Relationship between pharmacokinetics and pharmacodynamic responses in healthy smokers informs a once daily dosing regimen for nemiralisib

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Non-standard abbreviations:

ANOVA: analysis of variance; BAL: bronchoalveolar lavage; COPD: Chronic Obstructive Pulmonary Disease; CF: cellular fraction; CI: confidence interval; ELF: epithelial lining fluid; FTIH: first time in human; fMLP: N-formyl-L-methionyl-L-leucyl-L-phenylalanine; LLQ: lower limit of quantification; HLQ: higher limit of quantification; PD: Pharmacodynamic; PK: Pharmacokinetic; PIP3: phosphatidylinositol-trisphosphate; PI3Kδ: phosphoinositide 3-kinase delta

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Abstract

Nemiralisib (GSK2269557) is a potent inhaled inhibitor of phosphoinositide 3-kinase delta (PI3Kδ) which is being developed for the treatment of respiratory disorders including COPD (Chronic Obstructive Pulmonary Disease). Determining the pharmacokinetic (PK) and pharmacodynamic (PD) responses of inhaled drugs early during drug development is key to informing the appropriate dose and preferred dose regimen in patients. We set out to measure PD changes in induced sputum in combination with drug concentrations in plasma and bronchoalveolar lavage (BAL) taken from healthy smokers (n=56) treated for up to 14 days with increasing doses of inhaled nemiralisib (0.1 mg to 6.4 mg). Induced sputum analysis demonstrated a dose-dependent reduction in phosphatidylinositol-trisphosphate (PIP3, the product of PI3K activation), with a maximum placebo-corrected reduction of 23% (90% CI 11-34%) and 36% (90% CI 11-64%) following single dose or 14 days of treatment with nemiralisib respectively (2 mg, once daily). Plasma analysis suggested a linear PK relationship with an observed accumulation of ~3-4.5-fold (peak vs. trough) in plasma exposure following 14 days of nemiralisib treatment. BAL analysis at trough confirmed higher levels of drug in lung vs. plasma (32-fold in the BAL fluid component, and 214-fold in the BAL cellular fraction). Comparison of drug levels in plasma and reductions in sputum PIP3 show a direct relationship between exposure and PIP3 reduction. In conclusion, these results demonstrate target engagement upon treatment with inhaled nemiralisib and provide confidence for a once-daily dosing regimen.
Introduction

Inhaled drug therapeutics have the advantage of being delivered directly to the target organ, providing the opportunity for quick onset of action, and a low systemic exposure limiting potential unwanted effects in other organs (Lötvall, 1997; Lipworth, 1996). However, compared to systemically delivered therapies, the development of inhaled medicines has two key challenges; firstly, the need for understanding the pharmacokinetic (PK) profile of local concentrations, and secondly the need to understand any pharmacodynamic (PD) changes in the lung following inhaled dosing. Both are challenging to assess as sampling of the local compartment is not feasible in all patients or stages of disease. Addressing these challenges however, enables the determination of a PK/PD relationship and informs future development of novel therapeutics. A recent review has highlighted the importance of these factors in the interpretation of clinical study observations, and ultimately reducing the number of failed drug trials (Morgan et al., 2012).

Asthma and Chronic Obstructive Pulmonary Disease (COPD) are characterized by chronic inflammation in the airways and are currently treated with several inhaled treatments (Durham et al., 2016; Barnes, 2013). Phosphoinositide 3-kinase delta (PI3Kδ) is a lipid kinase expressed predominantly in leucocytes, where it regulates activation, proliferation and function of multiple cell types, thereby modulating immune responses (Down et al., 2015, Stark, 2015). Inhibitors of PI3Kδ have been proposed as therapeutics for asthma and COPD due to their potential to inhibit the recruitment of inflammatory cells and reduce the release of pro-inflammatory mediators such as cytokines, chemokines, reactive oxygen species, and proteolytic enzymes (Park, 2008, Stark et al., 2015; Cahn et al., 2017). In addition, targeting the PI3Kδ pathway improves innate immune responses to infections by promoting host defence (Down et al., 2015, Stark et al., 2015), and may restore steroid effectiveness under conditions of oxidative stress (Sriskantharajah et al., 2013; Marwick et al., 2010). Furthermore, individuals with activating mutations in PI3Kδ suffer from profound
immune defects, accompanied by bacterial colonisation, severe recurrent respiratory infections and progressive lung damage, suggesting the PI3Kδ pathway is involved in pathogen-driven responses (Angulo et al., 2013). Lastly, recent data suggest that targeting the PI3Kδ pathway could reduce the incidence of pathogen-induced exacerbations by improving immunity and enhancing viral clearance (Stark et al., 2015). Nemiralisib (GSK2269557) is a potent inhaled PI3Kδ inhibitor which is >1000-fold more selective at PI3Kδ than 250 other kinases, including the three other PI3K isoforms (Down, 2015). We postulate that an inhaled PI3Kδ inhibitor will provide localised anti-inflammatory action, while potentially avoiding any unwanted effects in other organs. Given our approach, an understanding of PK/PD in the lung early in the clinical phase was deemed critical for successful drug development.

