Received: 10 July 2024 | Revised: 8 October 2024 | Accepted: 12 October 2024

DOI: 10.1111/cts.70060

ARTICLE

# Safety, pharmacokinetics, and pharmacodynamics of sofnobrutinib, a novel non-covalent BTK inhibitor, in healthy subjects: First-in-human phase I study

**Kyoko Miyamoto**[1] | **Robert M. Miller**[2] | **Christine Voors-Pette**[3] | **Jart A. F. Oosterhaven**[4] | **Marieke van den Dobbelsteen**[5] | **Katsuhiro Mihara**[5] | **Marian Geldof**[5] | **Yuji Sato**[6] | **Naomi Matsuda**[6] | **Shirou Kirita**[6] | **Masaaki Sawa**[6] | **Akinori Arimura**[1,6]

[1]CarnaBio USA, Inc., South San Francisco, California, USA

[2]Artemida Pharma, Ltd., Hertfordshire, UK

[3]QPS Netherlands BV, Groningen, The Netherlands

[4]ICON plc, Groningen, The Netherlands

[5]Venn Life Sciences ED B.V., Breda, The Netherlands

[6]Carna Biosciences, Inc., Kobe, Japan

**Correspondence**
Akinori Arimura, Carna Biosciences, Inc., 1-5-5 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan.
Email: akinori.arimura@dd.carnabio.com

**Funding information**
Carna Biosciences, Inc.

## Abstract

Bruton's tyrosine kinase (BTK) is a potential therapeutic target for allergic and autoimmune diseases. This first-in-human phase I study evaluated safety, pharmacokinetic, and pharmacodynamic profiles of sofnobrutinib (formerly AS-0871), a highly selective, orally available, non-covalent BTK inhibitor, in healthy adult subjects. Single ascending doses (SAD; 5–900 mg) and multiple ascending doses (MAD; 50–300 mg twice daily [b.i.d.] for 14 days [morning dose only on Day 14]) of sofnobrutinib were tested. In the entire study, all adverse events (AEs) were mild or moderate, and no apparent dose-proportional trend in severity or frequency was observed. No serious treatment-emergent AEs, cardiac arrythmias, or bleeding-related AEs were reported. In the SAD part, sofnobrutinib exhibited approximately dose-dependent systemic exposures up to 900 mg with rapid absorption (median time to maximum concentration of 2.50–4.00 h) and gradual decline (mean half-lives of 3.7–9.0 h). In the MAD part, sofnobrutinib showed low accumulation after multiple dosing (mean accumulation ratios of ≤1.54) and reached a steady state on ≤Day 7. Single dosing of sofnobrutinib rapidly and dose-dependently suppressed basophil and B-cell activations in ex vivo whole blood assays. Multiple dosing of sofnobrutinib achieved 50.8%–79.4%, 67.6%–93.6%, and 90.1%–98.0% inhibition of basophil activation during the dosing interval of 50, 150, and 300 mg b.i.d., respectively. Based on pharmacokinetic-pharmacodynamic analysis, half-maximal inhibitory concentration ($IC_{50}$) of sofnobrutinib for basophil activation was 54.06 and 57.01 ng/mL in the SAD and MAD parts, respectively. Similarly, $IC_{50}$ for B-cell activation was 187.21 ng/mL. These data support further investigation of sofnobrutinib in allergic and autoimmune diseases.

**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

Bruton's tyrosine kinase (BTK) inhibitors are potential therapeutics for allergic and inflammatory autoimmune diseases. However, treatment-emergent adverse events limit their development in non-malignant diseases. Sofnobrutinib is a highly selective, orally available, non-covalent BTK inhibitor that exhibited significant inhibitory efficacies on inflammatory and autoimmune diseases in animal models.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

This first-in-human phase I study investigated the safety, pharmacokinetic, and pharmacodynamic profiles of single and multiple ascending doses of sofnobrutinib in healthy male and female subjects.

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

Sofnobrutinib was safe and well-tolerated after single or multiple exposures to healthy subjects. Sofnobrutinib demonstrated a strong inhibition of B-cell and basophil activations in ex vivo whole blood assays.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

The findings support the further investigation of sofnobrutinib in patients with allergic and autoimmune diseases.

## INTRODUCTION

Bruton's tyrosine kinase (BTK) is a cytoplasmic, non-receptor tyrosine kinase and expressed in hematopoietic cell lineage including B cells, basophils, mast cells, and macrophages.[1,2] BTK acts downstream of the high-affinity immunoglobulin (Ig)-E receptor, FcεRI, on activation of basophils and mast cells, resulting in the release of histamine and the production of inflammatory cytokines.[3–5] Moreover, BTK is involved in B-cell receptor and IgG Fc receptor (FcγR)-mediated signaling, which contributes to the activation, differentiation, and proliferation of B cells and the production of inflammatory cytokines in macrophages/monocytes.[2,6] Small molecule inhibitors of BTK are, therefore, expected to be new therapeutics for allergic diseases such as chronic spontaneous urticaria (CSU)[7,8] and autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), and systemic lupus erythematosus (SLE).[6,9,10]

First- to second-generation covalent BTK inhibitors, ibrutinib, acalabrutinib, and zanubrutinib, have been approved for the treatment of B-cell malignancies, but life-threatening adverse reactions such as bleeding and atrial fibrillation have limited their applications for non-malignant indications.[11–13] Recently, a selective, non-covalent BTK inhibitor, fenebrutinib, has been reported to alleviate the clinical signs and symptoms in patients with CSU and RA in phase II studies.[14,15] In addition, selective, covalent BTK inhibitors such as remibrutinib, tolebrutinib, and evobrutinib have also been shown to be effective in CSU, MS, and SLE.[16–19] However, a reversible elevation in liver transaminases was reported in the clinical studies of some of these selective inhibitors,[14–19] limiting their development in allergy and autoimmune diseases.

Sofnobrutinib (AS-0871) is a highly selective, orally available, non-covalent BTK inhibitor. Sofnobrutinib exhibited significant suppression of IgE-mediated type I allergy in a mouse passive cutaneous anaphylaxis model and RA-like symptoms in a mouse collagen-induced arthritis model.[20] From these results, sofnobrutinib is anticipated to be a new therapeutic option for the treatment of allergic and autoimmune diseases.

This first-in-human phase I study was conducted to characterize the safety, pharmacokinetic (PK), and pharmacodynamic (PD) profiles of single and multiple oral doses of sofnobrutinib in healthy male and female subjects.

## METHODS

### Study design

This phase I study in healthy subjects consisted of three parts: a single ascending dose (SAD) part with sofnobrutinib formulated as a suspension (hereafter, sofnobrutinib suspension), a relative bioavailability (rBA)/ food effect (FE) part with sofnobrutinib formulated

as a tablet (hereafter, sofnobrutinib tablet), and a multiple ascending dose (MAD) part with sofnobrutinib tablet. The SAD part was a first-in-human study conducted at QPS Netherlands BV (Groningen, The Netherlands) between June 22, 2020 and November 25, 2020. The rBA/FE and MAD parts were part of phase I study conducted at ICON plc (Groningen, The Netherlands) between December 16, 2021 and April 17, 2023.

## SAD part

The SAD part was a double-blind (i.e., blind to subjects and researchers), placebo-controlled, randomized, interleaved study with seven treatment periods. Eight subjects each in two cohorts (Cohorts A and B) were alternately dosed with six ascending dose levels (5, 25, 100, 300, 600, and 900 mg under fasted conditions) of sofnobrutinib suspension or placebo (projected treatment ratio of 6:2), with at least 7 days of washout period between dosing (Figure 1a). Subjects were randomized in such a way that for each dose level, different subjects received placebo, except for 1 subject who received placebo twice in Treatment Periods 1 and 6. Food effect on the PK was investigated at the 300-mg dose level; the subjects who had received sofnobrutinib or placebo under fasted conditions in Treatment Period 4 received the same treatment in Treatment Period 6 following the intake of high-fat breakfast. In all treatment periods, sofnobrutinib and the matching placebo in suspension with commercial vehicle (Flavor Blend™, Humco, Texas, USA) were orally administered, followed by drinking of an amount of the vehicle to rinse the cup and the mouth. The total volume of sofnobrutinib or placebo suspension and the vehicle for rinsing was 200 mL in Treatment Periods 1–3 and 160 mL in Treatment Periods 4–7 (Table S1). One more rinsing step in which subjects rinsed their mouth with 50 mL water and spat it out was added in Treatment Periods 4–7 to remove the taste of Flavor Blend™. Full PK profiles were determined up to 72 h after study drug administration. Blood samples for PD assessments were obtained at predefined time points up to 24 h after study drug administration. Holter electrocardiogram (ECG) recording was performed at 4 dose levels (100, 300 [fasted], 600, and 900 mg) for continuous 25 h (−1 to 24 h after study drug administration).

