, Kon OM, Holgate ST, Davies DE, Kotenko SV, Papi A, Johnston SL. Role of deficient type iii interferon-lambda production in asthma exacerbations. *Nat Med* 2006;12:1023-1026.
8. Sykes A, Edwards MR, Macintyre J, del Rosario A, Bakhsoliani E, Trujillo-Torralbo MB, Kon OM, Mallia P, McHale M, Johnston SL. Rhinovirus 16-induced ifn-alpha and ifn-beta are deficient in bronchoalveolar lavage cells in asthmatic patients. *J Allergy Clin Immunol* 2012;129:1506-1514.
9. Bosco A, Ehteshami S, Stern DA, Martinez FD. Decreased activation of inflammatory networks during acute asthma exacerbations is associated with chronic airflow obstruction. *Mucosal Immunol* 2010;3:399-409.
10. Edwards MR, Regamey N, Vareille M, Kieninger E, Gupta A, Shoemark A, Saglani S, Sykes A, Macintyre J, Davies J, Bossley C, Bush A, Johnston SL. Impaired innate interferon induction in severe therapy resistant atopic asthmatic children. *Mucosal Immunol* 2013;6:797-806.
11. Cakebread JA, Xu Y, Grainge C, Kehagia V, Howarth PH, Holgate ST, Davies DE. Exogenous ifn-beta has antiviral and anti-inflammatory properties in primary bronchial epithelial cells from asthmatic subjects exposed to rhinovirus. *J Allergy Clin Immunol* 2011;127:1148-1154.
12. Lopez-Souza N, Favoreto S, Wong H, Ward T, Yagi S, Schnurr D, Finkbeiner WE, Dolganov GM, Widdicombe JH, Boushey HA, Avila PC. In vitro susceptibility to rhinovirus infection is greater for bronchial than for nasal airway epithelial cells in human subjects. *J Allergy Clin Immunol* 2009;123:1384-1390.
13. Sykes A, Macintyre J, Edwards MR, Del Rosario A, Haas J, Gielen V, Kon OM, McHale M, Johnston SL. Rhinovirus-induced interferon production is not deficient in well controlled asthma. *Thorax* 2014;69:240-246.
14. Boxall C, Dudley S, Beegan R, Tear V, Hrebien S, Lunn K and Monk P. Effect of inhaled sng001 (interferon-beta 1a) on sputum and blood antiviral biomarkers following a respiratory virus infection in asthmatic subjects [abstract]. *Eur Respir J Suppl* 2013;42:4369.
15. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. *Respir Med* 2005;99:553-558.
16. Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. *AMA Arch Intern Med* 1958;101:267-278.
17. Predy GN, Goel V, Lovlin R, Donner A, Stitt L, Basu TK. Efficacy of an extract of north american ginseng containing poly-furanosyl-pyranosyl-saccharides for preventing upper respiratory tract infections: A randomized controlled trial. *CMAJ* 2005;173:1043-1048.
18. British Thoracic Society Scottish Intercollegiate Guidelines N. British guideline on the management of asthma. *Thorax* 2008;63 Suppl 4:iv1-121.
19. Sorkness RL, Gonzalez-Fernandez G, Billmeyer EE, Evans MD, Gern JE, Jarjour NN. The asthma index: A continuous variable to characterize exacerbations of asthma. *J Allergy Clin Immunol* 2008;122:838-840.
20. Walter MJ, Castro M, Kunselman SJ, Chinchilli VM, Reno M, Ramkumar TP, Avila PC, Boushey HA, Ameredes BT, Bleecker ER, Calhoun WJ, Cherniack RM, Craig TJ, Denlinger LC, Israel E, Fahy JV, Jarjour NN, Kraft M, Lazarus SC, Lemanske RF, Jr., Martin RJ, Peters SP, Ramsdell JW, Sorkness CA, Sutherland ER, Szefler SJ, Wasserman SI, Wechsler ME, National Heart L, Blood Institute's Asthma Clinical Research N. Predicting worsening asthma control following the common cold. *Eur Respir J* 2008;32:1548-1554.
21. Reddel HK, Taylor DR, Bateman ED, Boulet L-P, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, de Jongste JC, Kerstjens HAM, Lazarus SC, Levy ML, O'Byrne PM, Partridge MR, Pavord ID, Sears MR, Sterk PJ, Stoloff SW, Sullivan SD, Szefler SJ, Thomas MD, Wenzel SE, Control

Copyright © 2014 by the American Thoracic Society

obotATSERSTFoA, Exacerbations. An official american thoracic society/european respiratory society statement: Asthma control and exacerbations. *Am J Respir Crit Care Med* 2009;180:59-99.

22. Kieser M, Friede T. Simple procedures for blinded sample size adjustment that do not affect the type i error rate. *Stat Med* 2003;22:3571-3581.

23. Honda K, Takaoka A, Taniguchi T. Type i interferon [corrected] gene induction by the interferon regulatory factor family of transcription factors. *Immunity* 2006;25:349-360.

24. Serra-Batlles J, Plaza V, Morejon E, Comella A, Brugues J. Costs of asthma according to the degree of severity. *Eur Respir J* 1998;12:1322-1326.

25. Rodrigo GJ, Neffen H, Castro-Rodriguez JA. Efficacy and safety of subcutaneous omalizumab vs placebo as add-on therapy to corticosteroids for children and adults with asthma: A systematic review. *Chest* 2011;139:28-35.

26. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, Ortega H, Chanez P. Mepolizumab for severe eosinophilic asthma (dream): A multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;380:651-659.

27. Harrison TW, Oborne J, Newton S, Tattersfield AE. Doubling the dose of inhaled corticosteroid to prevent asthma exacerbations: Randomised controlled trial. *Lancet* 2004;363:271-275.

28. Lampl C, You X, Limmroth V. Weekly im interferon beta-1a in multiple sclerosis patients over 50 years of age. *Eur J Neurol* 2012;19:142-148.

29. De Stefano N, Sormani MP, Stubinski B, Blevins G, Drulovic JS, Issard D, Shotekov P, Gasperini C. Efficacy and safety of subcutaneous interferon beta-1a in relapsing-remitting multiple sclerosis: Further outcomes from the improve study. *J Neurol Sci* 2012;312:97-101.

30. Kappos L, Traboulsee A, Constantinescu C, Eralinna JP, Forrestal F, Jongen P, Pollard J, Sandberg-Wollheim M, Sindic C, Stubinski B, Uitdehaag B, Li D. Long-term subcutaneous interferon beta-1a therapy in patients with relapsing-remitting ms. *Neurology* 2006;67:944-953.

31. Panitch H, Goodin DS, Francis G, Chang P, Coyle PK, O'Connor P, Monaghan E, Li D, Weinshenker B, Efficacy ESGEoID-rENAC, University of British Columbia MSMRIRG. Randomized, comparative study of interferon beta-1a treatment regimens in ms: The evidence trial. *Neurology* 2002;59:1496-1506.

LEGENDS

Table 1. Patient characteristics in the mITT population. Patients who fulfilled either the Jackson or Predy criteria (16, 17) for a cold were included in the modified intention-to-treat (mITT) group. There were no clinically important differences between patients randomised to receive IFN-β or placebo in respect of any clinical criterion, either when assessing the mITT population as a whole or when subgrouped by asthma severity, using British Thoracic Society (BTS) Steps (18, 21).

*Atopy percentages may not always add up to 100% due to rounding.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

**Two patients taking 200µg/day inhaled corticosteroids (fluticasone equivalent) were taking four classes of asthma drug as part of their normal asthma medication and were, therefore, assigned to the BTS step 4 subgroup. One subject taking 200µg/day inhaled corticosteroids (fluticasone equivalent) was taking oral corticosteroids as part of their normal asthma medication and was assigned to the BTS Step 5 subgroup.

Table 2a Analysis of ACQ-6 during the treatment period by asthma severity (BTS treatment Step) in the mITT population. The ACQ-6 scores were recorded on days 1 (treatment baseline) and 8 (see methods).

Table 2b Analysis of PEF during the treatment period by asthma severity (BTS treatment Step) in the mITT population. Morning (am) PEF was measured daily at home and an area under the curve (AUC) analysis was calculated for change from day 2 during the treatment period (beginning on day 2 and ending on day 14). The AUC data were then divided by the number of days to provide a clinically meaningful outcome (AUC data are shown as L/min per day).

Figure 1. CONSORT flow diagram

Figure 2. Study design. During the pre-treatment phase, patients responded to daily text messages enquiring about symptoms of cold. If the pre-specified threshold for symptoms was reached, they visited the research unit within 24 hours to receive their first dose. Thereafter, patients received their daily treatment of IFN-β or placebo for a total of 14 days. The primary outcome, a validated shortened version of the Asthma Control Questionnaire

Copyright © 2014 by the American Thoracic Society

(ACQ-6)(15), was completed during screening, before treatment began on day 1, and seven days later. For details of biological samples collected, see methods section and Table E1 in the online data supplement.

Figure 3. Daily cold and asthma symptom scores in patients not receiving active treatment. Symptoms (mean ± SEM) were analysed in patients randomised to the placebo arm of the trial (n=54-69) to avoid any effects of active treatment. This showed that both cold (closed symbol) and asthma (open symbol) symptoms peaked at presentation, before treatment was initiated, and declined to baseline in a similar manner over a period of about 17 days (A). There was a similar trend in the IFN-β group (B). At presentation there was a highly significant monotonic relationship (Spearman rank correlation = 0.48, $p<0.0001$) between URTI (cold) and asthma symptoms (n=61) (C).

Figure 4. Effect of nebulized IFN-β on clinical outcomes. Analysis of the mITT population (A) showed that IFN-β treatment (n=58) did not significantly affect the change in ACQ-6 scores (LS mean ± SEM) at Day 8 (from treatment baseline) compared to placebo (n=65). Further analysis of the subgroup with difficult-to-treat asthma (i.e. BTS Step 4/5) (B) showed an increase (LS mean ± SEM) in ACQ-6 of 0.53 in the placebo group (n=30) and a decrease of 0.10 in the IFN-β group (n=24), a between-group difference of -0.63 (95% CI -1.05, -0.21; p=0.004). In the whole mITT population, treatment with IFN-β (closed symbols) resulted in faster recovery of morning peak expiratory flow (PEF) (C), compared to placebo (open symbols) measured daily at home (p=0.033 for AUC analysis; n=56 for placebo; n=58 for IFN-β, dashed line represents clinically relevant difference). This improvement was also seen in

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

the BTS Step 4/5 (D) subgroup of patients (p=0.029 for AUC; n=25 for placebo; n=22 for IFN-β). Analysis of sputum (E) obtained on day 4 from patients, in whom rhinovirus was detected in nasal lavage, showed a trend towards reduced rhinovirus load (p=0.063) in IFN-β treated patients (n=9) compared to placebo (n=14).