Here we present data from two independent clinical studies with nemiralisib in healthy volunteers who were cigarette smokers, in whom drug concentrations were measured in plasma and bronchoalveolar lavage (BAL) samples, and correlated directly with levels of phosphatidylinositol-3,4,5 trisphosphate (PIP3), the product of all isoforms of PI3K activity, from matching induced sputum samples. The results of our work demonstrate the ability of nemiralisib to engage the target in the lung and data provide confidence for a once-daily dosing regimen.
Materials and Methods

Subject population

Data from independent clinical trials are presented. The first (Study A, protocol PII115117, NCT01462617) was conducted at Quintiles, Guy’s Hospital London, U.K. from July 2011 to March 2012. The second (Study B, protocol PII116617, NCT01762878) was conducted at Hammersmith Medicines Research, London, U.K. from January 2013 to October 2013.

Across the two studies, male and female (non-childbearing potential) subjects between the ages of 18 and 55 were eligible to be recruited, if they were deemed to be healthy by an experienced physician, had a body mass index within the range 18 to 31 kg/m², and had blood chemistry, electrocardiogram and spirometry results within normal range (NB: only males were included in Study A). For pharmacodynamic assessment, subjects were recruited who were current smokers had a pack history of ≥5 pack year, and Study B specified that subjects were able to produce an adequate sputum sample of >100 mg at screening. Subjects were excluded if they had liver or heart disease or a history of asthma.

Written informed consent was obtained prior to study participation. Both studies were conducted in accordance with the Declaration of Helsinki and relevant ethics committee/institutional review boards and regulatory authorities reviewed and approved the study protocols. Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com

Study Design

Both studies (Study A: protocol PII115117, NCT01462617; Study B: protocol PII116617, NCT01762878) were single centre, double-blind, placebo-controlled, randomised studies in healthy volunteers, that included cigarette smokers, designed to determine the safety, tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) profile of single and repeat doses of nemiralisib.
Study A was performed using a nebulised formulation, and Study B was conducted using a dry powder formulation provided in Diskus. In each study, there was a matching placebo. Abstracts of data from both studies have previously been presented (Wilson et al., 2013; Worsley et al., 2013; Wilson et al., 2014).

Study A determined the PK/PD relationship of a single dose of nemiralisib in a two cohort, crossover design with active doses administered using a nebulised dosimeter to smokers. In the first cohort, 15 subjects received nemiralisib 0.4 mg or 6.4 mg single dose, or placebo in a 3-way crossover with a 7-day washout between treatments. Induced sputum samples were collected 3 h after treatment inhalation. In the second cohort, 12 subjects received nemiralisib 6.4 mg single dose or placebo in a 2-way crossover with 14 days washout between treatments. Bronchoscopy for the collection of bronchoalveolar lavage (BAL) samples was conducted 3 h after inhalation of treatment. In both cohorts, blood samples for PK analysis were collected after inhalation of treatment, and at 3 h (pre- and post-sputum induction or BAL), 6, 12 and 24 h post-inhalation.

Study B explored the PK/PD relationship of nemiralisib in a single-dose escalation phase in a total of 16 subjects all of whom were regular cigarette smokers, in a 4-way cross-over with each subject receiving placebo, 0.1 mg, 0.5 mg and 2 mg of a dry powder formulation of nemiralisib administered using the Diskus device, with a 14-day washout between treatments. Induced sputum samples were collected 3 and 24 h after treatment inhalation and blood samples for PK analysis were collected at pre-dose and at 5 min, 30 min, 45 min, 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose. A 14-day repeat dose arm was conducted in a further cohort where subjects received 2 mg nemiralisib (n=10) or placebo (n=3) in a parallel group design, also delivered as a dry powder formulation using the Diskus device. Induced sputum samples were collected pre-dose, 3 h post-dose on days 1 and 6 of treatment, and 3 h and 24 h post-dose on day 12 of treatment. Blood samples for PK analysis were collected pre-dose and 5 min post-dose on days 1 to 13 inclusive, and pre-dose, 5 min, 30 min, 45 min, 1,
2, 3, 4, 6, 8 and 12 h post-dose on day 14. Bronchoscopy for the collection of BAL samples was done at 24 h (trough) after the final (day 14) dose.
Study procedures

Sample collection

Pharmacokinetics blood sample

For the PK analysis of nemiralisib, 2mL of blood samples were collected into a K3 ethylenediaminetetraacetic acid tubes, inverted 8-10 times and placed on wet ice in an upright position. Blood samples were centrifuged for 10 min at approximately 1500g at 2-8°C to generate plasma. The resulting plasma samples were aliquoted into clean tubes and frozen at approximately -20°C within 1 h of collection and were stored at -20°C until shipment to the bioanalysis site.

