

First-in-Human Single and Multiple Ascending Dose Studies of Balinatunfib, a Small Molecule Inhibitor of TNFR1 Signaling in Healthy Participants

Nassr Nassr^{1,*} , Faiza Rharbaoui¹ , Dietmar Weitz¹ , Johann Gassenhuber¹ , Markus Rehberg¹ , Markus Kohlmann¹ , Fabienne Schumacher¹ , Amel Lahmar² , Andreas Kovar¹ , Laurent Perrin³ , Frank-Dietrich Wagner⁴ , Maria Wiekowski² , and Mai Anh Nguyen¹ 

Oral small molecule inhibitors of tumor necrosis factor alpha (TNF α) are emerging as attractive therapeutic agents for the treatment of various autoimmune diseases. Balinatunfib (SAR441566), a novel oral inhibitor of tumor necrosis factor receptor 1 (TNFR1) signaling, changes the configuration of the soluble TNF α (sTNF α) trimer and prevents its heterotrimerization with TNFR1 but not TNFR2, thereby blocking TNFR1 signaling. Herein, we report the results from a first-in-human (FIH) study that evaluated the safety, pharmacokinetics (PK), and pharmacodynamics (PD) following single ascending doses (SAD) and multiple ascending doses (MAD) of balinatunfib in healthy male participants. Single (5–600 mg) and multiple (100–600 mg total daily dose for up to 14 days) oral doses of balinatunfib were well-tolerated in all participants. Consistent PK data were obtained across the studies, with a median $t_{\max}$ of 2.5–5 hours, a mean terminal half-life of 22–30 hours, and a time to steady state of 5–6 days. A supra-proportional exposure increase was observed in both SAD and MAD studies, which was less pronounced at doses ≥ 180 mg. Food had no relevant effects on the PK characteristics of balinatunfib. As the main PD read-out, complete TNF α occupancy was shown at all tested time points after the treatment started. Balinatunfib, as the first clinically tested oral TNFR1 signal inhibitor, demonstrated a good safety profile along with favorable PK/PD characteristics that allowed both once and twice daily dosing, confirming a successful preclinical-to-clinical translation and guiding dose selection for further clinical efficacy studies.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

 Biologic tumor necrosis factor α (TNF- α) inhibitors have emerged as therapeutic agents for the treatment of various inflammatory diseases. However, severe adverse effects with biologics led to further research. Oral small molecule inhibitors of human TNF might evolve as novel therapeutic agents for the treatment of these diseases with a differentiated profile based on the selective TNFR1 inhibition and sparing of TNFR2 signaling.

WHAT QUESTION DID THIS STUDY ADDRESS?

 This study investigated the safety and PK and PD characteristics following the administration of oral single and multiple ascending doses of balinatunfib, the first selective inhibitor of TNFR1 signaling, in healthy participants.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

 Balinatunfib was well tolerated following a single dose up to 600 mg and repeated doses up to 300 mg BID. This study also demonstrated a favorable clinical PK and PD profile of balinatunfib.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

 The PK and PD properties observed in this study were in alignment with the expectations reported previously, confirming a successful preclinical-to-clinical translation; the target occupancy results also aid in making informed decisions on the dosage regimen in further clinical investigations of balinatunfib.

¹Sanofi, Frankfurt, Germany; ²Sanofi, Bridgewater, New Jersey, USA; ³Sanofi, Montpellier, France; ⁴Charité Research Organization, Berlin, Germany.

*Correspondence: Nassr Nassr (nassr.nassr@sanofi.com)

Received November 20, 2024; accepted March 12, 2025. doi:10.1002/cpt.3655

Tumor necrosis factor alpha (TNF α), a pleiotropic cytokine that regulates both proinflammatory and immunoregulatory functions, is crucial for the pathogenesis of many chronic autoimmune diseases, including psoriasis and rheumatoid arthritis.^{1–3} It is expressed as soluble TNF α (sTNF α) and membrane-bound TNF α (mTNF α), which regulate the functional activities of TNF α by engaging TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2), respectively.^{1,2,4,5} TNFR1 mediates cytotoxic and proinflammatory activities, while TNFR2 plays a role in the homeostatic activities of TNF.^{1,2,4–8} TNFR2 signal is also crucial for the sustained activation of regulatory T cells and tissue repair.⁹