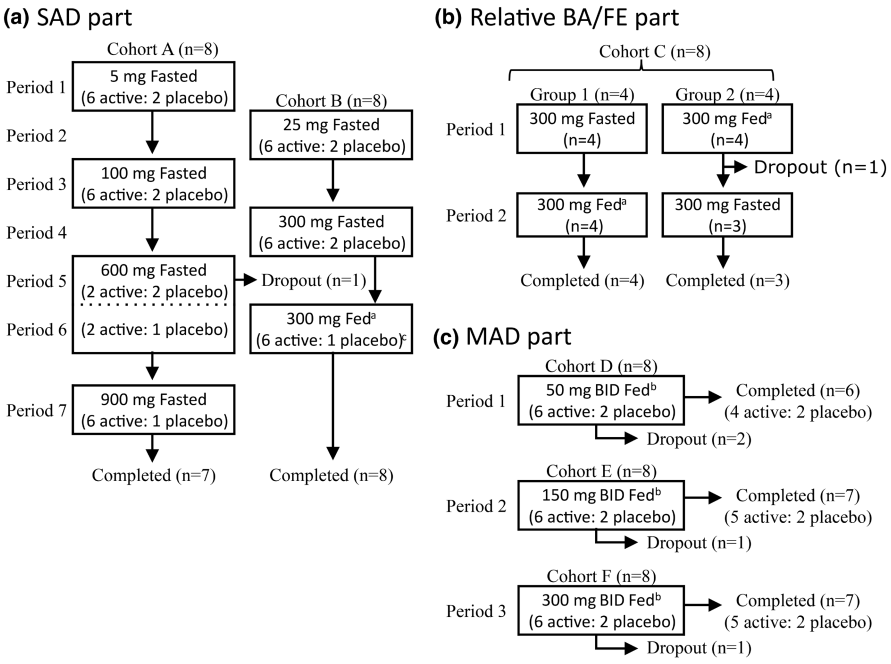


**FIGURE 1** Study design. (a) Single ascending doses of sofnobrutinib or placebo suspension were received by healthy subjects in two alternate cohorts (Cohorts A and B) with a washout period of at least 7 days. For 600-mg dose level, 4 subjects in Cohort A were treated in Period 5 and the other 3 in Period 6. Food effect was investigated in the same set of subjects (Cohort B). (b) Relative BA and FE of sofnobrutinib tablet were investigated in healthy subjects (Cohort C) with a washout period of at least 7 days between the conditions. Relative BA was determined using the different sets of subjects (Cohorts B vs. C), while FE was determined using the same set of subjects (Cohort C). (c) Multiple ascending doses of sofnobrutinib or placebo tablets were received by healthy subjects in three cohorts (Cohorts D–F). The treatment was given under fed conditions twice daily (b.i.d.) for 13 days and a morning dose only on Day 14. [a]High-fat breakfast; [b]Standardized diet; [c]One subject was excluded from the treatment due to logistic reasons. BA, bioavailability; b.i.d., *bis in die* (twice daily); FE, food effect; MAD, multiple ascending dose; *n*, number of subjects; SAD, single ascending dose.

### Relative BA/FE part

This part was an open-label, randomized, 2-period crossover design. Eight subjects (Cohort C) were randomized to two groups and received sofnobrutinib oral tablet at the dose of 300 mg under fasted and fed conditions (high-fat breakfast) in two treatment periods with a washout period of at least 7 days between dosing (Figure 1b). Full PK profiles were determined up to 72 h after study drug administration. Pharmacodynamic assessments were not performed for this part.

### MAD part

The MAD part was a double-blind (i.e., blind to subjects and researchers), placebo-controlled, randomized design, consisting of 3 cohorts (Cohorts D–F) of 8 subjects each, randomized 6:2 to active or placebo treatment. Three ascending doses (50, 150, and 300 mg twice daily [b.i.d.]) of sofnobrutinib tablet were tested (Figure 1c). Sofnobrutinib was orally administered b.i.d. for 14 days (morning dose only on Day 14) under fed conditions (standardized diet). Plasma PK profiles were determined up to 11 h after the first dose on Day 1 and up to 72 h after the last dose on Day 14. Additional plasma PK samples were taken before the morning doses on Days 7, 9, 11, and at the end-of-trial visit (6 ± 1 days after the last dose). Blood samples for PD assessment were collected at predefined time points up to 11 h after the first dose on Day 1 and up to 72 h after the last dose on Day 14. Holter ECG recording was performed at each dose level for 12 h on Day 1 (−1 to 11 h after the first dose), 25 h on Day 14 (−1 to 24 h after the last dose), and 1 h each on Days 16 and 17 (47 to 48 h and 71 to 72 h, respectively, after the last dose on Day 14). Telemetry was performed at each dose level for at least 16.5 h on Day −1 (baseline recording), 24 h each on Days 4 and 10 (−1 to 23 h after the morning dosing), and 24 h on Day 13 (−1.5 to 22.5 h after the morning dosing).

## Study end points

The primary end point was safety and tolerability of sofnobrutinib following single-dose and 14-day multiple-dose oral administration in healthy subjects. The secondary end point was PK, PD, and FE of sofnobrutinib in suspension formulation and the PK, PD, FE, and rBA of sofnobrutinib in tablet formulation. Exploratory end points (SAD and MAD parts only) were data collection for (future) investigation of potential QT effects of sofnobrutinib.

## Subjects

Eligible subjects were healthy males and females of non-childbearing potential, aged 18–64 years, with body mass index between 18.0 and 30.0 kg/m$^2$, and in good physical and mental health as established by medical history, physical examination, ECG and vital sign recording, and results of biochemistry, hematology, and urinalysis tests in the screening.

## Safety and tolerability assessments

The safety assessments included the regular monitoring of vital signs, physical examinations, 12-lead ECG, continuous 12-lead Holter ECG (SAD and MAD parts only), telemetry (MAD part only), and clinical laboratory tests (hematology, biochemistry, and urinalysis in all parts, and coagulation in the MAD part only). Adverse events (AEs) and serious AEs (SAEs) were recorded from signing of the informed consent form until the subject's last trial-related activity. Hemorrhage and bruising were predefined as AEs of special interest (AESIs) in the rBA/FE and MAD parts. All events were coded using the Medical Dictionary for Regulatory Activities version 23.0 (SAD part) or version 24.1 (rBA/FE and MAD parts), graded using Common Terminology Criteria for Adverse Events version 5.0, and evaluated for its relationship to the study drug administration.

## Pharmacokinetic assessments

For the SAD and rBA/FE parts, plasma samples were collected in each treatment period from all subjects at predose and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16 (SAD part only), 24, 48, and 72 h postdose. For the MAD part, plasma samples were collected in each treatment period from all subjects at predose and at 0.25, 0.5, 1, 2, 2.5, 3, 3.5, 4, 6, 8, and 11 h after the first dose on Day 1, at pre-morning dose on Days 7, 9, and 11, and at predose and at 0.25, 0.5, 1, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 24, 48, and 72 h after the last dose on Day 14.

Plasma concentrations of sofnobrutinib were determined by Ardena Bioanalysis BV (Assen, The Netherlands) using a validated liquid chromatography–tandem mass spectrometry method. The lower limit of quantitation of the assays was 1.00 ng/mL in plasma. Plasma samples from subjects assigned to placebo treatment were not analyzed.

## Pharmacodynamic assessments

BTK inhibition by sofnobrutinib was evaluated ex vivo by basophil activation assay (SAD and MAD parts) and

B-cell activation assay (SAD part only) using whole blood samples collected before and after dosing. Anti-IgE-induced CD63 upregulation in basophils and anti-IgD-induced CD69 upregulation in B cells were measured as the activation markers for basophils and B cells, respectively.

For the SAD part, whole blood samples were collected in each treatment period (except for the FE period) from all subjects at predose and 1, 2, 4, 6, 8, 12, and 24 h post-dose. For the MAD part, whole blood samples were collected in each treatment period from all subjects at predose and at 1, 2, 4, 6, 8, and 11 h after the first dose on Day 1 and at predose and at 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after the last dose on Day 14.

Anti-IgE-induced CD63 upregulation on basophils ($CD123^+/HLA\text{-}DR^-$) and anti-IgD-induced CD69 upregulation on naïve B cells ($CD19^+/CD27^-$) were determined by Sanquin Diagnostic Services (Amsterdam, The Netherlands) using the qualified ex vivo methods[21] with modifications as described in Supplementary Information.

## Statistical analysis

The safety analysis set consisted of all randomized subjects who received at least one dose of study drug. The numbers of subjects experiencing treatment-emergent AEs (TEAEs) were summarized by treatment.

The PK analysis set consisted of all randomized subjects who received at least one dose of study drug and of whom at least 1 plasma concentration was available. Plasma PK parameters were estimated from the individual plasma concentration-time data for all evaluable subjects using standard noncompartmental methods (Phoenix™ WinNonlin® version 8.1) and summarized by treatment using descriptive statistics. Subjects who vomited after dosing were excluded from the PK analysis. The dose proportionality of sofnobrutinib for $C_{max}/AUC_{\infty}$ (SAD) and $C_{max}/AUC_{12h}$ (MAD) was explored based on the power model (relating the log of the PK parameter linearly to the log of the dose).