Bronchoalveolar lavage (BAL) sample

Lung lavage was collected via the instillation of 3 x 50 mL aliquots of saline administered to the right middle lobe. Aliquots were aspirated (directly by syringe connected to bronchoscope) following each instillation the separate return volume was measured for each aspirate and kept separately on ice for analysis. Each separate wash was centrifuged at 4000g for 5 min and supernatant removed (containing epithelial lining fluid [ELF]) and the cell pellet kept separately. The cell pellet for concentration analysis was then re-suspended in deionized water (causing cell lysis) to an equivalent volume of 20% of the individual lavage return volume. The reconstituted pellet and supernatant (ELF) samples were stored at -80°C until shipment to the bioanalysis site.

Induced sputum sample

Induction was performed after each of up to three 5-min inhalations of saline (0.9%, 3%, 4%), following standard local procedures, and sputum samples were frozen at each site for subsequent phospholipid analysis.

Sample analyses
**Drug concentration measurements in plasma and BAL cells and fluid**

Derivation of concentrations in the lung ELF and cell pellet were conducted using the raw concentration data with the equations detailed in Supplemental Table 1. Human plasma, lung ELF supernatant and BAL cell pellet samples were analyzed for nemiralisib using a validated analytical method based on protein precipitation, followed by HPLC-MS/MS analysis. The lower limit of quantification (LLQ) was 20 pg/mL using a 25 µL aliquot of human plasma, lung ELF supernatant and BAL cell pellet with a higher limit of quantification (HLQ) of 10000 pg/mL.

Human plasma and lung ELF supernatant samples were analyzed for urea by a validated analytical method based on the Roch-Ramel enzymatic reaction, using urease and glutamate dehydrogenase methodology on the Siemens Advia 1800 chemistry analyser. In plasma, the lower limit of quantification (LLQ) was 0.2 mmol/L with a higher limit of quantification (HLQ) of 239.5 mmol/L, and in lung ELF supernatant the LLQ was 0.01 mmol/L with a HLQ of 2.8 mmol/L. Due to the invasive nature of the BAL procedure this was performed at a single time point at a single dose in our healthy volunteers.

**Pharmacokinetic analysis of concentration data**

Individual plasma nemiralisib concentration-time data using actual times was analysed using WinNonlin Professional Edition v5.3 or higher (Certara Inc) to derive PK parameters, including maximum observed plasma concentration ($C_{\text{max}}$), time to $C_{\text{max}}$ ($t_{\text{max}}$), trough concentration ($C_{\text{min}}$), and area under the plasma concentration-time curve (AUC$_{(0-t)}$). Assessment of dose proportionality was carried out for the plasma PK data using a power model (Supplemental Tables 2 and 3). An estimate of the mean slope of log$_e$ (dose) was reported for each relevant parameter, along with corresponding 90% confidence intervals.

Further exploratory analysis was performed using the Analysis of Variance (ANOVA) method after normalising the parameters by a chosen nominal dose of 100mcg. Adjusted geometric
means and 90% CIs were presented for each dose along with estimated treatment ratios and corresponding 90% CI.

Assessment of accumulation for the plasma PK data was carried out, using data from the single and repeat dose phases in Study B. Accumulation ratios were calculated for both Peak (5 min post-dose) and Trough (24 h post-dose) values, using day 14 and day 1 values. Peak:Trough ratios were also calculated for day 1 and day 14.

No formal assessment of steady-state was performed. Visual analysis of the concentration-time profiles taken daily during the studies was used to assess the achievement of steady-state concentration in the plasma of dosed subjects.

BAL samples (cells and supernatant) were analysed for nemiralisib concentrations for derivation of lung ELF and cell pellet concentrations (Study B only). Urea levels were measured in the BAL samples together with the corresponding time matched plasma sample for calculation of ELF dilution factor (using urea as a dilution marker). The dilution factor was used to multiply the BAL nemiralisib concentration to derive the volume of ELF within the sample and subsequently concentrations within ELF. The derived ELF concentrations of nemiralisib for each BAL wash were calculated separately. A pooled analysis was calculated per subject by calculating the total mass of nemiralisib in all three washes and dividing by the total volume of ELF within all three washes. The cell pellet concentration (Study B only) was derived using the resulting ELF concentration and the ratio of the raw (not urea corrected) sample results between supernatant and cell pellet (reconstituted and lysed).