Figure 5. Induction of innate immunity by IFN-β treatment. A more sustained rise (mean ± SEM) in serum concentrations of CXCL10 (A) was measured in patients treated with IFN-β (n=58-65; closed symbols) when compared to those on placebo (n=62-66; open symbols). Data were analysed on the log scale via analysis of covariance, including day 1 as a covariate (p=0.037, 0.001, <0.001 and <0.001 for days 4, 7, 10 and 13). By comparison with placebo (n=25) treatment, the concentrations of CCL4 (B) in the sputum fluid phase measured on day 7 was significantly (p=0.035, data were $\log_{10}$ transformed and analysed by unpaired T test) lower in patients on IFN-β (n=24) and there was a trend towards lower CXCL8 (p=0.109) (C). Gene expression of anti-viral biomarkers OAS1 (D), Mx1 (E) and CXCL10 (F) in sputum cells from patients treated with IFN-β (n=16) was significantly higher when compared to placebo (n=20) on day 7 (p=0.0003, 0.0001 and 0.0008 respectively). Data are from patients in the mITT population.

Figure 6. Up-regulation of the Type I IFN-β canonical pathway by IFN-β treatment. Analysis of circulating blood cell microarray data using Ingenuity Pathway Analysis software (Ingenuity® Systems [www.ingenuity.com], Mountain View, CA, USA) showed a significant up-regulation of elements (highlighted in red) of the IFN-β signalling pathway at both day 4 and day 7 after correction for multiple testing using a Benjamini-Hochberg procedure. Data are from patients in the BTS Step 4/5 mITT population.

Copyright © 2014 by the American Thoracic Society

Population		Whole mITT		BTS Step 2		BTS Step 3		BTS Step 4/5	
Treatment		Placebo (n=69)	IFN-β (n=65)	Placebo (n=15)	IFN-β (n=17)	Placebo (n=23)	IFN-β (n=21)	Placebo (n=31)	IFN-β (n=27)
Age	Years (range)	39.6 (19-64)	37.0 (19-64)	42.3 (25-58)	33.6 (21-61)	40.3 (19-64)	32.9 (19-57)	37.8 (19-61)	42.2 (23-64)
Gender	Male/ female (%)	30/70	37/63	33/67	24/76	30/70	43/57	29/71	41/59
Atopy status (skin prick test)	Atopic/ Non-atopic/ Not tested (%)*	74/25/1	80/17/3	67/33/0	65/24/12	74/22/4	86/14/0	77/23/0	85/15/0
Smoking status	Non-smoker/ smoker (%)	87/13	88/12	87/13	88/12	83/17	90/10	90/10	85/15
Smoking history	Pack years current smoker/ ex-smoker	13.3/8.2	7.8/5.8	22.0/16.3	0.3/2.3	8.1/3.1	14.0/2.0	14.3/3.5	8.5/9.9
Dose of Inhaled corticosteroids (prior to treatment baseline)	µg/day (Fluticasone equivalent) Median (range)	400 (100-2000)	400 (50-2000)	200 (100-400)	200 (50-400)	200 (100-400)	200 (100-400)	750 (200-2000)**	800 (200-2000)**
Pre-broncho-dilator FEV_1 at screening	% of predicted (mean (SD))	90.9 (20.4)	88.9 (19.0)	92.7 (22.7)	94.2 (19.5)	91 (18.8)	91.1 (18.7)	90.0 (21.0)	83.8 (18.5)
Pre-broncho-dilator FEV_1 at treatment baseline	% of predicted (mean (SD))	89.1 (18.5)	88.8 (18.4)	88.7 (19.5)	90.3 (19.9)	89.0 (18.8)	91.1 (17.6)	89.3 (18.5)	86.2 (18.5)
Post-broncho-dilator FEV_1 at screening	% of predicted (mean (SD))	98.3 (18.2)	95.6 (17.5)	99.4 (20.3)	101.5 (16.0)	97.0 (17.2)	97.8 (17.3)	98.7 (18.3)	90.1 (17.6)
Post-broncho-dilator FEV_1 at treatment baseline	% of predicted (mean (SD))	96.2 (16.7)	94.9 (16.4)	98.1 (16.9)	98.5 (14.1)	95.6 (16.6)	96.2 (15.9)	95.8 (17.1)	91.8 (17.9)
Jackson Cold Score at treatment baseline (max. 24)	Score (mean (SD))	8.2 (3.7)	9.1 (4.3)	7.2 (3.5)	8.9 (3.6)	8.8 (4.5)	10.1 (3.5)	8.5 (3.7)	8.1 (4.6)
ACQ-6 at screening (0-6)	Score (mean (SD))	1.26 (0.88)	1.20 (0.91)	1.08 (0.69)	0.89 (0.62)	1.19 (0.83)	0.90 (0.82)	1.41 (0.99)	1.63 (1.03)

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

ACQ-6 at treatment baseline (0-6)	Score (mean (SD))	1.57 (0.98)	1.44 (0.95)	1.24 (0.78)	1.23 (0.82)	1.45 (0.97)	1.07 (0.84)	1.82 (1.02)	1.87 (0.97)

Table 1

	Change in ACQ-6 from treatment baseline to day 8							
BTS Step Group	**Whole mITT**		**Step 2**		**Step 3**		**Step 4/5**	
Treatment group	**Placebo**	**IFN-β**	**Placebo**	**IFN-β**	**Placebo**	**IFN-β**	**Placebo**	**IFN-β**
N	65	58	14	17	21	17	30	24
Least Squares Mean	0.31	0.20	0.12	0.52	0.13	0.32	0.53	-0.1
Mean difference (95% CI)	-0.11 (-0.40,0.19)		0.41 (-0.15, 0.97)		0.19 (-0.31, 0.70)		-0.63 (-1.05, -0.21)	
p-value	0.469		0.15		0.45		0.004	

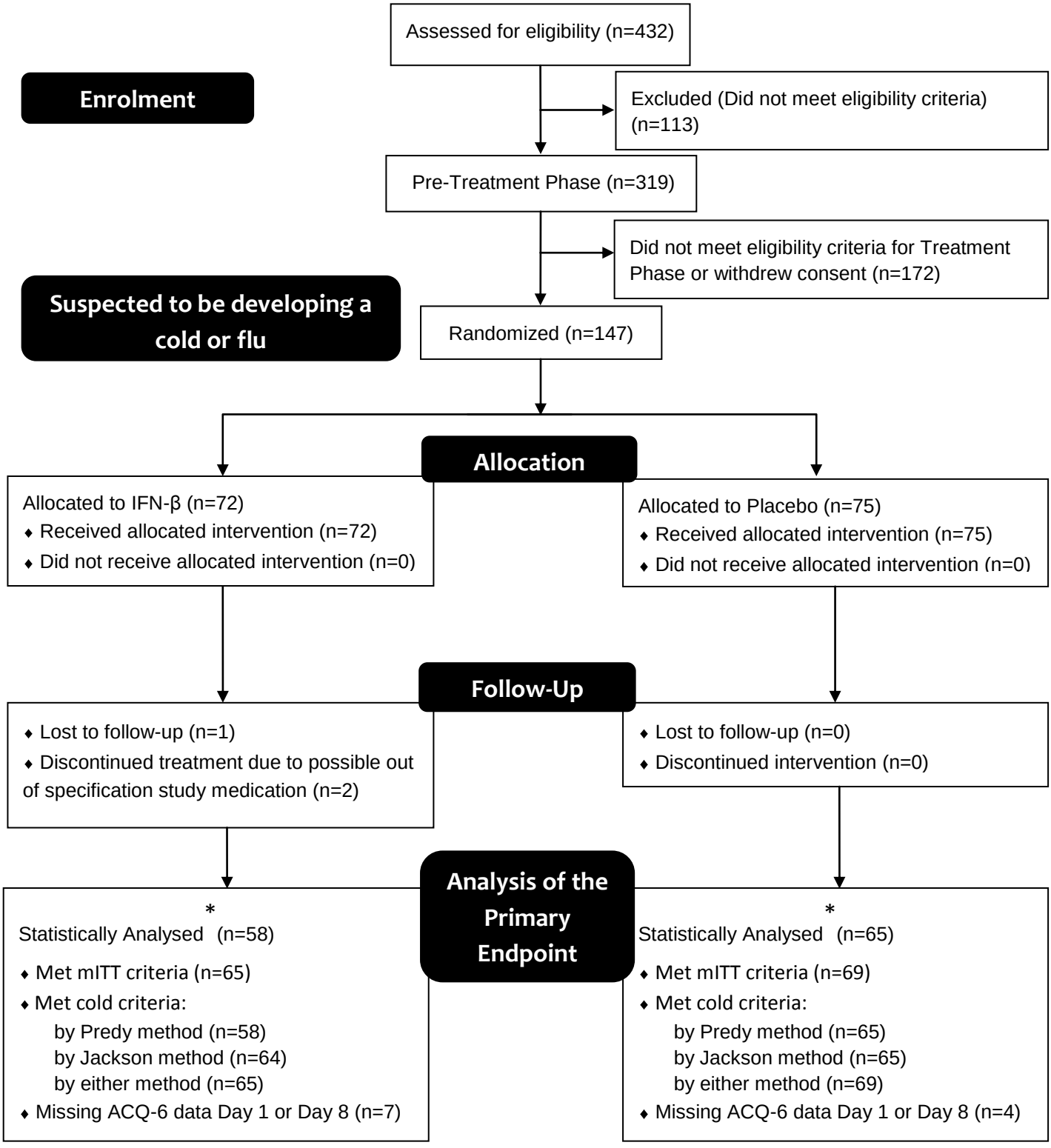
Table 2a

	Change in morning PEF (AUC) from Day 2 to 14							
BTS Step Group	**Whole mITT**		**Step 2**		**Step 3**		**Step 4/5**	
Treatment group	**Placebo**	**IFN-β**	**Placebo**	**IFN-β**	**Placebo**	**IFN-β**	**Placebo**	**IFN-β**
N	56	58	14	16	17	20	25	22
Least Squares Mean	-5.76	13.71	-3.28	2.93	-7.39	9.69	-6.13	25.29
Mean difference (95% CI)	19.47 (1.62,37.31)		6.21(-28.99,41.42)		17.08(-14.62,48.78)		31.42(3.21,59.63)	
p-value	0.033		0.727		0.288		0.029	