The PD analysis set consisted of all randomized subjects who received at least one dose of study drug and of whom at least one PD parameter was calculated. The percent changes from baseline in basophil CD63 and naïve B-cell CD69 upregulation were summarized by treatment. Subjects whose predose sample was not taken or who vomited after dosing were excluded from the PD analysis. Subjects whose predose sample showed <0.5% CD63 or CD69 upregulation for at least one treatment period in the SAD part or <1% CD63 upregulation on Day 1 in the MAD part were considered as non-responders and were excluded from the PD analysis. The subject whose Day 1 predose sample showed <250 basophil count in measurements was excluded from the analysis in the MAD part only. The change in the exclusion rules made from the MAD part was to increase data reliability.

Individual PK and PD data were subjected to a PK/PD analysis to determine PD characteristics. The relationship between %inhibition of CD63 or CD69 upregulation and plasma concentration of sofnobrutinib was graphically described, and the half-maximal inhibitory concentration ($IC_{50}$) with 90% confidence interval (CI) was determined using R (R Foundation). The following equation was used to fit and describe the PK/PD relationship based on top and bottom values of the curve fit, plasma concentration level (C), and derived Hill Slope.

$$\text{Inhibition} = \text{Bottom} + (\text{Top} - \text{Bottom})/\left[1 + 10^{((\log10(IC50) - \log10(C)) \cdot \text{HillSlope})}\right]$$

The exploratory statistical analyses were performed by Venn Life Sciences ED BV (Breda, The Netherlands). All summaries and statistical analyses were generated using Phoenix™ WinNonlin® version 8.1 (PK results) and SAS version 9.4 or higher (safety, PK and PD results).

## Ethics

The study protocols were reviewed by an independent ethics committee, and the studies were conducted in accordance with the ethical principles of the Declaration of Helsinki, International Conference of Harmonization Guideline for Good Clinical Practice (GCP), European Union GCP directives, and the applicable national regulatory requirements. All subjects provided written informed consent before beginning any study procedures.

# RESULTS

## Subject disposition and demographics

In total 48 healthy subjects were enrolled. Of these, 16 subjects were randomized to two cohorts of the SAD part, 8 subjects to one cohort with two groups in the rBA/FE part, and 24 subjects to three cohorts of the MAD part (Figure 1). In Treatment Period 5 of the SAD part, 1 subject was tested positive for COVID-19 on Day −1 and dropped out of the study, and 3 close-contact subjects went into quarantine in accordance with the governmental instructions. Thus, only 4 subjects were dosed in Treatment Period 5, and 3 straggler subjects, after quarantine, were dosed in the next treatment

period. Overall, 42 subjects completed the study. Of the 6 subjects who prematurely discontinued the study, 5 discontinuations were due to AEs (3 at least possibly related and 2 not related) and 1 due to a personal reason. Subject demographics and baseline characteristics were comparable between the cohorts and the treatments (Table S2).

## Safety and tolerability

All AEs reported during the study are listed in Table S3, and TEAEs are summarized in Tables 1–3. Treatment-emergent AEs were reported in 25 (47.2%) occasions (21 [52.5%] on active; 4 [30.8%] on placebo) in the SAD part, 4 (26.7%) occasions (2 [28.6%] under fasted; 2 [25.0%] under fed conditions) in the rBA/FE part, and 19 (79.2%) subjects (14 [77.8%] on active; 5 [83.3%] on placebo) in the MAD part. Of them, 20 (37.7%) occasions (19 [47.5%] on active; 1 [7.7%] on placebo) in the SAD part and 4 (16.7%) subjects (4 [22.2%] on active; none on placebo) in the MAD part were considered at least possibly related to study drug. All TEAEs were of mild (Grade 1) or moderate (Grade 2) intensity and were confirmed as recovered/resolved during the trial or by the follow-up contact, except for 2 unknown outcomes (Grade 1, not related) in the SAD part and 1 recovering/resolving (Grade 1, not related) in the rBA/FE part. No SAEs nor AESIs (i.e., hemorrhage and bruising) were reported. There were 5 TEAEs leading to study discontinuations: 2 COVID-19 infections (Grade 1, not related) in the SAD and MAD parts, 2 medical device site reactions (Grade 2, at least possibly related) both in the MAD part, and 1 ear inflammation (Grade 1, at least possibly related and possibly caused by ear wax) in the MAD part. Nausea and vomiting were frequently reported in the SAD part, and some of them were considered to be caused by the intense sweetness for drug taste masking and the large volume (200 mL in Treatment Periods 1–3; 160 mL in Treatment Periods 4–7) of the vehicle solution administered (Tables S1 and S4). The medical device site reaction was a skin irritation at the site of the ECG or telemetry electrodes, which was reported in the MAD part only and was considered to be caused by the long and repeated application of the electrode stickers. It was frequently reported during Treatment Period 1 (2 Grade 2 and 1 Grade 1 on active [50 mg b.i.d.]; 1 Grade 1 on placebo) and led to the study discontinuation of 2 subjects who had Grade 2 reactions. Therefore, for the following treatment periods, the electrodes were changed to the less irritating type, and the total time that the electrodes were applied to the skin was shortened. After these changes, medical device site reactions were still reported but of mild intensity (2 Grade 1 each on active [150 mg b.i.d.] and placebo) in Treatment Period 2, and not reported in Treatment Period 3 (Table S5). Overall, there were no apparent trends toward increased reporting or increased severity of TEAEs with increasing doses of sofnobrutinib.

No clinically relevant abnormalities nor apparent trends after dosing compared to baseline were observed in any of the clinical laboratory parameters for any of the treatments. One subject in the SAD part experienced transient elevations in liver enzymes after 600 and 900 mg dosing, which were of Grade 1 and were considered clinically irrelevant. No liver enzyme elevations were observed in the MAD part.

No clinically relevant abnormalities were observed in any of the safety ECGs (all parts), Holter ECG (SAD and MAD parts), or telemetry results (MAD part only) for any of the treatments. In particular, none of the subjects experienced a confirmed QT interval corrected for heart rate according to Fridericia (QTcF) prolongation, which was defined as QTcF value >500 ms or an increase from baseline >60 ms, during the trial.

Overall, sofnobrutinib was safe and well-tolerated at all evaluated doses. No clinically significant changes were observed in hematology, biochemistry, or urinalysis parameters or in cardiovascular function assessments, supporting the further clinical investigation of sofnobrutinib.

## Pharmacokinetics

After single oral dosing in the fasted conditions, sofnobrutinib was rapidly absorbed across all dose levels (5–900 mg) with a median time to reach the maximum plasma concentration ($t_{max}$) ranged from 2.50 to 4.00 h (Figure 2a and Table 4). After achieving the maximum plasma concentration ($C_{max}$), the plasma concentrations declined gradually with mean elimination half-life ($t_{1/2}$) values between 7.3 and 9.0 h in the dose range of 100–900 mg. The lower mean $t_{1/2}$ values were obtained at 5 mg (3.7 h) and 25 mg (5.6 h), which are likely related to the concentrations falling below limit of quantification early in the time course. Based on the power model assessment of PK parameters versus sofnobrutinib dose, $C_{max}$ was found to increase dose proportionally while area under the concentration-time curve (AUC) from time zero to infinity ($AUC_{\infty}$) appeared to increase more than dose-proportional (Figure S1). After intake of high-fat breakfast, sofnobrutinib showed a decreased absorption, as evidenced by lower $C_{max}$ and $AUC_{\infty}$ values than those under fasted conditions in most subjects, with a geometric mean ratio of fed to fasted of 0.553 (90% CI:

**TABLE 1** Summary of TEAEs in the SAD part.*Grade 1688552842345Grade 20000000011Grade 3 or higher0000000000*

| Dose level | 5 mg Fasted | 25 mg Fasted | 100 mg Fasted | 300 mg Fasted | 300 mg Fed[a] | 600 mg Fasted | 900 mg Fasted | Pooled active | Pooled placebo | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cohort** | **A** | **B** | **A** | **B** | **B** | **A** | **A** | **A and B** | **A and B** | **A and B** |
| **Number of subjects** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 4)** | **(*n* = 6)** | **(*n* = 40[b])** | **(*n* = 13[c])** | **(*n* = 53[d])** |
| Any TEAE | | | | | | | | | | |
| Subjects (%)[e] | 3 (50.0%) | 3 (50.0%) | 3 (50.0%) | 4 (66.7%) | 2 (33.3%) | 1 (25.0%) | 5 (83.3%) | 21 (52.5%) | 4 (30.8%) | 25 (47.2%) |
| Events | 6 | 8 | 8 | 5 | 5 | 2 | 8 | 42 | 4 | 46 |
| *Grade 1* | *6* | *8* | *8* | *5* | *5* | *2* | *8* | *42* | *3* | *45* |
| *Grade 2* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *1* | *1* |
| *Grade 3 or higher* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | 0 | *0* |
| TEAE at least possibly related to study drug | | | | | | | | | | |
| Subjects (%)[e] | 2 (33.3%) | 3 (50.0%) | 3 (50.0%) | 3 (50.0%) | 2 (33.3%) | 1 (25.0%) | 5 (83.3%) | 19 (47.5%) | 1 (7.7%) | 20 (37.7%) |
| Events | 2 | 6 | 5 | 3 | 5 | 2 | 7 | 30 | 1 | 31 |
| TEAE leading to study discontinuation | | | | | | | | | | |
| Subjects (%)[e] | 0 | 0 | 1 (16.7%) | 0 | 0 | 0 | 0 | 1 (2.5%) | 0 | 1 (1.9%) |
| COVID-19 | 0 | 0 | 1 (16.7%) | 0 | 0 | 0 | 0 | 1 (2.5%) | 0 | 1 (1.9%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* |
| Any SAE | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Any death | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Any AESI[f] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TEAE reported by ≥2 subjects or occasions in total | | | | | | | | | | |
| Headache | 2 (33.3%) | 1 (16.7%) | 1 (16.7%) | 1 (16.7%) | 1 (16.7%) | 1 (25.0%) | 3 (50.0%) | 10 (25.0%) | 0 | 10 (18.9%) |
| *Related* | *1 (16.7%)* | *1 (16.7%)* | *1 (16.7%)* | *1 (16.7%)* | *1 (16.7%)* | *1 (25.0%)* | *3 (50.0%)* | *9 (22.5%)* | *0* | *9 (17.0%)* |
| Nausea | 0 | 1 (16.7%) | 2 (33.3%) | 0 | 0 | 1 (25.0%) | 2 (33.3%) | 6 (15.0%) | 1 (7.7%) | 7 (13.2%) |
| *Related* | *0* | *1 (16.7%)* | *2 (33.3%)* | *0* | *0* | *1 (25.0%)* | *2 (33.3%)* | *6 (15.0%)* | *0* | *6 (11.3%)* |
| Vomiting | 0 | 1 (16.7%) | 1 (16.7%) | 0 | 0 | 0 | 1 (16.7%) | 3 (7.5%) | 0 | 3 (5.7%) |
| *Related* | *0* | *1 (16.7%)* | *1 (16.7%)* | *0* | *0* | *0* | *1 (16.7%)* | *3 (7.5%)* | *0* | *3 (5.7%)* |
| Diarrhea | 0 | 1 (16.7%) | 1 (16.7%) | 0 | 0 | 0 | 0 | 2 (5.0%) | 0 | 2 (3.8%) |
| *Related* | *0* | *1 (16.7%)* | *1 (16.7%)* | *0* | *0* | *0* | *0* | *2 (5.0%)* | *0* | *2 (3.8%)* |
| Pyrexia | 1 (16.7%) | 1 (16.7%) | 0 | 0 | 0 | 0 | 0 | 2 (5.0%) | 0 | 2 (3.8%) |
| *Related* | *1 (16.7%)* | *1 (16.7%)* | *0* | *0* | *0* | *0* | *0* | *2 (5.0%)* | *0* | *2 (3.8%)* |
| Fatigue | 0 | 0 | 0 | 0 | 2 (33.3%) | 0 | 0 | 2 (5.0%) | 0 | 2 (3.8%) |
| *Related* | *0* | *0* | *0* | *0* | *2 (33.3%)* | *0* | *0* | *2 (5.0%)* | *0* | *2 (3.8%)* |

(Continues)

**TABLE 1** (Continued)

| Dose level | 5 mg Fasted | 25 mg Fasted | 100 mg Fasted | 300 mg Fasted | 300 mg Fed[a] | 600 mg Fasted | 900 mg Fasted | Pooled active | Pooled placebo | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cohort** | **A** | **B** | **A** | **B** | **B** | **A** | **A** | **A and B** | **A and B** | **A and B** |
| **Number of subjects** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 4)** | **(*n* = 6)** | **(*n* = 40[b])** | **(*n* = 13[c])** | **(*n* = 53[d])** |
| Back pain | 1 (16.7%) | 0 | 1 (16.7%) | 0 | | 0 | 0 | 2 (5.0%) | 0 | 2 (3.8%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* |
| Paraesthesia | 0 | 0 | 0 | 1 (16.7%) | 0 | 0 | 0 | 1 (2.5%) | 1 (7.7%) | 2 (3.8%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* | *0* |

Abbreviations: AESI, adverse event of special interest; COVID-19, coronavirus disease 2019; *n*, number of subjects; related, at least possibly related to study drug; SAD, single ascending dose; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

[a]High-fat breakfast.

[b]40 treatment occasions in 16 subjects.

[c]13 treatment occasions in 11 subjects.

[d]53 treatment occasions in 16 subjects.

[e]For pooled or total data, the subject who experienced any TEAE in multiple treatment periods was counted multiple times.

[f]AESIs were not predefined in SAD part, therefore, the data were retrospectively analyzed.

0.411–0.744) for $C_{max}$ and 0.634 (90% CI: 0.461–0.873) for $AUC_{\infty}$ (Figure 2a and Table 4).

Single oral dosing of the sofnobrutinib tablet showed comparable PK profiles to those of sofnobrutinib suspension at the same dose level (300 mg) (Figure 2b,c, and Table 4). In contrast to the suspension, however, sofnobrutinib tablet showed a positive FE in most subjects, as evidenced by higher $C_{max}$ and $AUC_{\infty}$ values in fed conditions than those in fasted conditions, with a geometric mean ratio of fed to fasted of 1.27 (90% CI: 0.918–1.77) for $C_{max}$ and 1.36 (90% CI: 1.11–1.66) for $AUC_{\infty}$. Relative BA of sofnobrutinib tablet, as determined by the ratio of geometric mean $AUC_{\infty}$ values of tablet to suspension, was 0.535 (90% CI: 0.396–0.725) in fasted conditions and 1.11 (90% CI: 0.834–1.48) in fed conditions. From these results, sofnobrutinib tablet in the fed conditions, which is expected to result in higher exposure along with better drug compliance, was selected as the dosing condition for the MAD part.

Multiple oral b.i.d. dosing of sofnobrutinib tablet showed similar PK profiles to those of single oral dosing (the first dose on Day 1). Low accumulation was observed at all three dose levels on Day 14, with the mean of individual accumulation ratios ranging from 1.15 to 1.40 for $C_{max}$ and from 1.18 to 1.54 for AUC from 1 to 12 h ($AUC_{12h}$) (Figure 2d and Table 4). Sofnobrutinib achieved steady-state exposure on Day 7 or before, as indicated by the stable trough concentration ($C_{trough}$) between Days 7 and 14 (Figure 2d-middle). Based on the power model assessment of PK parameters versus sofnobrutinib dose, $C_{max}$ and $AUC_{12h}$ appeared to increase more than dose proportional on Day 1 while dose proportional on Day 14 (Figure S2).

## Pharmacodynamics

Single oral dosing of sofnobrutinib suspension suppressed the basophil CD63 and B-cell CD69 upregulations at the lowest dose of 5 mg sofnobrutinib upwards in a dose-dependent manner (Figure 3a,b). Maximum inhibition of basophil and B-cell activation was reached immediately after dosing of 100 mg or higher sofnobrutinib, with the duration extended dose-dependently.

In the MAD part where the tablet formulation was used, basophil activation was suppressed at all three dose levels in a dose-dependent manner (Figure 3c). Maximum inhibition was achieved at 150 mg or higher dose after a single and repeated dosing. During the b.i.d. dosing interval (i.e., Day 14 predose to 12 h postdose), basophil activation was persistently inhibited in the range of 50.8%–79.4% at 50 mg, 67.6%–93.6% at 150 mg, and 90.1%–98.0% at 300 mg b.i.d. (Table S6).

**TABLE 2** Summary of TEAEs in the rBA/FE part.

| Dose level | 300 mg Fasted | 300 mg Fed[a] | Total |
|---|---|---|---|
| **Cohort** | **C** | **C** | **C** |
| **Number of subjects** | **(*n* = 7)** | **(*n* = 8)** | **(*n* = 15[b])** |
| Any TEAE | | | |
| Subjects (%)[c] | 2 (28.6%) | 2 (25.0%) | 4 (26.7%) |
| Events | 4 | 4 | 8 |
| *Grade 1* | *4* | *4* | *8* |
| *Grade 2* | *0* | *0* | *0* |
| *Grade 3 or higher* | *0* | *0* | *0* |
| TEAE at least possibly related to study drug | 0 | 0 | 0 |
| TEAE leading to study discontinuation | 0 | 0 | 0 |
| Any SAE | 0 | 0 | 0 |
| Any death | 0 | 0 | 0 |
| Any AESI | 0 | 0 | 0 |
| TEAE reported by ≥2 subjects or occasions in total | | | |
| Headache | 2 (28.6%) | 0 | 2 (13.3%) |
| *Related* | *0* | *0* | *0* |
| Abdominal discomfort | 1 (14.3%) | 1 (12.5%) | 2 (13.3%) |
| *Related* | *0* | *0* | *0* |

Abbreviations: AESI, adverse event of special interest; FE, food effect; *n*, number of subjects; rBA, relative bioavailability; related, at least possibly related to study drug; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

[a]High-fat breakfast.