**PIP2 and PIP3 detection and analysis in sputum samples**

PIP3 peak area proportion (PIP3 peak area/[PIP3 peak area+PIP2 peak area]) was calculated from the mass spectrometer peak areas for PIP3 and PIP2 (derived using established methodology (Clark et al., 2011)). The PIP3 proportion, using PIP2, was calculated to correct for changes in cell numbers between different sputum samples, assuming PIP2 was correlated with cell number (Clark et al., 2011). The time of freeze of
each sputum sample was recorded and the time elapsed between collection and freeze was calculated. For clinical study samples, $\log_\text{e} (\text{PIP3 peak area Proportion})$ was analysed using mixed effects, repeated measures analyses including time to freeze as a covariate. Treatment ratios (and 90% CI) of adjusted geometric means for nemiralisib versus placebo are presented. Statistical models were used to determine Bayesian posterior probabilities (assuming a non-informative prior); a Student's $t$-cumulative distribution function was used to obtain the probabilistic statements.

**Results**

**Demographics and Safety Data for Study A & Study B**

For pharmacokinetic and pharmacodynamic assessment, 56 subjects were randomised in the two studies (Table 1). The ages, heights and weights of the subjects in both studies were broadly similar. All subjects were current smokers, with the mean number of cigarettes smoked per day ranging from 14 to 19 across the two studies, and mean pack years ranging from 10 to 18 (Table 1). The reported adverse effects were minor, with the most common being headache, none of which were attributed to being related to test agent (Supplemental Table 4).

**Single Dose PK (plasma)**

Following single inhaled administration of doses of 0.1 to 6.4 mg of nemiralisib, peak plasma nemiralisib concentrations ($C_{\text{max}}$) were achieved rapidly; typically, in the initial sample taken post dose at approx. 5 min (Figure 1). Concentrations declined rapidly and in a bi-exponential manner with a more protracted terminal elimination phase. Minor fluctuations in the concentration time profiles post dose were observed and were reflected in the median data particularly at the low dose. Exposures increased in an approximately proportional manner in both Study A and Study B, with geometric mean values presented in Table 2.
Repeat Dose PK (plasma)

Following once daily administration of 2 mg nemiralisib for 14 days in Study B, there was an accumulation of approximately 4.5-fold on trough concentrations and 3-fold on peak values (Figure 2). Peak to trough ratios were approximately 3.2 on day 1 and 2.2 on day 14. Based on peak and trough concentrations, steady state was reached by day 7, with levels comparable, although variable, between days 7 through to day 14. Exposure data can be seen in Table 2.

BAL PK (BAL ELF and cell fraction)

In Study A, following single nebulised dosing at 6.4 mg, concentrations of nemiralisib in ELF derived from BAL data obtained 3 h post-dosing was approximately 598 ng/mL of ELF. Using the time matched plasma concentration of nemiralisib (approximately 1.1 ng/ml) a lung ELF to plasma ratio was approximately 540:1.

In Study B, exposure of the lung to nemiralisib, following repeat 2 mg daily dosing, was derived from BAL samples obtained 24 h post day 14. Lung ELF and cell pellet concentrations at 24 h post day 14 dose were 55 ng/mL (ELF:plasma ratio 32:1) and 366 ng/mL (cell:plasma ratio 213:1), respectively (Table 3).

PIP3 levels in induced sputum

In Study A, PIP3 proportion after 3 h dosing, was reduced from 0.00382 (0.00341, 0.00428) after receiving placebo, to 0.00306 (0.00273, 0.00343) following a single 0.4 mg dose, and to 0.00294 (0.00262, 0.00330) following a single 6.4 mg dose of nemiralisib (Figure 3, Panel A). The ratios of adjusted geometric means were 0.801 (0.691, 0.928) for 0.4 mg vs placebo, and 0.770 (0.663, 0.895) for 6.4 mg vs placebo, representing reductions of 20% and 23% respectively. Therefore, from these data, the probability that there truly is a decrease from placebo is 99.1% for 0.4 mg and 99.6% for 6.4 mg.
In Study B, nemiralisib reduced PIP3 levels measured 3h after inhalation in a dose-dependent manner from 0.00320 (0.00292, 0.00350) after receiving placebo to 0.00260 (0.00236, 0.00286) following a single 2 mg dose (Figure 3, Panel B). The ratio of adjusted geometric means for 2 mg vs placebo was 0.813 (0.723, 0.915), representing a 19% decrease in the levels of PIP3 compared to placebo, with the probability that there truly is a decrease from placebo of 99.8%. The reduced levels of PIP3 were maintained at 24 h after receiving a single 2 mg dose; however broadly returned to placebo levels in the subjects dosed with 0.1 mg and 0.5 mg.