Table 2b

Copyright © 2014 by the American Thoracic Society

CONSORT Flow Diagram



* NB: patients who did not have missing data

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

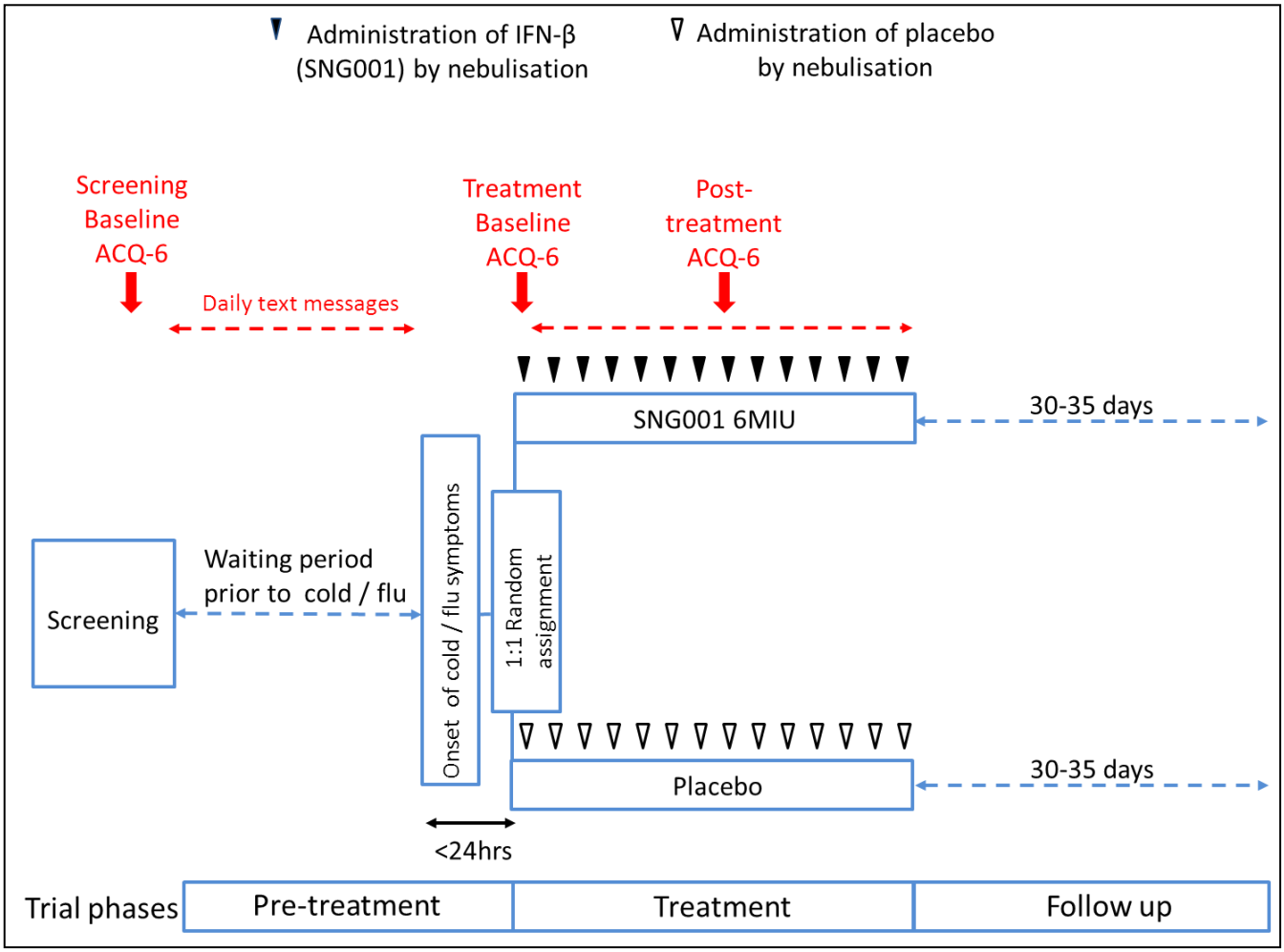


Figure 2

Copyright © 2014 by the American Thoracic Society

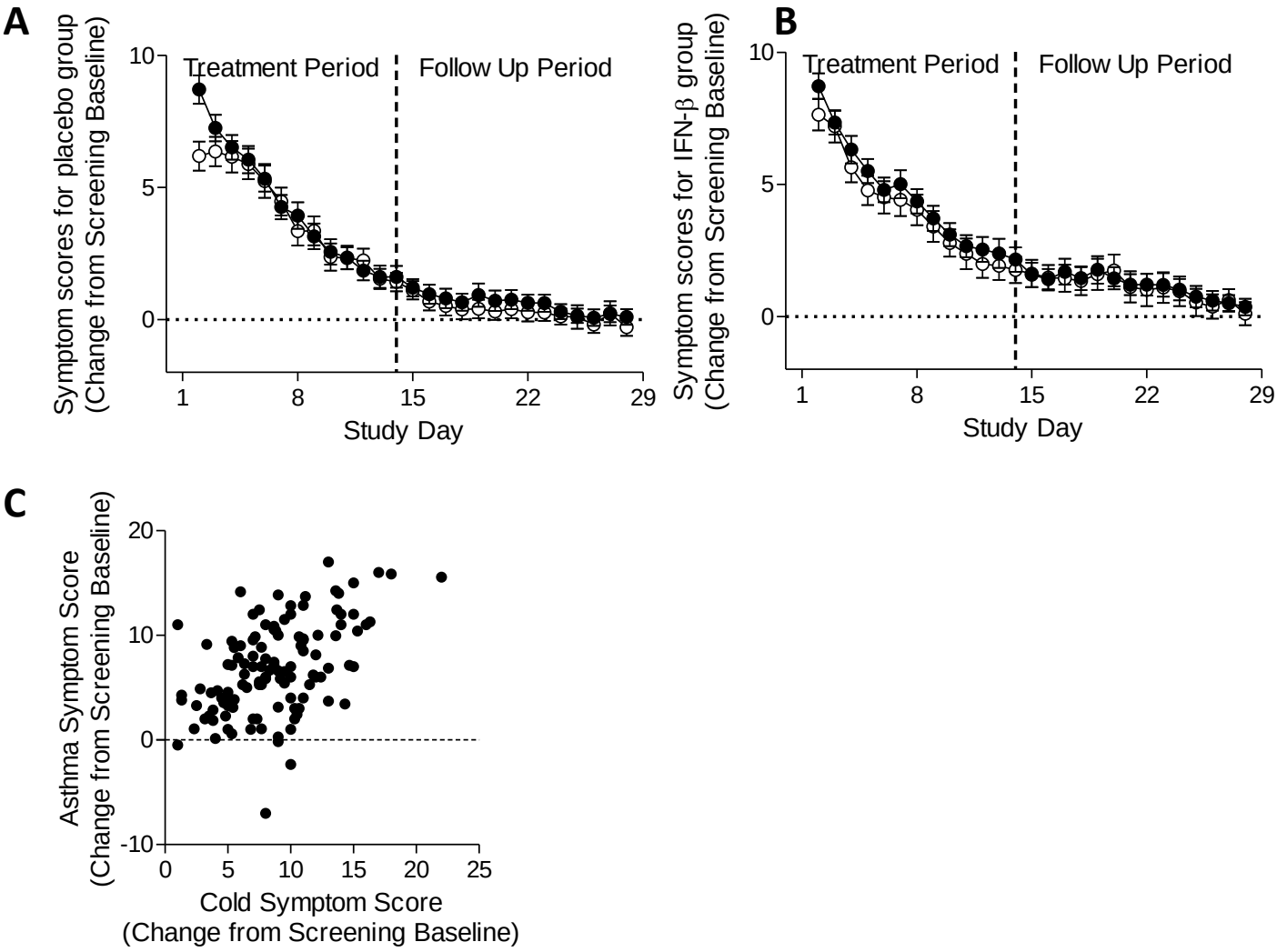


Figure 3 A-C

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

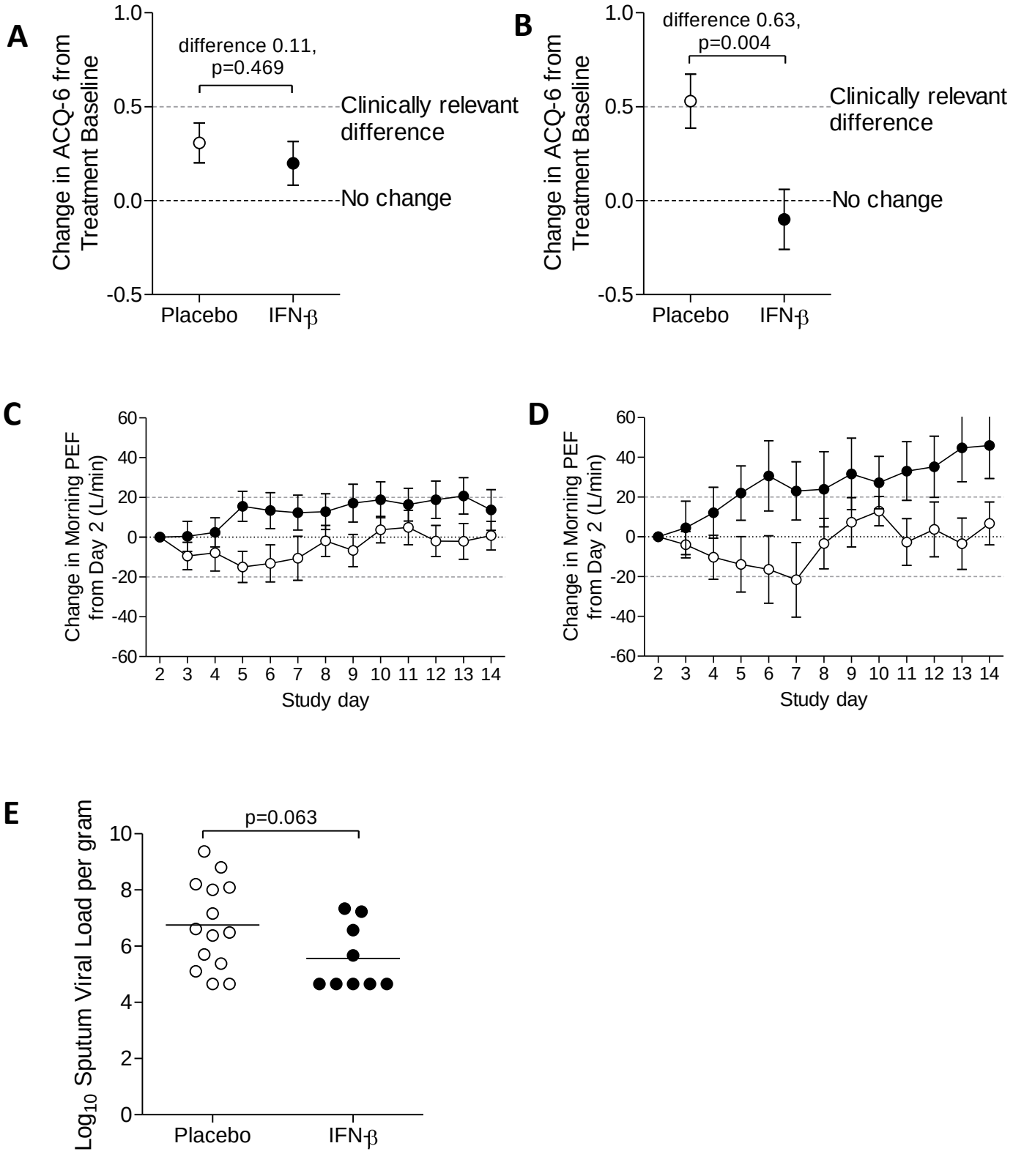


Figure 4 A-E

Copyright © 2014 by the American Thoracic Society

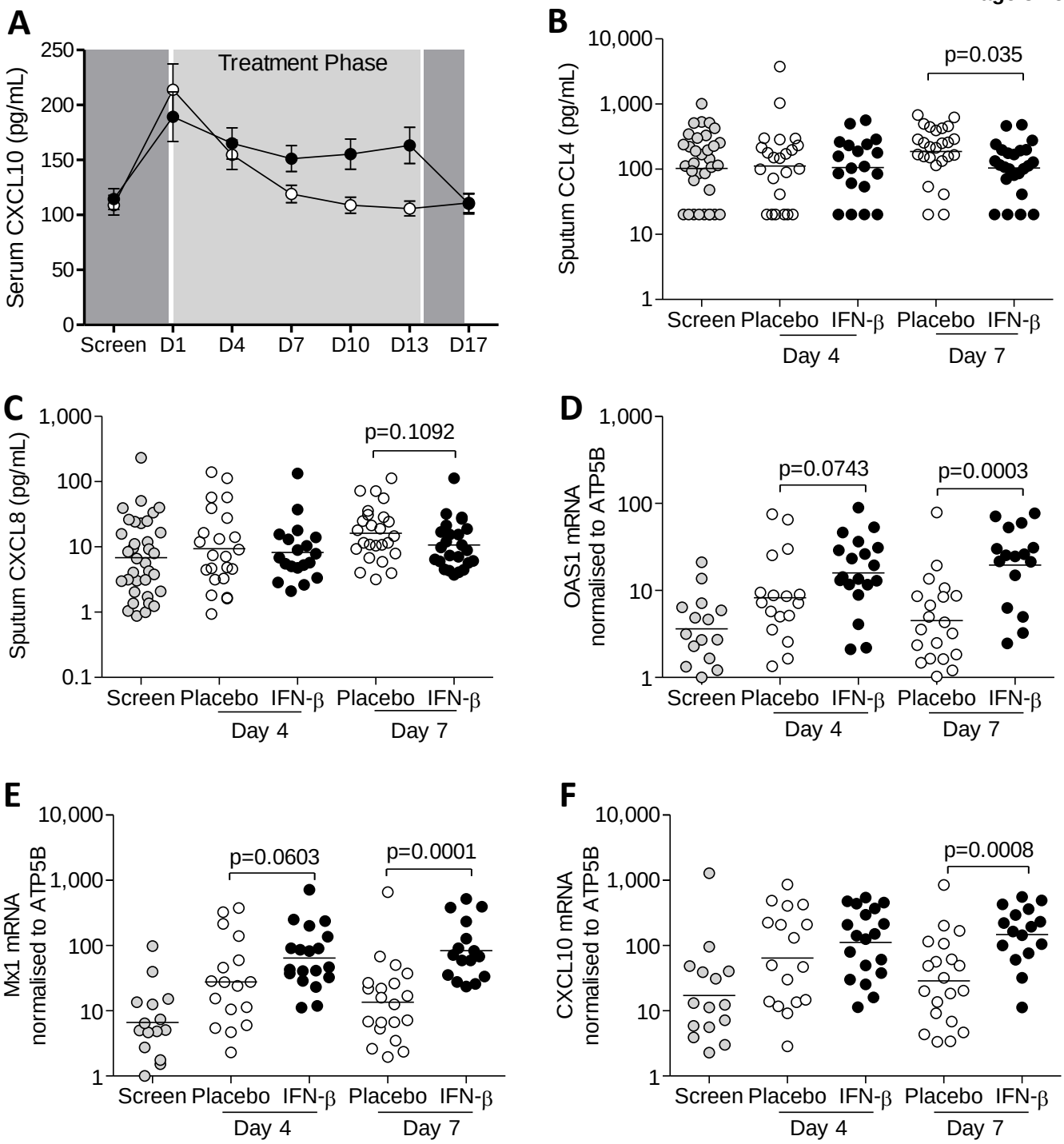


Figure 5 A-F

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

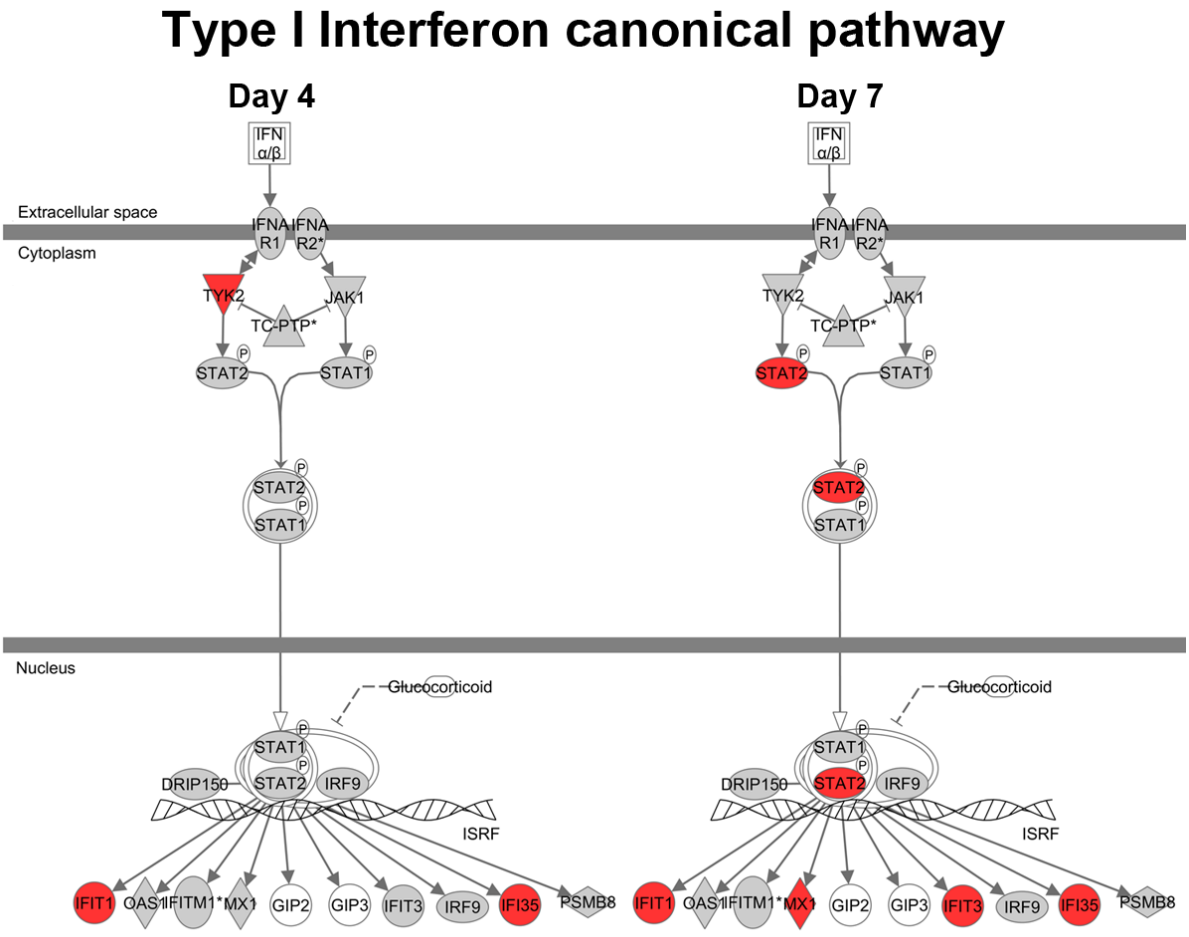


Figure 6

Copyright © 2014 by the American Thoracic Society

The effect of inhaled interferon-beta on worsening of asthma symptoms caused by viral infections: a randomised trial

Online data supplement

Authors:

Ratko Djukanović, M.D.[1], Tim Harrison, M.D.[2], Sebastian L. Johnston, M.D.[3], Flic Gabbay, M.D.[4] Peter Wark, M.D.[5], Neil C Thomson, M.D.[6], Robert Niven, M.D.[7], Dave Singh, M.D.[8], Helen K Reddel, PhD[9], Donna E. Davies, PhD[1], Richard Marsden, B.A.[10], Christine Boxall, PhD[10], Sarah Dudley, PhD[10], Vincent Plagnol, PhD[11], Stephen T. Holgate, M.D.[1], Phillip Monk, PhD[10] and the **INTERCIA** study group*

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

*Members of the **INTERCIA** (**INTER**feron-beta treatment of **C**old-**I**nduced **A**sthma exacerbations) group

Ratko Djukanović, M.D., NIHR Southampton Respiratory Biomedical Research Unit, Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, United Kingdom.

Donna E Davies, PhD, M.D., NIHR Southampton Respiratory Biomedical Research Unit, Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, United Kingdom.