[b]15 treatment occasions in 8 subjects.

[c]For total data, the subject who experienced any TEAE in multiple treatment periods was counted multiple times.

## Pharmacokinetics/pharmacodynamics relationship

Based on the PK/PD analysis, the $IC_{50}$ values of sofnobrutinib for basophil CD63 upregulation were 54.06 ng/mL (90% CI: 43.54–64.58 ng/mL) with Hill Slope of −0.82 in the SAD part and 57.01 ng/mL (90% CI: 43.64–70.38 ng/mL) with Hill Slope of −1.92 in the MAD part. Similarly, the $IC_{50}$ value of sofnobrutinib for B-cell CD69 upregulation in the SAD part was 187.21 ng/mL (90% CI: 171.60–202.82 ng/mL) with Hill Slope of −1.70.

## DISCUSSION

This first-in-human phase I study investigated the safety, tolerability, PK, and PD of a highly selective, non-covalent BTK inhibitor, sofnobrutinib, in healthy subjects. Single ascending doses of sofnobrutinib in oral suspension up to 900 mg and multiple ascending doses as a tablet formulation up to 300 mg b.i.d. for 14 days (morning dose only on Day 14) were evaluated and found to be safe and well-tolerated with a favorable PK profile and promising PD results. No clinically relevant, treatment-related abnormalities were observed throughout this study.

During the trial, 6 subjects prematurely discontinued the study, but the study was continued in a blind manner (for SAD and MAD parts) and completed without any replacement. Among the 5 TEAEs leading to the study discontinuation, 2 events of Grade 2 medical device site reaction (i.e., skin irritation at the site of Holter ECG or telemetry electrodes) and 1 event of Grade 1 ear inflammation possibly caused by ear wax were considered at least possibly related to the study drug. Skin irritation was reported only in the MAD part and was probably a contact hypersensitivity to materials of the electrode stickers by the repeated and prolonged exposure.[22,23] There was no trend toward increased reporting of skin irritation with increasing dose of sofnobrutinib. Follow-up contact was made after the study discontinuation, and both the skin irritation and ear inflammation cases were confirmed as recovered/resolved.

BTK inhibitors such as fenebrutinib, remibrutinib, evobrutinib, and tolebrutinib have been reported to

**TABLE 3** Summary of TEAEs in the MAD part.

| Dose level | 50 mg b.i.d. | 150 mg b.i.d. | 300 mg b.i.d. | Pooled active b.i.d. | Pooled placebo b.i.d. | Total |
|---|---|---|---|---|---|---|
| **Cohort** | **D** | **E** | **F** | **D–F** | **D–F** | **D–F** |
| **Number of subjects** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 6)** | **(*n* = 18)** | **(*n* = 6)** | **(*n* = 24)** |
| Any TEAE | | | | | | |
| Subjects (%) | 4 (66.7%) | 4 (66.7%) | 6 (100.0%) | 14 (77.8%) | 5 (83.3%) | 19 (79.2%) |
| Events | 13 | 5 | 14 | 32 | 10 | 42 |
| *Grade 1* | *11* | *5* | *14* | *30* | *10* | *40* |
| *Grade 2* | *2* | *0* | *0* | *2* | *0* | *2* |
| *Grade 3 or higher* | *0* | *0* | *0* | *0* | *0* | *0* |
| TEAE at least possibly related to study drug | | | | | | |
| Subjects (%) | 2 (33.3%) | 2 (33.3%) | 0 | 4 (22.2%) | 0 | 4 (16.7%) |
| Events | 2 | 2 | 0 | 4 | 0 | 4 |
| TEAE leading to study discontinuation | | | | | | |
| Subjects (%) | 2 (33.3%) | 1 (16.7%) | 1 (16.7%) | 4 (22.2%) | 0 | 4 (16.7%) |
| COVID-19 | 0 | 0 | 1 (16.7%) | 1 (5.6%) | 0 | 1 (4.2%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* |
| Medical device site reaction | 2 (33.3%) | 0 | 0 | 2 (11.1%) | 0 | 2 (8.3%) |
| *Related* | *2 (33.3%)* | *0* | *0* | *2 (11.1%)* | *0* | *2 (8.3%)* |
| Ear inflammation | 0 | 1 (16.7%) | 0 | 1 (5.6%) | 0 | 1 (4.2%) |
| *Related* | *0* | *1 (16.7%)* | *0* | *1 (5.6%)* | *0* | *1 (4.2%)* |
| Any SAE | 0 | 0 | 0 | 0 | 0 | 0 |
| Death | 0 | 0 | 0 | 0 | 0 | 0 |
| Any AESI | 0 | 0 | 0 | 0 | 0 | 0 |
| TEAE reported by ≥2 subjects in total | | | | | | |
| Medical device site reaction | 3 (50.0%) | 2 (33.3%) | 0 | 5 (27.8%) | 3 (50.0%) | 8 (33.3%) |
| *Related* | *2 (33.3%)* | *1 (16.7%)* | *0* | *3 (16.7%)* | *0* | *3 (12.5%)* |
| Headache | 2 (33.3%) | 0 | 1 (16.7%) | 3 (16.7%) | 0 | 3 (12.5%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* |
| Nasopharyngitis | 2 (33.3%) | 0 | 1 (16.7%) | 3 (16.7%) | 0 | 3 (12.5%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* |
| Dry skin | 0 | 1 (16.7%) | 0 | 1 (5.6%) | 2 (33.3%) | 3 (12.5%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* |
| Abdominal discomfort | 1 (16.7%) | 0 | 1 (16.7%) | 2 (11.1%) | 0 | 2 (8.3%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* |
| Constipation | 1 (16.7%) | 0 | 1 (16.7%) | 2 (11.1%) | 0 | 2 (8.3%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* |
| Flatulence | 1 (16.7%) | 1 (16.7%) | 0 | 2 (11.1%) | 0 | 2 (8.3%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* |
| Dizziness | 0 | 0 | 2 (33.3%) | 2 (11.1%) | 0 | 2 (8.3%) |
| *Related* | *0* | *0* | *0* | *0* | *0* | *0* |

Abbreviations: AESI, adverse event of special interest; b.i.d., *bis in die* (twice daily); COVID-19, coronavirus disease 2019; MAD, multiple ascending dose; *n*, number of subjects; related, at least possibly related to study drug; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