The degree of reduction in PIP3 levels was enhanced after 12 days repeat dosing of 2 mg of nemiralisib, to 0.00205 (0.00175, 0.00241) representing a 36% decrease at 3h compared to placebo, with the probability that there truly is a decrease of 98.1% (Table 4). The levels of PIP3 were still reduced at 24 h after the final 14-day dose at 0.00208 (0.00186, 0.00234). Therefore, both studies demonstrated a reduction in the levels of PIP3 in sputum samples following single and repeat dosing with nemiralisib, compared to placebo (Table 4).

**Relationship between nemiralisib exposure and pharmacodynamic effect**

Figure 4 shows a post-hoc analysis of the combined PIP3 proportion values from both studies plotted against the respective time matched plasma concentration from each individual subject treated with nemiralisib. When evaluating this data, it can be observed that as the plasma concentration of nemiralisib increased, the PIP3 proportion decreased (range 0.0016 to 0.0063), with a correlation of -0.3518 and an unadjusted p value of 0.0012. The range of PIP3 proportion following placebo dosing was 0.0020 to 0.0064.

**Discussion**
The value of inhaled therapeutics includes quick onset of action, target organ deposition and potential avoidance of systemic consequences. The research described here aimed to address the need to understand lung exposure and the consequence of target engagement whilst in early stage clinical development. Here, we present the pharmacokinetics (PK) of nemiralisib (GSK2269557), a novel inhaled inhibitor of PI3Kδ, in blood and lung samples, and compare the data with alterations in a pharmacodynamic (PD) marker in induced sputum samples taken at the same time. We have successfully demonstrated PI3Kδ target engagement in these samples as indicated by consistent reductions in sputum PIP3 levels following single and repeat dosing of nemiralisib. This was observed in two clinical studies with different formulations, and the combined data show that as the levels of inhaled drug exposure increase (measured in plasma), the proportion of PIP3 in sputum decreases in a linear fashion, and data are consistent with a once daily dosing interval.

Smoking is described to directly activate the PI3Kδ pathway (Daijo et al., 2016; To et al., 2010), and although the PIP3 reductions (~36%) may seem modest in our studies, this is likely because only the PI3Kδ-dependent PIP3 component of total PIP3 will have been inhibited due to the high selectivity of nemiralisib over other PI3K isoforms. To try and understand the observed degree of reduction in PIP3 in sputum, we performed an additional in vitro experiment. Here we measured the reduction of PIP3 levels in unstimulated isolated human neutrophils in the presence of nemiralisib and a pan-PI3K inhibitor (Supplemental Figure 1). In this experiment the reduction with nemiralisib was similar to that achieved with the pan-PI3K, suggesting the change was entirely PI3Kδ driven (Supplemental Figure 1).

Interestingly, the degree of reduction in PIP3 levels in isolated neutrophils is similar to that observed in sputum following dosing of nemiralisib, suggesting this change is the maximal achievable inhibition through inhibition of PI3Kδ alone. We have recently demonstrated that inhalation of nemiralisib also results in reduction of inflammatory cytokines in sputum from stable COPD patients dosed for 14 days (Cahn et al., 2017).
The reduction in PIP3 levels was greater in subjects dosed for 14-days compared to a single dose, demonstrating that the effects of nemiralisib are increased upon repeat dosing, potentially due to greater exposure. Notably, reductions in PIP3 levels were still observed 24 h after dosing (see Table 4). The absolute PIP3 values, together with the degree of change in PIP3 proportion observed were broadly consistent across the two clinical studies, indicating that this is a robust biomarker of PI3Kδ activity.

The nemiralisib plasma concentration-time profiles were slightly variable, consistent with a drug taken by inhalation, and were characterized by two peaks; a first one occurring at 5 min after dosing and a second smaller one at approximately 2 h, thus resulting in a median \( t_{\text{max}} \) ranging between 5 min to 2.0 h. The first peak representing rapid absorption from the lung and the second peak representing an oral absorption component as recently characterised (Wilson et al., 2018). The plasma concentrations subsequently decrease less rapidly, with the profile showing a more protracted elimination route.

Exposure to nemiralisib increased with increasing dose in an approximately proportionate manner, with some deviations in derived \( C_{\text{max}} \) and AUC parameters between individuals within a dose level, and between doses and cohorts. Definitive kinetics, in particular calculation of the terminal elimination phase, was difficult to show in both studies due to the slow rate of observed elimination of compound from plasma. This meant that the sampling regimen (and in particular the last time point) were influential in the ability to accurately determine the terminal half-life and this also impacted the ability to calculate AUC to infinity. In a recent study in healthy Japanese volunteers (Ino et al., 2019), the terminal half-life appeared to be approximately 42 h and is being further characterised in an ongoing human ADME study (NCT03315559).