Stephen T Holgate, M.D., NIHR Southampton Respiratory Biomedical Research Unit, Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, United Kingdom.

Tim Harrison, M.D., Nottingham Respiratory Biomedical Research Unit, University of Nottingham, Nottingham City Hospital, Nottingham, United Kingdom.

Christopher Brightling, M.D., Institute for Lung Health, Department of Infection, Inflammation and Immunity, Glenfield Hospital, University of Leicester, Leicester, United Kingdom.

Sebastian Johnston, M.D., Airway Disease Infection Section, National Heart & Lung Institute and Centre for Respiratory Infections, Imperial College, MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, Imperial College NHS Trust NIHR Comprehensive Biomedical Research Centre, London, United Kingdom.

Robert Niven, M.D., Manchester Academic Health Sciences Centre, University of Manchester and University Hospital of South Manchester UHSM, Manchester United Kingdom.

Copyright © 2014 by the American Thoracic Society

Helen Reddel, M.D., Woolcock Institute of Medical Research, Glebe, Australia.

Ferdinandus de Looze, M.D., Trialworks Clinical Research and the School of Medicine, University of Queensland, Brisbane, Australia.

Peter Wark, M.D., Priority Research Centre for Asthma and Respiratory Diseases, Hunter Medical Research Institute and The University of Newcastle, Newcastle, New South Wales, Australia.

Phillip Bardin, M.D., Respiratory and Sleep Medicine, Monash Medical Centre, Melbourne, Australia

Neil C Thomson, M.D., Institute of Infection, Immunity and Inflammation, University of Glasgow and Gartnavel General Hospital, Glasgow, United Kingdom.

Liam Heaney, M.D., Centre for Infection and Immunity, Belfast City Hospital, Queen's University Belfast, Belfast, United Kingdom.

Lorcan McGarvey, M.D., Centre for Infection and Immunity, Belfast City Hospital, Queen's University Belfast, Belfast, United Kingdom.