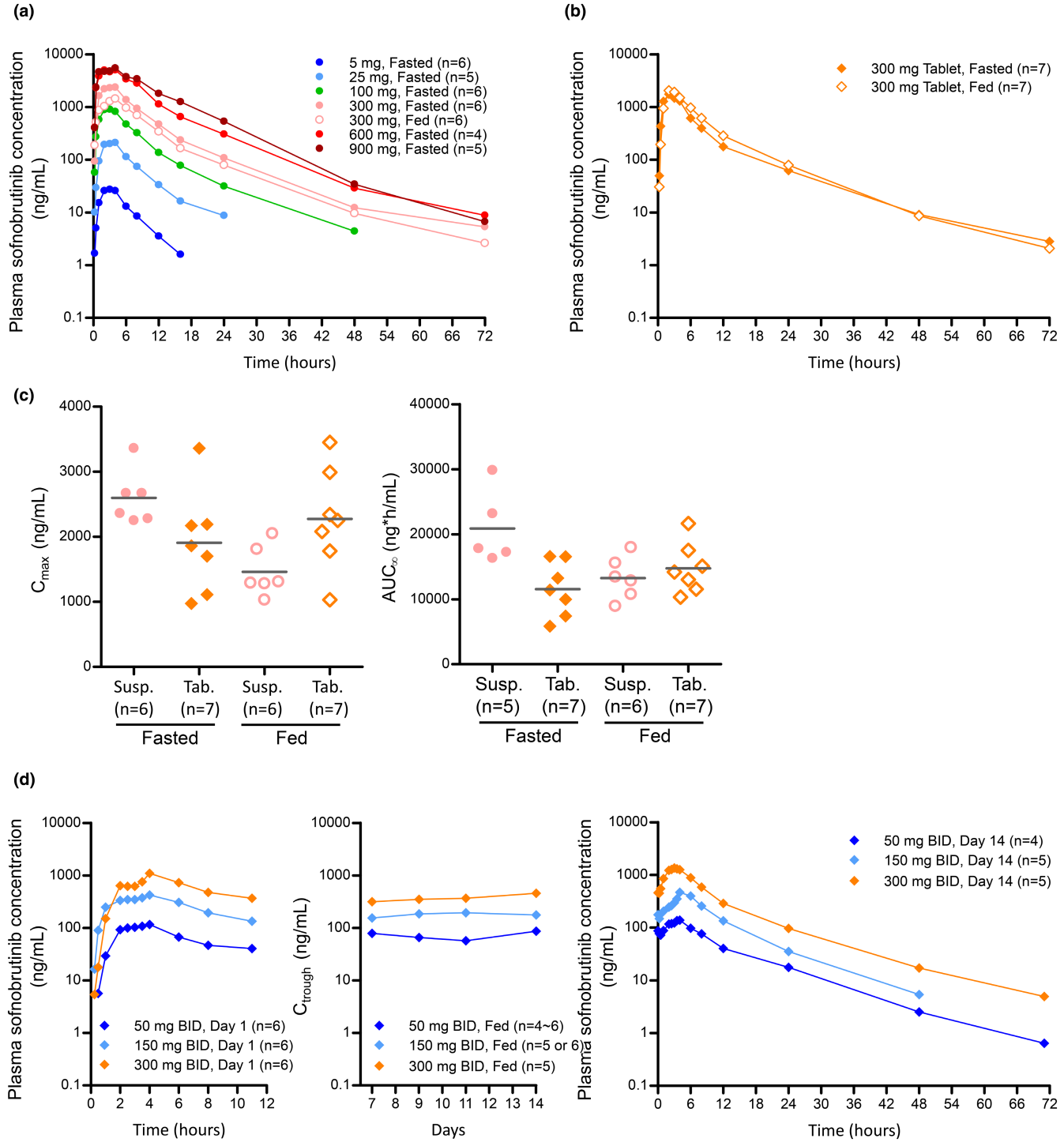


**FIGURE 2** Pharmacokinetic profiles of sofnobrutinib. (a) Single ascending doses of sofnobrutinib oral suspension administered in the fasted conditions and FE at 300-mg dose level. Mean concentration-time profiles are shown in semilogarithmic scale. (b) Food effect of sofnobrutinib oral tablet at 300-mg dose level. Mean concentration-time profiles are shown in semilogarithmic scale. (c) Relative BA of sofnobrutinib oral tablet compared to sofnobrutinib oral suspension in the fasted and fed conditions. Individual $C_{max}$ and $AUC_{\infty}$ values were plotted. The bar indicates the mean value. (d) Multiple ascending doses of sofnobrutinib oral tablet administered in the fed conditions, Day 1 after the first dose (left), Days 7, 9, 11, and 14 before the morning dose (middle), and Day 14 after the last dose (right). Mean concentration-time profiles are shown in semilogarithmic scale. When more than half of the values at a single time point were below quantification limit (<1.0 ng/mL), the mean value was reported as below quantification limit, which was treated as zero and is missing in the semilogarithmic plots. Subjects who vomited after dosing (1 subject each at 25 and 900 mg in the SAD part and 1 subject in the fed condition in the rBA/FE part) were excluded from the analyses. $AUC_{\infty}$, area under the plasma concentration-time curve from time zero to time infinity; BA, bioavailability; b.i.d., *bis in die* (twice daily); $C_{max}$, maximum plasma concentration; $C_{trough}$, concentration just prior to the beginning of a dosing interval; FE, food effect; *n*, number of subjects; rBA, relative bioavailability; SAD, single ascending dose; Susp., suspension; Tab., tablet.

**TABLE 4** Plasma pharmacokinetic parameters.

| Single-Dose | Condition | Cohort | *n* | $C_{max}$ (ng/mL) | $t_{max}$ (h) | $AUC_{last}$ (ng h/mL) | $AUC_{\infty}$ (ng h/mL) | $t_{1/2}$ (h) | CL/F (L/h) | $V_z$/F (L) |
|---|---|---|---|---|---|---|---|---|---|---|
| Oral suspension | | | | | | | | | | |
| 5 mg | Fasted | A | 6 | 28.6 (12.1) | 3.01 (2.00–4.03) | 177 (85.5) | 186 (88.1) | 3.7 (0.8) | 32.6 (16.0) | 166 (71.7) |
| 25 mg | Fasted | B | 5 | 219 (67.0) | 4.00 (2.00–4.00) | 1577 (562) | 1606 (553) | 5.6 (1.9) | 16.9 (5.00) | 127 (14.2) |
| 100 mg | Fasted | A | 6 | 980 (193) | 2.50 (2.00–4.00) | 7086 (1585) | 7118 (1587) | 8.2 (2.1) | 14.5 (2.70) | 168 (35.6) |
| 300 mg | Fasted | B | 6[a] | 2598 (417) | 2.52 (1.00–4.01) | 20,402 (5159) | 20,889 (5672) | 9.0 (1.2) | 15.1 (3.50) | 197 (57.7) |
| 300 mg | Fed[b] | B | 6 | 1462 (385) | 4.01 (1.00–4.01) | 13,228 (3255) | 13,270 (3243) | 8.6 (1.6) | 23.8 (6.07) | 295 (93.9) |
| 600 mg | Fasted | A | 4 | 5365 (2569) | 3.00 (1.03–4.02) | 50,274 (34,283) | 50,387 (34,442) | 8.2 (0.9) | 15.7 (7.88) | 186 (96.1) |
| 900 mg | Fasted | A | 5 | 5746 (2020) | 4.00 (1.00–4.03) | 64,105 (35,253) | 64,179 (35,310) | 7.3 (1.2) | 19.1 (12.0) | 212 (156) |
| Oral tablet | | | | | | | | | | |
| 300 mg | Fasted | C | 7 | 1909 (797) | 2.00 (1.00–3.00) | 11,508 (4201) | 11,595 (4200) | 9.7 (3.0) | 29.5 (12.3) | 422 (230) |
| 300 mg | Fed[b] | C | 7 | 2274 (788) | 2.00 (1.00–4.00) | 14,755 (3921) | 14,801 (3929) | 8.2 (1.3) | 21.4 (5.22) | 258 (90.7) |

| Multiple dose | Condition | Day | *n* | $C_{trough}$ (ng/mL) | $C_{max}$ (ng/mL) | $t_{max}$ (h) | $AUC_{12h}$[c] (ng h/mL) | $t_{1/2}$ (h) |
|---|---|---|---|---|---|---|---|---|
| Oral tablet | | | | | | | | |
| 50 mg b.i.d. | Fed[d] | 1 | 6 | – | 124 (41.2) | 3.74 (2.50–4.00) | 743 (229) | – |
| | | 14 | 4 | 86.7 (38.2) | 153 (46.5) | 3.52 (2.52–4.00) | 1075 (360) | 9.8 (4.0) |
| 150 mg b.i.d. | Fed[d] | 1 | 6[e] | – | 509 (244) | 4.01 (2.00–6.00) | 3070 (1491) | |
| | | 14 | 5 | 176 (89.0) | 526 (152) | 4.00 (1.00–6.00) | 3342 (862) | 8.4 (2.2) |
| 300 mg b.i.d. | Fed[d] | 1 | 6[f] | – | 1384 (497) | 4.01 (2.00–6.00) | 7033 (639) | – |
| | | 14 | 5 | 457 (141) | 1583 (779) | 3.00 (2.00–6.00) | 9550 (4031) | 10.2 (1.9) |

*Note*: $t_{max}$ is presented as median (minimum – maximum). The remaining parameters are presented as mean (SD), unless otherwise indicated.

Abbreviations: $AUC_{12h}$, area under the plasma concentration-time curve from 0 to 12 h; $AUC_{24h}$, area under the plasma concentration-time curve from 0 to 24 h; $AUC_{\infty}$, area under the plasma concentration-time curve from time zero to time infinity; $AUC_{last}$, area under the plasma concentration-time curve from time zero to the last measurable concentration; b.i.d., *bis in die* (twice daily); CL/F, apparent drug clearance; $C_{max}$, maximum plasma concentration; $C_{trough}$, concentration just prior to the beginning of a dosing interval; *n*, number of subjects; SD, standard deviation; $t_{1/2}$, elimination half-life; $t_{max}$, time to reach the maximum plasma concentration; $V_z$/F, apparent volume of distribution at the terminal phase.

[a] $n = 5$ for $AUC_{\infty}$, $t_{1/2}$, CL/F, and $V_z$/F.

[b] High-fat breakfast.

[c] Calculated with extrapolation from the 11-h sample for Day 1.

[d] Standardized diet.

[e] $n = 5$ for $AUC_{12h}$ and $AUC_{24h}$; $n = 3$ for $AUC_{\infty}$ and $t_{1/2}$.

[f] $n = 5$ for $AUC_{12h}$ and $AUC_{24h}$.