In our studies, plasma accumulation was observed upon repeat administration of nemiralisib consistent with a long terminal elimination rate, with steady-state being achieved by day 7. This is a consistent time to steady-state for a molecule with an elimination rate of at least 36
h, which would be expected to achieve steady-state by day 6 following once daily administration.

The data derived from BAL demonstrated a high concentration of nemiralisib in the lungs as compared to the plasma exposure, as would be expected following topical delivery. At 3 h post-dose of a single nebulised solution, ELF levels were approximately 540 times higher than those in plasma. Upon repeat administration of a dry powder formulation to steady-state however, the ELF levels at 24 h post last dose were 32 times that of plasma. However, given that the cellular concentration was 213 times higher when compared to plasma, collectively these data demonstrates that the compound was distributed into the cellular compartment. The measured total cell fraction may be an over-estimation of available drug as it reflects a combination of intracellular, cell-bound and undissolved drug captured during the BAL process. Therefore, the intracellular concentration capable of inhibiting the kinase could be lower.

Measuring cellular lung trough concentration is extremely valuable for translating in vitro to in vivo data, and suggests a 2 mg dose would be sufficient to engage the target for a period of 24 h, indicating a once daily therapeutic dosing interval. Cellular levels however, may not be truly reflective of drug within the cell and could represent drug which is intracellular and bound to the outside, or undissolved drug material. However, while we cannot be certain if the drug is intracellular or cell bound, the BAL concentration measured in our study (see Table 3) equates to approximately 10nM. This concentration of nemiralisib has been shown to generate robust effects across multiple in vitro assays (Down et al., 2015). This demonstrates the importance of combining target pharmacokinetics with pharmacodynamics over a range of doses and exposures in order to characterise the shape of the PK/PD response.

Although the formulation was different between the two studies explored here (solution versus dry powder), due to the high solubility of nemiralisib it is anticipated that the dry
powder will rapidly dissolve upon deposition in the lung and therefore would be expected to
demonstrate similar PK properties to the nebulized solution. It is also assumed that
distribution of drug in the lung would be broadly similar for both formulations, although this is
difficult to experimentally establish. Recently we have demonstrated that the PK profile in
stable COPD patients closely matches that presented here in healthy volunteers, suggesting
a similar exposure (Cahn et al., 2017).

The combination of plasma PK profile, with the PD reductions in sputum PIP3 demonstrate a
direct relationship between increasing plasma exposure and reducing PIP3 proportion in
sputum with no evidence of hysteresis. This suggests a good correlation between plasma
exposures at the times measured (3 h and 24 h post dose) and exposure in the effect
compartment driving the response. Plasma PK are absorption/distribution rate limiting
following delivery of nemiralisib to the lungs. It is assumed (although not proven) that the
sputum response is reflective of pharmacology within the ELF and lung cellular
compartment.

In conclusion, we demonstrate that nemiralisib has acceptable tolerability, with a well-
defined PK profile, showing significantly higher levels in lung compared to plasma.
Nemiralisib appears to engage PI3Kδ in the target organ, as demonstrated by the reduction
of PIP3 in sputum, with a linear relationship observed between plasma exposure and
reduction in PIP3 levels. The generation of a well-defined PK/PD relationship adds to our
confidence to progress the development of a molecule and facilitates interpretation of
subsequent clinical observations.

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Footnotes

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Figures legend

Figure 1: Nemiralisib 24 h plasma PK profile

Median plasma concentration of nemiralisib over 24 h following single dosing in Study A (Panel A 0.4 and 6.4 mg only) and single dosing (SD) and 14-day repeat dosing (RD) in Study B (Panel B 0.1, 0.5 and 2 mg SD and 2 mg RD).