Ian Sabroe, M.D., Department of Infection and Immunity, Royal Hallamshire Hospital, University of Sheffield, Sheffield, United Kingdom

Bernard Higgins, M.D., Respiratory Medicine, Freeman Hospital, Newcastle upon Tyne, United Kingdom.

Mark Arya, M.D., Marouba Medical Centre, Marouba, Australia.

Christopher Strang, M.D., Mortimer Surgery, Mortimer, United Kingdom.

Najib Rahman, M.D., Oxford Centre for Respiratory Medicine, National Institute for Health Research Biomedical Research Centre, Churchill Hospital, Oxford, United Kingdom

Babatunde Oyesile, M.D., Synexus Midlands Dedicated Research Centre, Birmingham, United Kingdom

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

Hawys Thomas, M.D., Synexus Wales Dedicated Research Centre, Cardiff, United Kingdom

Essam Hakim, M.D., Synexus Merseyside Dedicated Research Centre, Liverpool, United Kingdom

Phillip Monk, PhD, Synairgen Research Limited, Southampton General Hospital, Southampton, United Kingdom.

Jody Brookes, BSc, Synairgen Research Limited, Southampton General Hospital, Southampton, United Kingdom.

Christine Boxall, PhD, Synairgen Research Limited, Southampton General Hospital, Southampton, United Kingdom.

Sarah Dudley, PhD, Synairgen Research Limited, Southampton General Hospital, Southampton, United Kingdom.

Rona Beegan, Synairgen Research Limited, Southampton General Hospital, Southampton, United Kingdom.

Joanna Samways, MSc, Synairgen Research Limited, Southampton General Hospital, Southampton, United Kingdom.

Copyright © 2014 by the American Thoracic Society

SUMMARY OF TRIALS LEADING TO THE PHASE II TRIAL REPORTED IN THE ARTICLE

The phase II clinical trial reported in the article was preceded by two double-blind, randomised, placebo controlled, dose-escalating trials: a phase I trial (SG003), assessing the safety and tolerability of inhaled interferon-β (IFN-β) in atopic, non-asthmatic individuals with no airways hyperresponsiveness, as verified by absence of responsiveness to methacholine at 8 mg/mL, and a second phase Ib trial (SG004), assessing the safety and tolerability of inhaled IFN-β in moderately severe asthmatics treated with inhaled corticosteroids.

The first trial (SG003) was a dose-escalating study, starting with a single low dose of IFN-β ($0.48x10^6$ IU) and increasing the doses ($0.48x10^6$ IU, $2.4x10^6$ IU and $4.8x10^6$ IU) based on tolerability and safety. Thirty-six patients with evidence of atopic sensitisation to common aero-allergens (as determined by skin prick tests) were studied in four cohorts of nine subjects, within which seven were given active treatment and two placebo. This population was chosen because they share some of the features of immune responses (e.g. high IgE production and T lymphocyte responses) to common aero-allergens with atopic asthmatics. They can also have raised eosinophil counts in the airways, albeit at levels below those in asthmatics[E1]. Eosinophils are an important feature of all allergic diseases, including asthma, and their numbers tend to rise in the airways during allergic reactions in the airways[E2]. However, in contrast to asthmatics, atopic non-asthmatics do not have bronchial airways hyperresponsiveness, another hallmark of asthma, which makes asthmatics susceptible to developing bronchoconstriction when exposed to asthma triggers. Thus, any inflammatory reaction to IFN-β that might exacerbate asthma and involves eosinophils was not expected

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

to result in bronchospasm but could be readily detected by the tests that were conducted during the trial (e.g. sputum induction).

Of relevance to the current trial, the SG003 trial showed that IFN-β delivered via the same nebuliser (I-neb) was well tolerated by patients and had no effect on the primary outcomes, sputum eosinophilia and exhaled nitric oxide (eNO) concentrations measured 24 hours post-dosing. This was equally true for the low dose ($0.48x10^6$ IU) and high dose ($4.8x10^6$ IU) IFN-β. There were also no changes in lung function, including spirometry and diffusing capacity of the lung for carbon monoxide (DLCO).

The second trial (SG004) was also a dose-escalating study ($0.4x10^6$ IU, $1.5x10^6$ IU and $6x10^6$ IU) to assess the general safety and tolerability of IFN-β in controlled asthmatic patients when administered as a single dose and as multiple, escalating doses over a 14 day period, using the same I-neb device. This trial showed that nebulised IFN-β was tolerated in the type of patient in whom IFN-β would be tested for efficacy and further safety and tolerability assessment. This trial was also used to assess the value of biomarkers in providing evidence that the nebulised IFN-β was reaching the lungs in sufficient quantities to have a biological effect and to guide the schedule of drug administration.

As shown in figure E1, administration of nebulised IFN-β resulted in a rise in sputum and blood markers of innate immunity which are known to be inducible by IFN-β[E3], some of which we have shown previously to be induced by *ex vivo* rhinovirus infection of epithelial cells[E4]. Markers remained elevated in sputum samples collected at visits that fell 24 hours but not 72 hours post-dosing, thus supporting a once-a-day dosing regimen (data not shown).

Copyright © 2014 by the American Thoracic Society

STUDY DESIGN OF THE REPORTED TRIAL

The trial reported in the main article was a randomised, double-blind, parallel, placebo-controlled trial of IFN-β (SNG001) (Figure 1 and 2 in the main article). The primary objective was to evaluate whether inhaled IFN-β can prevent or attenuate worsening asthma symptoms (defined as a rise in Asthma Control Questionnaire-6 [ACQ-6])[E6] caused by respiratory viruses if administered within 24 hours after onset of a cold. This was also set as the primary outcome variable (see below). The trial consisted of screening, pre-treatment, treatment and follow-up phases.

Eligible patients (criteria given in the main article) were enrolled into the pre-treatment phase based on a history of upper respiratory tract infection (URTI)-induced asthma exacerbations that had occurred within the past 24 months. Thereafter, patients were requested to respond daily to a questionnaire sent to them by automated text messaging from a central computer which they received via their cell-phone. The aim of this monitoring was to pick up symptoms suggestive of an URTI and to allow investigators to arrange a visit to their research units within 24 hours of onset of symptoms so as to begin treatment as soon as possible and, thereby, prevent and/or reduce the impact of the cold on lower airways symptoms. Patients were asked to respond to the following questions via SMS text: 1) Do you have a sore throat? 2) Do you have more nasal symptoms than normal? and 3) Do you think you might have a cold or flu? Patients were asked to confirm the presence or absence of symptoms with ‘yes’ (score of 1) or ‘no’ (score of 0). If the aggregate score was 2 or more, or the patient believed they might have a cold or flu, a member of the study team had to telephone the patient to ascertain whether they should be considered for entry into the treatment phase. Patients were enrolled into the treatment phase if they had

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

continued inhaled corticosteroid use since screening and for safety reasons, had a post-bronchodilator $FEV_1 \geq 35\%$ of predicted.

Upon entry into the treatment phase, patients attended the research units within 24 hours (i.e. day 1, treatment baseline) to begin treatment with inhaled IFN-β or placebo given as a single daily dose for 14 days. Patients were reviewed at study sites on days 4, 7, 10, 13 and 17 and, in addition, recorded daily at home their upper and lower respiratory tract symptoms and peak expiratory flows (PEF). The rest of the schedule is outlined in table E1.

Visit Number:	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**
	Pre-Treatment Phase	**Treatment Phase**						
Assessment Days (All days after Day 1 are +/- 1 day)	**Screening**	**Day 1** (within 24hr of cold symptoms)	**Day 4**	**Day 7**	**Day 10**	**Day 13**	**Day 17**	**30-35 days post-EOT**
Signed informed consent	X							
Medical history	X							
Physical examination	X	X	X	X	X	X	X	
Patient Demography	X							
Vital signs	X	X	X	X	X	X	X	
Height and weight	X						X	
12 lead ECG	X							
Skin allergy test	X							

Copyright © 2014 by the American Thoracic Society

FE_{NO}	X	X	X	X	X	X	X	
Clinic spirometry (FEV_1, FVC and PEF)	X	X	X	X	X	X	X	
DLCO	X	X					X	
Bronchial hyper-responsiveness test	X							
Download home monitoring		X	X	X	X	X	X	X
Blood sampling: Safety screen	X	X					X	
Blood sampling: Biomarkers & Serum pharmacokinetics	X	X	X	X	X	X	X	X
Blood sampling: Immunogenicity	X							X
Urinalysis: Urine dipstick analysis and pregnancy test	X	X					X	
Sputum induction	X		X	X				
Nasal lavage		X	X	X				
Throat swab			X	X				
Inclusion / Exclusion criteria	X	X						
Dose administration		X	X	X	X	X		
Adverse events, asthma medication and general concomitant medication	X	X	X	X	X	X	X	X

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

Table E1. Study schedule for the IFN-β trial (also see figure 1 in main article). EOT: end of treatment.

RECRUITMENT AND LOCATION OF STUDY SITES

The trial started on the 31st March 2010 and was completed on the 17th January 2012. The trial involved 21 sites in the UK and Australia. The study was approved by the ethics committees in the UK (Southampton and South West Hampshire Research Ethics Committee) and Australia (Bellberry, Southern Health (A) and Hunter New England Human Research Ethics Committees) and all participants provided informed written consent. The trial ended once sufficient subjects had met the mITT criteria and had contributed data to the primary endpoint (ACQ-6).

NEBULISER DEVICE

IFN-β was delivered via an I-neb (Philips Respironics, Chichester, UK), a breath-actuated (Adaptive Aerosol Delivery (AAD) System), hand-held, portable nebuliser which utilises Vibrating Mesh Technology (VMT) to generate aerosol. It is designed to administer a small volume of liquid medication for inhalation. Since the AAD system only delivers drug when the patient is inhaling through the mouthpiece, drug loss to the atmosphere is almost entirely eliminated. All patients who were enrolled into the trial had a training session on the use of the device and only qualified for trial participation if they showed proficiency in use of the I-neb system.

RANDOMISATION

Copyright © 2014 by the American Thoracic Society

Patients were randomised by site staff to one of two treatment groups (IFN-β or placebo) in a 1:1 ratio according to a pre-specified randomisation schedule generated by an independent data management organization, MDSL International (Maidenhead, Berkshire, United Kingdom). The randomisation schedules were generated as PDF documents via a validated computer programme. Treatments were randomly ordered within blocks of length 4 (2 x IFN-β, 2 x placebo), stratified by site. Randomisation numbers were allocated sequentially according to the randomisation list at each site. If a subject withdrew after receiving any study medication then the randomisation number was not re-assigned. If for any reason the subject had been randomised but had not received any study drug, this randomisation number could be re-assigned.