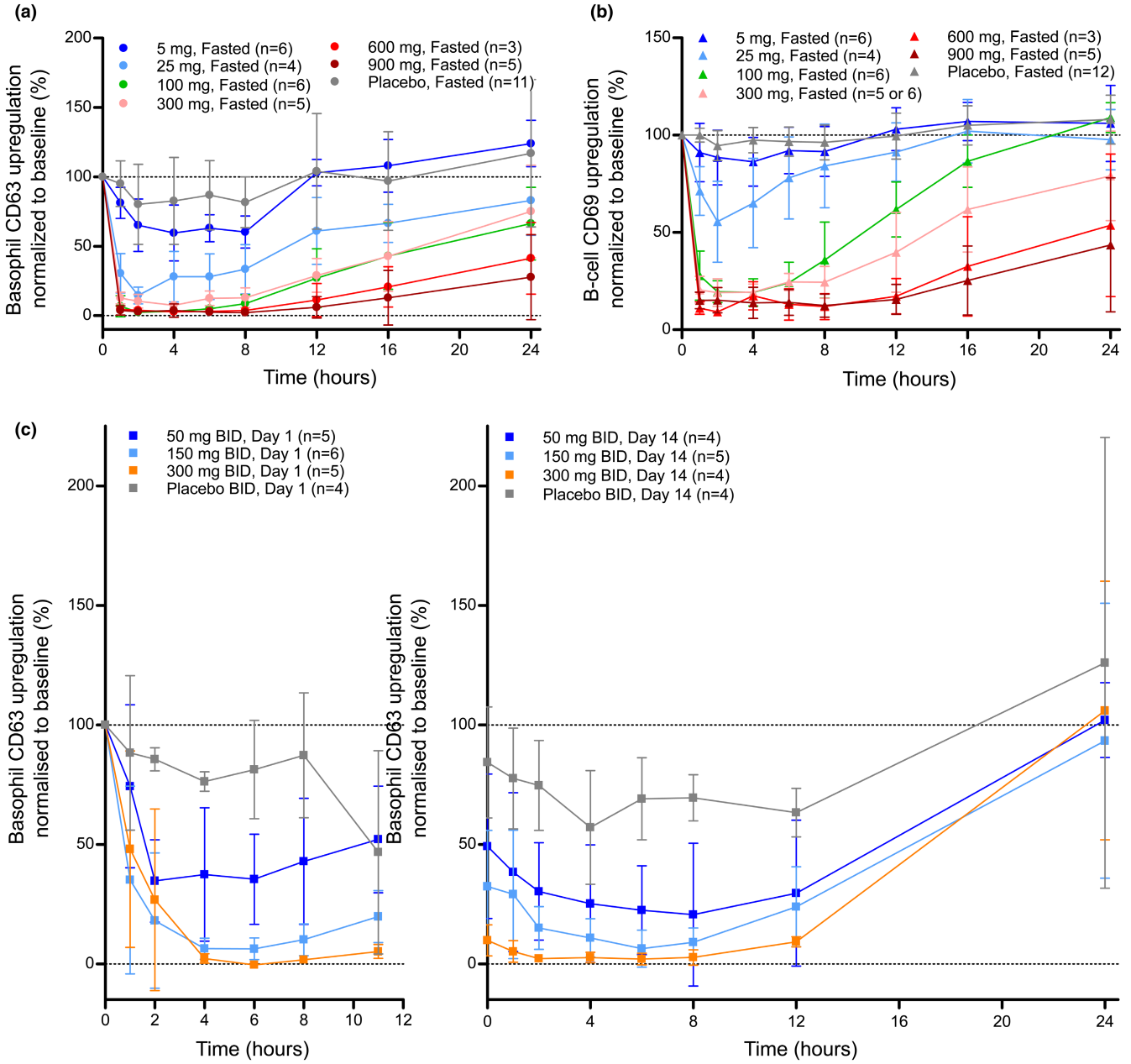


**FIGURE 3** Pharmacodynamics of sofnobrutinib. (a) Anti-IgE-induced basophil CD63 upregulation normalized to baseline in SAD part. Individual data were normalized to baseline values, and the mean (±standard deviation) of normalized values is presented. (b) Anti-IgD-induced B-cell CD69 upregulation normalized to baseline in SAD part. Individual data were normalized to baseline values, and the mean (±standard deviation) of normalized values is presented. (c) Anti-IgE-induced basophil CD63 upregulation normalized to baseline in MAD part after the morning dose on Day 1 (left) and after the morning dose on Day 14 (right). Individual data were normalized to each baseline (Day 1 predose) values, and the mean (±standard deviation) of normalized values is presented. The data at 48 and 72 h after the morning dose on Day 14 were taken but not shown. Subjects whose predose sample was not taken (1 subject at 25 mg in the SAD part), who vomited after the dosing (1 subject each at 25 and 900 mg in the SAD part), whose Day 1 predose sample showed <250 basophil count in measurements (1 subject at Placebo in the MAD part), or who was considered as a non-responder as defined in the method (1 basophil non-responder at 300 mg in the SAD part and 3 basophil non-responders [1 each at 50, 300 mg, and placebo b.i.d.] in the MAD part) were excluded from the analyses. b.i.d., *bis in die* (twice daily); IgD, immunoglobulin D; IgE, immunoglobulin E; MAD, multiple ascending dose; *n*, number of subjects; SAD, single ascending dose.

be effective for the treatment of allergic diseases and autoimmune inflammatory disorders including CSU, RA, SLE, and MS, but a reversible elevation in liver transaminases was reported in these inhibitors.[14–19] Therefore, liver transaminases were specified in the exclusion, dose escalation, and withdrawal criteria of this

study, and carefully monitored during the trial. One subject in the SAD part showed a transient increase in liver enzymes after 600 and 900 mg dosing, but the abnormalities were of Grade 1 (mild) and were not considered clinically relevant. In addition, as bleeding and cardiac arrhythmias were frequently reported AEs with BTK inhibitor treatment,[11–13,24,25] they were monitored specifically in this study. Bleeding-related events (i.e., bruising and hemorrhage) were set as AESIs for the rBA/FE and MAD parts of this study, and additional monitoring with telemetry and coagulation assessments were performed (MAD part). Holter monitoring, in addition to 12-lead safety ECGs, was performed in the SAD and MAD parts, and QTcF was specifically monitored. However, there were no bleeding-related events or clinically relevant abnormalities in telemetry, coagulation, Holter monitoring, or safety ECGs observed during the trial. Bleeding-related AEs of ibrutinib are considered to be caused by suppression of platelet aggregation through on-target inhibition of BTK and off-target inhibition of Tec and Src-kinases,[24,25] and ibrutinib-mediated atrial fibrillation has been reported to be caused by off-target inhibition of C-terminal Src kinase (CSK).[26] In contrast to ibrutinib, sofnobrutinib has little or no inhibitory effect against Src-kinases and CSK,[20] which may contribute to the low risk of bleeding and cardiac arrhythmias. Overall, sofnobrutinib was safe and well-tolerated up to 900 mg in single oral doses and up to 300 mg b.i.d. in multiple doses.

Sofnobrutinib tablets exhibited a favorable PK profile with a positive FE (geometric mean ratio of 1.27 for $C_{max}$ and 1.36 for $AUC_{\infty}$) and a higher exposure than a suspension formulation (geometric mean ratio of 1.11 for $AUC_{\infty}$) in the fed condition. After multiple dosing, low accumulation was observed with the accumulation ratios of 1.15–1.40 for $C_{max}$ and 1.18–1.54 for $AUC_{12h}$. These results support the rationale that sofnobrutinib as a tablet formulation and administered under fed conditions will be suitable for the further clinical development and the future clinical usage.

Previous studies showed that the inhibitory potency of BTK inhibitors against basophil activation was associated with their clinical efficacy for IgE-dependent allergic responses, such as allergic rhinitis, nut allergy, and CSU.[14,16,21,27–29] Interestingly, phase II study of remibrutinib, a covalent BTK inhibitor, was reported to alleviate the clinical signs and symptoms of CSU at all dose levels tested (10–100 mg once daily [q.d.] and 10–100 mg b.i.d.) with no clear dose–response relationship; the intermediate dose of 25 mg b.i.d. was more effective than the higher doses of 100 mg q.d. and 100 mg b.i.d.[16] Taken together with the result of phase I remibrutinib study in which full inhibition of basophil CD63 upregulation was achieved at 50 mg q.d. or higher doses,[29] sub-maximum inhibition of basophil activation may be sufficient or even optimal to obtain the clinical efficacy in CSU. In the MAD part of this study, sofnobrutinib at steady state maintained suppression of the basophil activation during the b.i.d. dosing interval in the range of 51%–79%, 68%–94%, and 90%–98% inhibition at 50, 150, and 300 mg, respectively, suggesting that b.i.d. dosing of sofnobrutinib at or around the tested dose levels are anticipated to be clinically effective for the treatment of CSU, and potentially other allergic and autoimmune diseases. Moreover, unlike covalent BTK inhibitors, BTK inhibition by sofnobrutinib was completely reversed within 24 h after the last dose. This shorter reversal time may be preferable from the safety perspective since it may contribute to a faster recovery from any unexpected side effects caused by BTK inhibition should the treatment need to be interrupted.

In conclusion, this first-in-human phase I study of sofnobrutinib in healthy subjects demonstrated a favorable safety profile and strong inhibition of basophil activation in ex vivo whole blood assay. These results support the further investigation of sofnobrutinib in patients with allergic and autoimmune diseases.

## AUTHOR CONTRIBUTIONS

Ky.M., A.A., R.M.M., M.v.d.D., Ka.M., and M.S. wrote the manuscript. Ky.M., A.A., R.M.M., Y.S., N.M., S.K., C.V.-P., and J.A.F.O. designed the research. C.V.-P. and J.A.F.O. performed the research. M.v.d.D., Ka.M., and M.G. analyzed the data.