Figure 2: Daily $C_{\text{max}}$ and $C_{\text{min}}$ plasma values following 14-day repeat dosing of nemiralisib in study B

Geometric mean (with min and max values) of plasma concentrations of nemiralisib at 5 min ($C_{\text{max}}$), and 24 h ($C_{\text{min}}$) post dose following 14-day daily dosing

Figure 3: PIP3 levels in induced sputum 3 h post dose

Nemiralisib reduced the PIP3 levels in induced sputum samples in healthy smokers who were given single dose administration of a nebulised (Panel A), or dry power formulation (Panel B)

Figure 4: Relationship between exposure and pharmacodynamic effect

Post-hoc analysis of the combined data from both studies reveal a direct relationship between the increase in plasma exposure of nemiralisib and the decrease in proportion of PIP3 levels was observed. All data from Study A (triangles), and Study B (squares) are included. PIP3: phosphatidylinositol-trisphosphate; SD: single dosing; RD: repeat dosing
Table 1: Demographics and baseline lung function of the subjects who smoked cigarettes in Study A (nebulised formulation) and Study B (dry powder formulation)

|                          | Study A |                          | Study B |                          |
|--------------------------|---------|--------------------------|---------|--------------------------|
|                          | Single Dose Cross Over | Repeat Dose Parallel Group |         |                         |
|                          | Cohort 1 | Cohort 2 | Placebo | 2 mg |
| N                        | 15       | 12       | 16      | 16   |
| Age in Years, Mean (SD)  | 34.2 (9.85) | 34.8 (11.48) | 39.3 (9.69) | 31.1 (6.51) |
| Sex, n (%):              |          |          |         |      |
| Female:                  | 0 (0%)   | 0 (0%)   | 1 (6%)  | 0 (0%) |
| Male:                    | 15 (100%) | 12 (100%) | 15 (94%) | 3 (100%) |
| BMI (kg/m2), Mean (SD)   | 25.15 (3.22) | 23.37 (2.58) | 25.06 (3.338) | 23.53 (2.458) |
| Height (cm), Mean (SD)   | 175.7 (6.66) | 177.2 (6.10) | 177.1 (6.42) | 176.3 (10.21) |
| Weight (kg), Mean (SD)   | 77.45 (9.46) | 73.46 (10.05) | 78.34 (9.085) | 73.07 (8.406) |
| History of smoking use:  |          |          |         |      |
| Current smoker, n (%)    | 15 (100%) | 12 (100%) | 16 (100%) | 3 (100%) |
| Cigarettes smoked per day, Mean (SD) | 14.1 (4.20) | 19.2 (4.69) | 14.4 (4.49) | 18.3 (2.89) |
| Years Smoked, Mean (SD)  | 14.7 (8.00) | 18.7 (11.27) | 18.0 (9.24) | 14.0 (8.00) |
| Number of pack years, Mean (SD) | 10.0 (5.4) | 18.0 (12.31) | 13.0 (8.21) | 12.0 (5.29) |
| FEV1 (L), Mean (SD)      | 3.903 (0.4081)* | 3.843 (0.3835)* | 3.413 (0.5740)* | 4.063 (1.0053) |

*Values obtained when receiving placebo dose
Table 2 – Summary of plasma PK from Study A and Study B following single
and repeat dosing with nemiralisib

| Parameter (units) | Dose (mg) | Day | N   | Geometric mean (90% CI) |
|-------------------|-----------|-----|-----|-------------------------|
|                   |           |     |     |                         |
| **Study A (Nebulised)** |           |     |     |                         |
| C<sub>max</sub> (pg/mL) | 0.4       | 1   | 12  | 225 (188-271)           |
|                   | 6.4       | 1   | 13  | 3276 (2468-4348)        |
|                   | 6.4 (BAL cohort) | 1   | 12  | 2575 (2134-3106)        |
| **Study B (Dry Powder Inhaler)** |           |     |     |                         |
| C<sub>max</sub> (pg/mL) | 0.1       | 1   | 16  | 44.4 (34.1-57.8)        |
|                   | 0.5       | 1   | 15  | 291 (230-370)           |
|                   | 2         | 1   | 21  | 1266 (1006-1593)        |
|                   | 2         | 14  | 9   | 3682 (3154-4299)        |
| AUC<sub>(0-24)</sub> (pg.h/mL) | 0.1       | 1   | 16  | 142 (94-214)            |
|                   | 0.5       | 1   | 15  | 2763 (2107-3625)        |
|                   | 2         | 1   | 21  | 14272 (10690-19054)     |
|                   | 2         | 14  | 9   | 50612 (42689-60004)     |

C<sub>max</sub>: maximum observed plasma concentration; AUC<sub>(0-24)</sub>: plasma concentration-time curve over 0 to 24 h
**Table 3: BAL PK (plasma, ELF and cell pellet PK) at day 14 trough**

| Study A: Single 6.4 mg nebulised BAL at 3 h* post dose | Concentration (ng/mL, 90% CI) |
|------------------------------------------------------|-------------------------------|
| Plasma pre-BAL                                        | 1.11 (0.83-1.48)              |
| Plasma post-BAL                                       | 1.02 (0.72-1.46)              |
| BAL ELF                                               | 598 (385-927)                 |

| Study B: Repeat daily 2 mg dry powder BAL at 24 h post Day 14 dose | Concentration (ng/mL, 90% CI) |
|------------------------------------------------------------------|-------------------------------|
| Plasma                                                           | 1.72 (1.41-2.09)              |
| BAL ELF                                                          | 55.3 (39.8-77.1)              |
| Cell Fraction                                                    | 366 (214-628)                 |