BLINDING

The study was subject- and investigator-blinded. Blinding codes could only be broken in emergency situations for reasons of subject safety and the investigator was required to contact the Medical Monitor of the trial. If the blinding code was to be broken, the reason had to be fully documented and entered in the case report form. The treatment allocation for each subject was provided in a sealed envelope to the study site pharmacist or the person delegated by the Investigator. The location of these envelopes had to be communicated to relevant study staff and documented in the Investigator Site File. A treatment assignment could only be unblinded in case of emergency where the knowledge of the double-blind treatment may influence further care of the subject. If medically feasible, every attempt had to be made to discuss the situation with the Medical Monitor prior to breaking the blind. If a treatment code was unblinded for any reason, the Investigator was required to notify the Sponsor and a record would be kept of who unblinded the code, the

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

reason for doing so and the date and time. In the case of an emergency, where knowledge of the double-blind treatment could influence the further care of the subject, the Investigator's first priority was to ensure the safety of the subject. They then had to attempt to contact the Sponsor. In the event that this was not possible, the Investigator was able to access the subject randomisation list 24 hours a day, as a sealed copy of treatment envelopes were maintained in the study site pharmacy and a second suitable location known to all study site staff.

PROTOCOL AMENDMENTS

Refer to Table E2 for a summary of the most important protocol amendments after trial commencement.

Amendment number and date	Wording in previous protocol	Wording in updated protocol	Rationale for change
Inclusion and Exclusion Criteria			
Amend 1 – May 2010	'Diagnosis of asthma at least 2 years prior to the Screening Visit confirmed by medical history and ≥12% or 200mL bronchodilator reversibility or PD_{20}'.	'Symptoms of asthma for at least 2 years prior to the Screening Visit, confirmed at visit 1 by a medical history and ≥12% and 200mL bronchodilator reversibility or evidence of bronchial hyper-responsiveness at screening or documented in the past 5 years'.	Wording clarified in accordance with the ATS/ERS guidelines.
Amend 10 – March 2011	'Symptoms of asthma for at least 2 years prior to the Screening Visit, confirmed at visit 1 by a medical history and ≥12% and 200mL bronchodilator reversibility or evidence of bronchial hyper-responsiveness at screening or documented in the past 5 years'.	Symptoms of asthma for at least 2 years prior to the Screening Visit, confirmed by a medical history and (a) ≥12% and 200mL bronchodilator reversibility at screening or documented in the past, OR, (b) evidence of bronchial hyper-responsiveness at screening or documented in the past, OR, (c) a documented hospital admission (including an Accident and Emergency admission) for asthma	A number of subjects were failing bronchial hyper-responsiveness challenges (such as methacholine), despite having a convincing history of asthma. Advice from a number of respiratory physicians suggested that this is a common finding due to inhaled medications having improved over the last 5 years. The inclusion criteria was therefore changed to allow subjects who have a convincing medical history and documented worsening of asthma symptoms during a viral illness to be included into the study (following confirmation from their GP via a questionnaire).

Copyright © 2014 by the American Thoracic Society

		since the age of 18, OR. (d) documented evidence that they have attended their GP surgery, out-of-hours clinic (or alternative health care provider) for worsening of asthma symptoms, since the age of 18	
Amend 3 – August 2010	N/A	'Regular use of oral steroids (prescribed for asthma)'.	It was intended that subjects taking oral steroids on a regular basis should not be included into the study. This was therefore added as an exclusion criteria.
Amend 3 – August 2010	'Current smokers (or ex-smokers who have given up smoking in the last 12 months)'.	Exclusion criteria has been removed: *Smokers and ex-smokers are now allowed to enter the study.*	Smokers account for approximately 20% of asthmatics and are over represented in the population that frequently exacerbate and are hospitalised.
Amend 10 – March 2011	'Regular use of oral steroids (prescribed for asthma)'.	Exclusion criteria has been removed: *Subjects on low dose oral steroids will now be accepted into the study.* *Inclusion criteria added:-* *'The subject has already started taking, or has increased their oral steroids for an asthma exacerbation'.*	It is anticipated that interferon will be marketed in a severe asthma population as this group are often hospitalised by virus induced exacerbations. This criteria was amended when over 40 subjects had been dosed and no unexpected adverse events had been reported, it was then considered reasonable to enter a more severe population of asthmatic.
Study Schedule			
Amend 6 – Feb 2011	N/A	Safety bloods were added to visit 2.	This set of bloods was the subject's additional baseline bloods as due to the nature of the study, safety bloods taken at visit 1 could have been taken several months ago. This was also a recommendation of the DSMC.
Amend 14 – July 2011	N/A	Addition of throat swabs to detect viral load at visit 3 and 4	Throat swabs were added as an additional way of detecting viral load.
Objectives			
Amend 6 – Feb 2011	'To compare the effect of inhaled SNG001 to placebo when administered to asthmatic subjects in the reduction of viral load on Days 4 and 7 in sputum'.	'To compare the effect of inhaled SNG001 to placebo when administered to asthmatic subjects on viral load on Days 4 and 7 in sputum'.	This secondary objective was re-worded to make it less ambiguous.

Table E2: Protocol amendments

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

SAMPLE COLLECTION, PROCESSING AND ANALYSIS

NASAL LAVAGE COLLECTION AND PROCESSING

Nasal lavage samples were collected for pathogen detection from all subjects during the trial on days 1, 4 and 7 during the treatment phase. 2.5mL of normal (0.9%) saline was used to repeatedly flush each nostril (5 times) using a syringe attached to an appropriately sized nasal olive placed half way up the patient's nostril. The lavage fluid from each nostril was then expelled from the syringe into a centrifuge tube, resulting in approximately 5mL in total. This was kept on ice until processed. The nasal lavage fluid was passed through a 100 µm cell strainer, centrifuged at 400g for 10 minutes and the resulting supernatant aliquotted and frozen ready for analysis.

PATHOGEN DETECTION IN NASAL LAVAGE

Viral nucleic acids were extracted from 900µL nasal lavage supernatant using column technology (QIAamp MinElute kit, Qiagen). The nucleic acid was assayed against a panel of 21 respiratory pathogens using a CE marked one-step multiplex qPCR kit (Respiratory pathogens 21, Fast Track Diagnostics) according to manufacturer's instructions, and rhinovirus (RV) using the genesig rhinovirus (all subtypes) PCR kit (PrimerDesign). The FTD respiratory pathogens 21 kit included primers and probes for the following pathogens: Influenza A, Influenza B, Rhinovirus, Influenza A H1N1 (swine flu), Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Coronavirus 43, Coronavirus 63, Coronavirus 229, Coronavirus HKU, Respiratory syncytical virus A & B, Parechovirus, Adenovirus, Enterovirus, Human metapneumovirus A & B, Mycoplasma Pneumoniae and

Copyright © 2014 by the American Thoracic Society

Bocavirus. The PCR was performed on a CFX96 Real-Time system with C1000 Thermal cycler (Bio-Rad). The data was analysed using CFX Manager 2.0 (Bio-Rad).

SPUTUM COLLECTION AND PROCESSING

Sputum induction was attempted at selected sites at screening and on treatment days 4 and 7 for virus load quantification and biomarker analysis. Not all subjects produced sputum samples; in sputum producers, samples were not obtained on all occasions. Sputum was induced by inhalation of 4.5% hypertonic saline for up to 20 minutes in patients with >60% predicted FEV_1; a more cautious approach (starting with a lower concentration of saline (0.9%) was used for patients with <60% predicted FEV_1. In brief, the induced sputum was selected from the saliva and incubated with 5mM dithioerythritol (DTE) and protease inhibitors for 30 minutes. The fluid phase was then separated from the cells and centrifuged to remove remaining mucus. The fluid phase was aliquotted and stored frozen. The cell pellets were lysed in RLTplus buffer (Qiagen) and stored frozen until nucleic acid extraction was performed.

RHINOVIRUS LOAD DETERMINATION IN SPUTUM SUPERNATANT

Viral nucleic acids were extracted from 140µL DTE processed sputum supernatant using column technology (QIAamp Viral RNA kit, Qiagen). Rhinovirus viral RNA was measured by two-step qPCR using the genesig rhinovirus (all subtypes) PCR kit (PrimerDesign) and quantified against the standard supplied. The PCR was performed on a CFX96 Real-Time system with C1000 Thermal cycler (Bio-Rad). The data was analysed using CFX Manager 2.0

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

(Bio-Rad). Data were normalised to the weight of the selected, i.e. mucoid part of the sputum.

EXPRESSION OF ANTI-VIRAL GENES IN SPUTUM CELLS

Nucleic acid from lysed sputum cells from treated subjects were extracted using column technology (Qiagen AllPrep DNA/RNA Mini Kit) according to manufacturer's instructions. Complementary DNA (cDNA) was generated using nanoScript reverse transcription (RT) kits (PrimerDesign) following manufacturer's instructions. Anti-viral gene expression (Mx1, OAS1 and CXCL10) was measured using primers and probes (PerfectProbe, PrimerDesign) on a CFX96 Real-Time system with C1000 Thermal cycler (Bio-Rad). The data were analysed using CFX Manager 2.0 (Bio-Rad).

MEASUREMENT OF PRO-INFLAMMATORY MEDIATORS IN SPUTUM FLUID PHASE

Sputum fluid phase samples from treated subjects were analysed using a custom Luminex multiplex kit (Life Technologies) for CXCL8, CCL4, G-CSF, IL-1ra, IL-6, IL-15, CXCL10 and IFN-α. Samples were assayed at 1:5 dilution (final concentration 1mM DTE). Manufacturer's instructions were followed with the exception that samples were read against a standard curve diluted in DTE (1mM final concentration). Samples were analysed on the BioPlex 200 (Bio-Rad) using BioPlex Manager software version 6.1.

SERUM CXCL10 MEASUREMENT

Blood was collected (at all clinic visits) into blood collection tubes containing clot activator and incubated at room temperature protected from light for 30-45 minutes to allow the

Copyright © 2014 by the American Thoracic Society

blood to clot. The blood was centrifuged (2,500g for 10 minutes) and the resulting serum was aliquotted and frozen. Serum CXCL10 (IP-10) was measured using a commercially available sandwich enzyme immunoassay (R&D Systems, Catalogue #DIP100). The ELISA was carried out according to manufacturer's instructions.

GENE EXPRESSION OF CIRCULATING BLOOD CELLS

Blood was collected directly into PAXgene tubes (PreAnalytiX) at all sites prior to and during the dosing period but analysis was restricted to samples from the BTS Step 4/5 mITT subgroup; samples were all stored frozen prior to analysis. Nucleic acid was extracted using PAXgene Blood RNA kits (Qiagen). Genome-wide gene expression analysis was performed by Oxford Gene Technology using Agilent SurePrint G3 Human Gene Expression 8x60K two colour microarrays. Samples were subjected to quality control prior to running the arrays and prior to analysis. Only RNA samples with a RIN number ≥5.9 were run on the arrays. Data were processed using protocol GE2_107_Sep09 using the Agilent Feature extraction software (v10.7). Gene expression from each sample was normalised to human reference RNA (Stratagene).