## ACKNOWLEDGMENTS

The authors thank all persons who participated in this study and the investigators and research staff at the study sites.

## FUNDING INFORMATION

This study was funded by Carna Biosciences, Inc.

## CONFLICT OF INTEREST STATEMENT

Ky.M. is an employee of CarnaBio USA, Inc., a subsidiary of Carna Biosciences, Inc. Y.S., N.M., S.K. are employees of Carna Biosciences, Inc., the company which is developing sofnobrutinib. M.S. is a Chief Scientific Officer and a Board member of Carna Biosciences, Inc. A.A. is a Chief Development Officer of CarnaBio USA, Inc. and Carna Biosciences, Inc., and a Board member of Carna Biosciences, Inc. R.M.M. is a medical monitor of Carna Biosciences, Inc. C.V.-P. is a former employee of QPS Netherlands BV, Clinical Research Organization

sponsored by Carna Biosciences, Inc. for the execution of this study. J.A.F.O. is an employee of ICON, Clinical Research Organization sponsored by Carna Biosciences, Inc. for the execution of this study. M.v.d.D., Ka.M., and M.G. are employees of Venn Life Sciences ED B.V., Consultant of Carna Biosciences, Inc.

## ORCID

*Kyoko Miyamoto* https://orcid.org/0009-0001-3157-2824
*Robert M. Miller* https://orcid.org/0000-0003-2030-1778
*Jart A. F. Oosterhaven* https://orcid.org/0000-0001-7214-7528
*Katsuhiro Mihara* https://orcid.org/0009-0005-8746-619X
*Masaaki Sawa* https://orcid.org/0000-0002-0077-2188
*Akinori Arimura* https://orcid.org/0009-0009-0167-3868

## REFERENCES

1. Liang C, Tian D, Ren X, et al. The development of Bruton's tyrosine kinase (BTK) inhibitors from 2012 to 2017: a mini-review. *Eur J Med Chem*. 2018;151:315-326.
2. Pal Singh S, Dammeijer F, Hendriks RW. Role of Bruton's tyrosine kinase in B cells and malignancies. *Mol Cancer*. 2018;17(1):57.
3. Dispenza MC, Krier-Burris RA, Chhiba KD, Undem BJ, Robida PA, Bochner BS. Bruton's tyrosine kinase inhibition effectively protects against human IgE-mediated anaphylaxis. *J Clin Invest*. 2020;130(9):4759-4770.
4. Hata D, Kawakami Y, Inagaki N, et al. Involvement of Bruton's tyrosine kinase in FcεRI-dependent mast cell degranulation and cytokine production. *J Exp Med*. 1998;187(8):1235-1247.
5. Smiljkovic D, Blatt K, Stefanzl G, et al. BTK inhibition is a potent approach to block IgE-mediated histamine release in human basophils. *Allergy*. 2017;72(11):1666-1676.
6. McDonald C, Xanthopoulos C, Kostareli E. The role of Bruton's tyrosine kinase in the immune system and disease. *Immunology*. 2021;164(4):722-736.
7. Dispenza MC. The use of Bruton's tyrosine kinase inhibitors to treat allergic disorders. *Curr Treat Options Allergy*. 2021;8(3):261-273.
8. Bernstein JA, Maurer M, Saini SS. BTK signaling—a crucial link in the pathophysiology of chronic spontaneous urticaria. *J Allergy Clin Immunol*. 2024;153(5):1229-1240.
9. Arneson LC, Carroll KJ, Ruderman EM. Bruton's tyrosine kinase inhibition for the treatment of rheumatoid arthritis. *Immunotargets Ther*. 2021;10:333-342.
10. Airas L, Bermel RA, Chitnis T, et al. A review of Bruton's tyrosine kinase inhibitors in multiple sclerosis. *Ther Adv Neurol Disord*. 2024;17:1-17.
11. Tam CS, Thompson PA. BTK inhibitors in CLL: second generation drugs and beyond BTK inhibitors in CLL: second generation drugs and beyond. *Blood Adv*. 2024;8(9):2300-2309.
12. Boriani G, Menna P, Morgagni R, Minotti G, Vitolo M. Ibrutinib and Bruton's tyrosine kinase inhibitors in chronic lymphocytic leukemia: focus on atrial fibrillation and ventricular tachyarrhythmias/sudden cardiac death. *Chemotherapy*. 2023;68(2):61-72.
13. Quartermaine C, Ghazi SM, Yasin A, et al. Cardiovascular toxicities of BTK inhibitors in chronic lymphocytic leukemia: JACC: CardioOncology state-of-the-art review. *JACC CardioOncol*. 2023;5(5):570-590.
14. Metz M, Sussman G, Gagnon R, et al. Fenebrutinib in H1 antihistamine-refractory chronic spontaneous urticaria: a randomized phase 2 trial. *Nat Med*. 2021;27(11):1961-1969.
15. Cohen S, Tuckwell K, Katsumoto TR, et al. Fenebrutinib versus placebo or adalimumab in rheumatoid arthritis: a randomized, double-blind, phase II trial. *Arthritis Rheumatol*. 2020;72(9):1435-1446.
16. Maurer M, Berger W, Giménez-Arnau A, et al. Remibrutinib, a novel BTK inhibitor, demonstrates promising efficacy and safety in chronic spontaneous urticaria. *J Allergy Clin Immunol*. 2022;150(6):1498-1506.e2.
17. Montalban X, Arnold DL, Weber MS, et al. Placebo-controlled trial of an oral BTK inhibitor in multiple sclerosis. *N Engl J Med*. 2019;380(25):2406-2417.
18. Reich DS, Arnold DL, Vermersch P, et al. Safety and efficacy of tolebrutinib, an oral brain-penetrant BTK inhibitor, in relapsing multiple sclerosis: a phase 2b, randomised, double-blind, placebo-controlled trial. *Lancet Neurol*. 2021;20(9):729-738.
19. Wallace DJ, Dörner T, Pisetsky DS, et al. Efficacy and safety of the Bruton's tyrosine kinase inhibitor Evobrutinib in systemic lupus erythematosus: results of a phase II, randomized, double-blind, placebo-controlled dose-ranging trial. *ACR Open Rheumatol*. 2023;5(1):38-48.
20. Kawahata W, Asami T, Kiyoi T, et al. Design and synthesis of novel amino-triazine analogues as selective Bruton's tyrosine kinase inhibitors for treatment of rheumatoid arthritis. *J Med Chem*. 2018;61(19):8917-8933.
21. Herman AE, Chinn LW, Kotwal SG, et al. Safety, pharmacokinetics, and pharmacodynamics in healthy volunteers treated with GDC-0853, a selective reversible Bruton's tyrosine kinase inhibitor. *Clin Pharmacol Ther*. 2018;103(6):1020-1028.
22. Uter W, Schwanitz H. Contact dermatitis from propylene glycol in ECG electrode gel. *Contact Dermatitis*. 1996;34(3):230-231.
23. Wu H, Yang G, Zhu K, et al. Materials, devices, and systems of on-skin electrodes for electrophysiological monitoring and human–machine interfaces. *Adv Sci*. 2021;8(2):2001938.
24. von Hundelshausen P, Siess W. Bleeding by bruton tyrosine kinase-inhibitors: dependency on drug type and disease. *Cancers (Basel)*. 2021;13(5):1-33.
25. Estupiñán HY, Berglöf A, Zain R, Smith CIE. Comparative analysis of BTK inhibitors and mechanisms underlying adverse effects. *Front Cell Dev Biol*. 2021;9:630942.
26. Xiao L, Salem JE, Clauss S, et al. Ibrutinib-mediated atrial fibrillation attributable to inhibition of C-terminal Src kinase. *Circulation*. 2020;142(25):2443-2455.
27. Regan JA, Cao Y, Dispenza MC, et al. Ibrutinib, a Bruton's tyrosine kinase inhibitor used for treatment of lymphoproliferative disorders, eliminates both aeroallergen skin test and basophil activation test reactivity. *J Allergy Clin Immunol*. 2017;140(3):875-879.e1.
28. Dispenza MC, Pongracic JA, Singh AM, Bochner BS. Short-term ibrutinib therapy suppresses skin test responses and eliminates IgE-mediated basophil activation in adults with peanut or tree nut allergy. *J Allergy Clin Immunol*. 2018;141(5):1914-1916.e7.

29. Kaul M, End P, Cabanski M, et al. Remibrutinib (LOU064): a selective potent oral BTK inhibitor with promising clinical safety and pharmacodynamics in a randomized phase I trial. *Clin Transl Sci*. 2021;14(5):1756-1768.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Miyamoto K, Miller RM, Voors-Pette C, et al. Safety, pharmacokinetics, and pharmacodynamics of sofnobrutinib, a novel non-covalent BTK inhibitor, in healthy subjects: First-in-human phase I study. *Clin Transl Sci*. 2024;17:e70060. doi:10.1111/cts.70060