*Plasma sample taken either side of BAL procedure with actual time range 2.7 to 3 h and 3.2 to 3.5 h for pre- and post-dose respectively with pre-BAL used for ratio to plasma estimate. BAL: bronchoalveolar lavage; ELF: epithelial lining fluid. Methodology for Cell Fraction drug level detection were not available during Study A.
Table 4: PIP3 levels in induced sputum

| Study A | Dose | Day  | Time point | N  | PIP3 Proportion (90% CI) | Ratio of Geometric Mean vs. Placebo (90% CI) | % Reduction in PIP3 Proportion | Probability (ratio <1) |
|---------|------|------|------------|----|-------------------------|---------------------------------------------|---------------------------------|---------------------|
| Placebo | 1    | 3 h  | 11         | 0.00382 (0.00341, 0.00428) | 801 (0.691, 0.928) | 20% | 99.1% |
| 0.4 mg  | 1    | 3 h  | 10         | 0.00306 (0.00273, 0.00343) | 0.801 (0.691, 0.928) | 20% | 99.1% |
| 6.4 mg  | 1    | 3 h  | 11         | 0.00294 (0.00262, 0.00330) | 0.770 (0.663, 0.895) | 23% | 99.6% |

| Study B | Dose | Day  | Time point | N  | PIP3 Proportion (90% CI) | Ratio of Geometric Mean vs. Placebo (90% CI) | % Reduction in PIP3 Proportion | Probability (ratio <1) |
|---------|------|------|------------|----|-------------------------|---------------------------------------------|---------------------------------|---------------------|
| Placebo | 1    | 3 h  | 18         | 0.00320 (0.00292, 0.00350) |                                              |                                |                                  |                     |
|         | 1    | 24 h | 18         | 0.00321 (0.00292, 0.00352) |                                              |                                |                                  |                     |
|         | 12   | 3 h  | 3          | 0.00319 (0.00240, 0.00426) |                                              |                                |                                  |                     |
|         | 12   | 24 h | 3          | 0.00255 (0.00213, 0.00304) |                                              |                                |                                  |                     |
| 0.1 mg  | 1    | 3 h  | 15         | 0.00298 (0.00267, 0.00334) | 933 (0.835, 1.043) | 7% | 84.8% |
|         | 1    | 24 h | 16         | 0.00334 (0.00301, 0.00371) | 1.043 (0.940, 1.158) | -4% | 25.0% |
| 0.5 mg  | 1    | 3 h  | 15         | 0.00299 (0.00263, 0.00326) | 0.916 (0.816, 1.027) | 9% | 89.8% |
|         | 1    | 24 h | 15         | 0.00312 (0.00281, 0.00346) | 0.973 (0.874, 1.083) | 3% | 66.4% |
| 2 mg    | 1    | 3 h  | 21         | 0.00260 (0.00236, 0.00286) | 0.813 (0.723, 0.915) | 19% | 99.8% |
| SD      | 1    | 24 h | 11         | 0.00270 (0.00238, 0.00307) | 0.843 (0.729, 0.974) | 16% | 97.4% |
| 2 mg    | 12   | 3 h  | 9          | 0.00205 (0.00175, 0.00241) | 0.643 (0.460, 0.899) | 36% | 98.1% |
| RD      | 12   | 24 h | 9          | 0.00208 (0.00186, 0.00234) | 0.819 (0.672, 0.998) | 18% | 95.2% |

PIP3: phosphatidylinositol-trisphosphate; SD: single dosing; RD: repeat dosing
Figure 1: Nemiralisib 24hr plasma PK profile

A

Figure 1A: Concentration (pg/ml) vs. Time (Hours) for different doses of Nemiralisib. The graph shows the plasma PK profile for 6.4mg, 6.4mg (BAL Cohort), and 0.4mg.

B

Figure 1B: Concentration (pg/ml) vs. Time (Hours) for different doses and timepoints of Nemiralisib. The graph shows the plasma PK profile for 2mg (Day 14), 2mg (Day 1), 0.5mg (Day 1), and 0.1mg (Day 1).
**Figure 2** – Daily $C_{\text{max}}$ and $C_{\text{min}}$ plasma values following 14-day repeat dosing of nemiralisib in Study B
Figure 3: PIP3 levels in induced sputum, 3hrs post dose
Figure 4: Relationship between exposure and pharmacodynamic effect

$r = -0.3518$
$p = 0.0012$