Differentially expressed gene lists for IFN-β versus placebo treatment were generated for days 4 and 7 by selecting probes that were significant at $p<0.05$ and had a fold change of >1.25 following analysis of covariance, including day 1 as a covariate. Data (submitted as GEO accession number GSE50943) were analysed using Ingenuity Pathway Analysis software (Ingenuity® Systems [www.ingenuity.com], Mountain View, CA, USA) applying a correction for multiple testing (Benjamini-Hochberg).

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

STATISTICAL ANALYSIS

Primary analysis was conducted on data from patients who fulfilled the Jackson [E7] or Predy [E8] criteria for a cold and, therefore, constituted the modified intention to treat (mITT) population. All the statistical analyses defined by the Statistical Analysis Plan were performed by an independent data management organisation, MDSL International (Maidenhead, Berkshire, United Kingdom), along with additional exploratory analyses (such as the sub-group analyses on the BTS Step 4/5 patients) and post-hoc analyses requested by the sponsor company. Statistical analyses were conducted using SAS (version 9.1.3 or higher) and were governed by a comprehensive quality assurance system following ICH and other applicable regulatory guidelines. The analysis methods used (analysis of covariance and unpaired t-tests for continuous data and Fishers exact test for categorical data) are standard statistical tests. The exploration of the cold and asthma symptom scores in patients randomised to the placebo arm of the trial, as well as the summary of the sputum viral loads, not originally in the plan, were performed by the scientists in the sponsor company and checked by the Chief Investigator.

The primary hypothesis was that IFN-β is superior to placebo in respect of the change from treatment baseline (Day 1) to day 8 in the ACQ-6 in the mITT population which was tested via analysis of covariance, including terms for pooled site and baseline value. Sample size calculation was performed on data from a prospective multi-centre study of asthma control associated with a cold [E9] and assuming a similar patient population. This determined that 56 patients per treatment-arm were required to detect a mean treatment difference of 0.5 in the change from baseline in ACQ-6, 7 days after onset of a cold [E10]. After the data for the primary endpoint for approximately 30 to 40% of patients had become available, the

Copyright © 2014 by the American Thoracic Society

statistical analysis plan allowed for an assessment of the required sample size to be recalculated using a blinded sample size adjustment method [E11] and accepted that it may be necessary to alter the number of patients in the mITT population. This planned sample size re-calculation was performed in a blind manner mid-study and showed that the sample size remained adequate.

The secondary objectives included the evaluation in the mITT population of the effect of IFN-β on the asthma index [E12], the frequency of moderate and severe exacerbations in the mITT population (analysed via Fisher's exact test) and lung function (FEV_1 and PEF, analysed via analysis of covariance) and the effect of treatment on ACQ-6 in the per protocol (PP) population. The per protocol population was defined as a subset of the mITT population, excluding all patients with major protocol deviations and excluding all patients that received less than three complete doses of study drug in the first seven days (study Day 1 to study Day 7 inclusive. Additional secondary objectives were to compare the effects on sputum viral load and concomitant medication use and to evaluate the safety and pharmacodynamic profile of inhaled IFN-β. The statistical analysis plan also stated that exploratory analyses of the primary and secondary objectives in patient subgroups defined by asthma severity may be investigated. Post-hoc exploratory analysis of serum CXCL10, not initially planned, was performed to assess the effect of treatment on induction of this biomarker of innate immunity. Serum CXCL10 changes were analysed in both the whole mITT population and in the difficult-to-treat subgroup (BTS step 4/5, mITT), refer to Figure 5A and Figure E2 respectively.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

MULTIPLE REGRESSION ANALYSIS

In order to further understand and explore the results for the primary endpoint change from treatment baseline to Day 8 in ACQ-6, some additional exploratory regression analyses were performed. These analyses were not pre-defined in the protocol and included a large number of hypothesis tests with no adjustments for multiplicity and should, therefore, be considered as hypothesis generating only.

Forty pre-treatment assessments (including demographics, asthma history and severity and a variety of screening and baseline lung function tests) were identified and screened via a linear regression model with treatment, the baseline assessment, the interaction between baseline assessment and treatment and ACQ-6 at baseline included in the model. The numbers of exacerbations in the last 24 months, screening lung function, BTS Step, use of long acting β-agonists, baseline cold and asthma scores were all identified as potentially related to the change in ACQ-6. Based on a subsequent stepwise regression model the asthma score on day 2 ($p = 0.0005$) and the interaction between treatment and BTS Step ($p = 0.0067$) appeared to have the most influence on the change in ACQ-6. Taking asthma symptoms at the start of treatment into account the estimated difference in change in ACQ-6 between IFN-β and placebo reduced in BTS Step 2 and 3 and increased in favour of IFN-β in the BTS Step 4/5 group (Refer to Analysis 2 in Table E3).

Copyright © 2014 by the American Thoracic Society

BTS Step Group	Step 2		Step 3		Step 4/5	
Treatment group	**Placebo**	**IFN-β**	**Placebo**	**IFN-β**	**Placebo**	**IFN-β**
N	14	17	21	17	30	24
a) Change in ACQ-6 from treatment baseline to day 8 (Analysis 1)*						
Least Squares Mean	0.12	0.52	0.13	0.32	0.53	-0.10
Mean difference (95% CI)	0.41 (-0.15, 0.97)		0.19 (-0.31, 0.70)		-0.63 (-1.05, -0.21)	
p-value	0.15		0.45		0.004	
b) Change in in ACQ-6 from treatment baseline to day 8 (Analysis 2) +						
Least Squares Mean	0.15	0.46	0.11	0.20	0.65	-0.11
Mean difference (95% CI)	0.30(-0.27,0.87)		0.09(-0.42,0.60)		-0.76(-1.19,-0.32)	
p-value	0.29		0.73		0.0008	

Table E3. Analysis of ACQ-6 during the treatment period by asthma severity (BTS treatment Step) in the mITT population.

The ACQ-6 scores were recorded on days 1 (treatment baseline) and 8 (see methods). The data was originally analysed (*) via an analysis of covariance model with terms with terms for treatment, BTS Step subgroup, the interaction between treatment and BTS Step subgroup and the baseline value. The data was further analysed ($^{+}$) via an analysis of covariance model with terms with terms for treatment, BTS Step subgroup, the interaction between treatment and BTS Step subgroup, daily asthma symptom score on day 2 and the baseline value.

SIDE EFFECTS

Treatment emergent adverse events (TEAE) in the system organ class (SOC) Respiratory, Thoracic and Mediastinal Disorders group occurred most frequently and occurred in almost

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

the same frequency in the two groups (31/75 (41.3%) of patients on placebo and 29/72 (40.3%) of patients on IFN-β), refer to Table E4. TEAE in the Nervous System Disorders, Infections and Infestations, and General Disorders and Administration Site Conditions SOC all occurred at a frequency of approximately 20% in each treatment group, with little difference between placebo and IFN-β. This was also the case for the other SOC where there were fewer TEAE with the one exception of Cardiac Disorders where 5 events were experienced in 5 patients in the IFN-β group. There were no events in the cardiac disorders class for placebo. The events were all assessed as mild in nature and all occurred transiently in the Treatment phase, none lasting for more than 2 days.

Copyright © 2014 by the American Thoracic Society

System Organ Class	Placebo (N=75)		IFN-β (N=72)	
	N	%	N	%
At least one TEAE	62	82.7	61	84.7
Respiratory, Thoracic and Mediastinal Disorders	31	41.3	29	40.3
Nervous System Disorders	21	28.0	17	23.6
Infections and Infestations	18	24.0	17	23.6
General Disorders and Administration Site Conditions	16	21.3	15	20.8
Gastrointestinal Disorders	15	20.0	14	19.4
Musculoskeletal and Connective Tissue Disorders	7	9.3	8	11.1
Skin and Subcutaneous Tissue Disorders	6	8.0	6	8.3
Investigations	3	4.0	5	6.9
Ear and Labyrinth Disorders	3	4.0	3	4.2
Metabolism and Nutrition Disorders	3	4.0	3	4.2
Injury, Poisoning and Procedural Complications	3	4.0	3	4.2
Cardiac Disorders	0	0	5	16.9
Immune System Disorders	3	4.0	2	2.8
Psychiatric Disorders	1	1.3	3	4.2
Blood and Lymphatic System Disorders	1	1.3	1	1.4

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

Reproductive System and Breast Disorders	2	2.7	1	1.4
Eye Disorders	1	1.3	1	1.4
Social Circumstances	1	1.3	0	0
Neoplasms Benign, Malignant and Unspecified (including cysts and polyps)	0	0	1	1.4
Renal and Urinary Disorders	1	1.3	0	0

Table E4: Treatment Emergent Adverse Events (TEAE) by System Organ Class and Preferred Term N = No. of patients with event, (No. of patients with event/No. of patients receiving that treatment). % = percentage of total number of patients in each treatment arm.

Table E5 shows the TEAE by SOC and Preferred Term that were deemed by the Investigator to be related (possibly, probably or definitely) to study medication. The frequency of TEAE were evenly split between the groups (29/75 (38.7%) of placebo patients and 28/72 (38.9%) of IFN-β patients). The SOC with the highest frequency of related events was General Disorders and Administration Site Conditions, with 12 patients reporting events in the placebo group and 13 patients on IFN-β. Related events of fatigue occurred slightly more frequently on IFN-β (5/72 (6.9%) compared to placebo 2/75 (2.7%)). Related events of headaches occurred more frequently in the placebo group than the treatment group (8 on placebo and 4 on IFN-β). 9 patients in each group had respiratory symptoms related to study medication. Nausea was assessed as related to study medication for one patient on placebo and three patients on treatment. Only 2 of the 5 events of palpitation were reported as related to study medication in the group.

Copyright © 2014 by the American Thoracic Society

	Placebo (N=75)		IFN-β (N=72)	
System Organ Class / Preferred Term	**N**	**%**	**N**	**%**
At Least One Study Medication Related TEAE	29	38.7	28	38.9
General disorders and administration site conditions	**12**	**16.0**	**13**	**18.1**
Administration related reaction	10	13.3	9	12.5
Fatigue	2	2.7	5	6.9
Malaise	0	0	1	1.4
Nervous system disorders	**11**	**14.7**	**9**	**12.5**
Headache	8	10.7	4	5.6
Dizziness	1	1.3	2	2.8
Lethargy	1	1.3	2	2.8
Dysgeusia	1	1.3	1	1.4
Loss of consciousness	0	0	1	1.4
Paraesthesia	0	0	1	1.4
Respiratory, thoracic and mediastinal disorders	**9**	**12.0**	**9**	**12.5**
*Asthma	3	4.0	4	5.6
Cough	1	1.3	3	4.2
Dyspnoea	1	1.3	2	2.8
Wheeze	2	2.7	0	0
Wheezing	2	2.7	0	0

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

	Placebo (N=75)		IFN-β (N=72)	
System Organ Class / Preferred Term	**N**	**%**	**N**	**%**
Dry throat	1	1.3	0	0
Epistaxis	1	1.3	0	0
Productive cough	1	1.3	0	0
Rhonchi	1	1.3	0	0
Gastrointestinal disorders	**9**	**12.0**	**5**	**6.9**
Diarrhoea	4	5.3	1	1.4
Nausea	1	1.3	3	4.2
Aphthous stomatitis	1	1.3	1	1.4
Dry mouth	1	1.3	0	0
Dyspepsia	1	1.3	0	0
Mouth ulceration	1	1.3	0	0
Stomatitis	1	1.3	0	0
Ear and labyrinth disorders	**1**	**1.3**	**1**	**1.4**
Ear pain	1	1.3	1	1.4
Ear congestion	1	1.3	0	0
Tinnitus	0	0	1	1.4
Skin and subcutaneous tissue disorders	**2**	**2.7**	**1**	**1.4**
Eczema	1	1.3	0	0
Rash	1	1.3	0	0
Skin sensitisation	0	0	1	1.4

Copyright © 2014 by the American Thoracic Society

	Placebo (N=75)		IFN-β (N=72)	
System Organ Class / Preferred Term	**N**	**%**	**N**	**%**
Musculoskeletal and connective tissue disorders	**0**	**0**	**3**	**4.2**
Muscle twitching	0	0	1	1.4
Musculoskeletal stiffness	0	0	1	1.4
Myalgia	0	0	1	1.4
Cardiac disorders	**0**	**0**	**2**	**2.8**
Palpitations	0	0	2	2.8
Infections and infestations	**0**	**0**	**2**	**2.8**
Nasopharyngitis	0	0	2	2.8
Psychiatric disorders	**0**	**0**	**2**	**2.8**
Abnormal dreams	0	0	1	1.4
Sleep disorder	0	0	1	1.4
Blood and lymphatic system disorders	**1**	**1.3**	**0**	**0**
Anaemia deficiencies	1	1.3	0	0
Metabolism and nutrition disorders	**1**	**1.3**	**0**	**0**
Decreased appetite	1	1.3	0	0

Table E5: Treatment Emergent Study Medication Related Adverse Events by System Organ Class and Preferred Term (Safety population)

* Asthma related conditions. N = No. of patients with event, (No. of patients with event/No. of patients receiving that treatment). %= percentage of total number of patients in each treatment arm.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

REFERENCES

E1. Djukanovic R, Lai CK, Wilson JW, et al. Bronchial mucosal manifestations of atopy: a comparison of markers of inflammation between atopic asthmatics, atopic nonasthmatics and healthy controls. Eur Respir J 1992;5:538-44.

E2. Grainge CL, Lau LC, Ward JA, et al. Effect of bronchoconstriction on airway remodeling in asthma. N Engl J Med 2011;364:2006-15.

E3. Stark GR, Kerr IM, Williams BR, et al. How cells respond to interferons. Annu Rev Biochem 1998;67:227-64.

E4. Wark PA, Bucchieri F, Johnston SL, et al. IFN-gamma-induced protein 10 is a novel biomarker of rhinovirus-induced asthma exacerbations. J Allergy Clin Immunol 2007;120:586-93.

E5. Djukanovic R, Sterk PJ, Fahy JV, et al. Standardised methodology of sputum induction and processing. Eur Respir J Suppl 2002;37:1s-2s.

E6. Juniper EF, Svensson K, Mork AC, et al. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. Respir Med 2005;99:553-8.

E7. Jackson GG, Dowling HF, Spiesman IG, et al. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. AMA Arch Intern Med 1958;101:267-78.

E8. Predy GN, Goel V, Lovlin R, et al. Efficacy of an extract of North American ginseng containing poly-furanosyl-pyranosyl-saccharides for preventing upper respiratory tract infections: a randomized controlled trial. CMAJ 2005;173:1043-8.

E9. Walter MJ, Castro M, Kunselman SJ, et al. Predicting worsening asthma control following the common cold. Eur Respir J 2008;32:1548-54.

E10. Reddel HK, Taylor DR, Bateman ED, et al. An Official American Thoracic Society/European Respiratory Society Statement: Asthma Control and Exacerbations. American Journal of Respiratory and Critical Care Medicine 2009;180:59-99.

Copyright © 2014 by the American Thoracic Society

E11. Kieser M, Friede T. Simple procedures for blinded sample size adjustment that do not affect the type I error rate. Stat Med 2003;22:3571-81.

E12. Sorkness RL, Gonzalez-Fernandez G, Billmeyer EE, et al. The asthma index: a continuous variable to characterize exacerbations of asthma. J Allergy Clin Immunol 2008;122:838-40.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

FIGURE LEGENDS

Figure E1. Biomarkers of an IFN-β effect on lung and blood innate immune responses. Patients participating in the trial underwent sputum induction using standard protocols and processing methods approved by the ERS Task Force for the use of induced sputum method[E5]. Samples were assessed for relevant markers of innate immunity: IFNγ inducible protein 10 (IP-10; CXCL10), myxoma resistance protein 1 (Mx1), neopterin and 2'-5' oligoadenylate synthetase (OAS1). This showed induction of CXCL10 protein and neopterin detected by ELISA and OAS1, Mx1 and CXCL10, as measured by qPCR.

Figure E2. A more sustained rise (mean ± SEM) in serum concentrations of CXCL10 was measured in BTS Step 4/5 patients (mITT population) treated with IFN-β (n=24-27; closed symbols) when compared to those on placebo (n=27-30; open symbols). Data were analysed on the log scale via analysis of covariance, including day 1 as a covariate ($p=0.004$, <0.001 and <0.001 for days 7, 10 and 13; analysed on the log scale via analysis of covariance, including day 1 as a covariate).

Copyright © 2014 by the American Thoracic Society

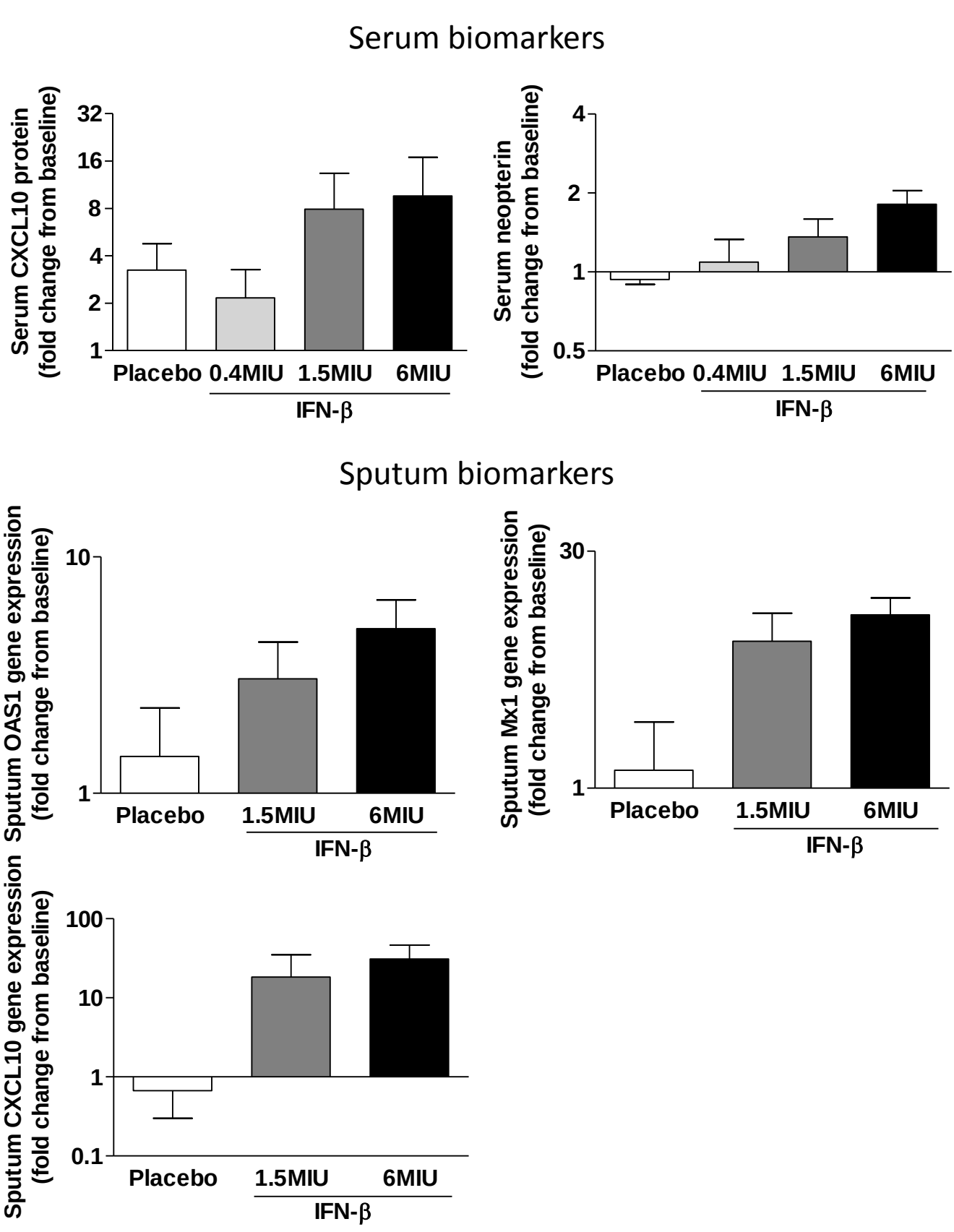


Figure E1

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 17-June-2014 as 10.1164/rccm.201312-2235OC

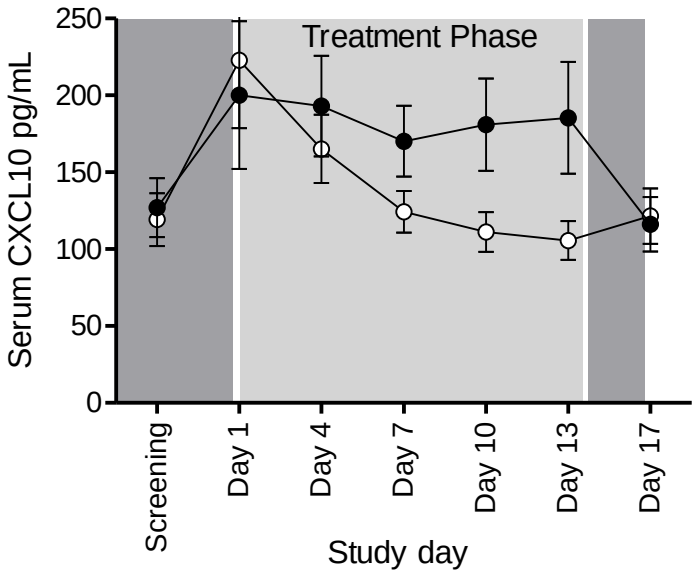


Figure E2

Copyright © 2014 by the American Thoracic Society