Anti-TNF α biologics represent a class of drugs that changed the clinical outcome for many patients with autoimmune diseases.^{3,9,10} However, biologic TNF blockers cause side effects, such as injection site reactions, the development of anti-drug antibodies limiting the duration of clinical response, and an increased risk of opportunistic and serious infections requiring an extended wash-out time in case of safety concerns, and they are perceived as inconvenient and uncomfortable as injectables.^{11,12} This led to further investigations into developing an orally available, small molecule to target the TNF α pathway.^{6,13}

He *et al.*¹⁴ were the first to describe the small molecule inhibitors of TNF α in 2005. In 2019, O'Connell *et al.*¹³ described a series of small molecules based on the TNF trimer disruption mechanism,¹⁴ wherein balinatunfib (SAR441566) was identified as a promising drug candidate.^{13,15} These small molecules might offer advantages by avoiding biologic- and immunogenicity-related adverse effects and loss of clinical efficacy, as well as by allowing a more convenient route of administration.^{15,16} Development of neutralizing anti-drug antibodies against anti-TNF injectables is one of the main reasons for their loss of response in patients on those therapies.¹⁶ As small molecules are expected to overcome immunogenicity-related concerns, balinatunfib may exhibit a durable effect with less probability of loss of response compared to commercially available anti-TNF agents.

Balinatunfib binds to a pocket in the center of the sTNF α trimer.^{13,15} This results in the allosteric stabilization of a naturally occurring, asymmetric form of sTNF α that has an impaired capacity to engage TNFR1 and thereby inhibits TNFR1 signaling.^{13,15} Balinatunfib demonstrated complete TNF occupancy and strong TNF inhibition *in vitro*, as well as antibody-like efficacy in a collagen-induced arthritis mouse model *in vivo* within a consistent range of balinatunfib concentrations (16 nM was the concentration leading to 90% occupancy of TNF α [OCC₉₀], 35 nM the 50% inhibitory concentration [IC₅₀], and 24 nM the average free efficacious exposure in mice).¹⁵ In preclinical studies, balinatunfib demonstrated optimal pharmacokinetic (PK) properties including moderate to high bioavailability across animal species, low metabolic clearance (in human hepatocytes) with a direct intestinal elimination of parent compound (in a mass balance study), high tissue distribution, and high fraction unbound, making it a promising candidate for therapeutic use.¹⁵ The preclinical pharmacology and toxicology data supported further advancement of balinatunfib into clinical trials as the first oral inhibitor of TNFR1 signaling to be administered in humans.

Herein, we report the safety, PK, and PD properties of balinatunfib following its first-in-human (FIH) administration of single and multiple ascending doses in healthy adult participants.

METHODS

Study design

This was a single-center, phase I, FIH, double-blind, randomized, placebo-controlled SAD and MAD trial (EudraCT: 2020-004416-10), including a food interaction study. The SAD (in fasted condition) and MAD (in fasted and fed conditions) assessed the safety, tolerability, PK, and PD properties of balinatunfib. The effect of food on the PK of balinatunfib was also investigated in a two-period, one-sequence, non-randomized, open-label crossover design.

Population

This study included healthy male participants, with ages 18–55 years and a body mass index of 18–30 kg/m². Only male volunteers were included in SAD and MAD due to pending characterization on reproductive and embryo/fetal development toxicity potential of balinatunfib at the time of study initiation. The participants who had normal vital signs, standard 12-lead electrocardiogram (ECG) parameters at screening, and laboratory parameters within the normal range were included.

Randomization and treatment

SAD study. The participants ($N=48$) were randomized at a 3:1 ratio (six verum and two placebo in each cohort) into six SAD cohorts to receive either balinatunfib (5–600 mg) or placebo orally in fasted conditions (**Figure S1a**).

Food effect study. Eight participants received 200 mg of balinatunfib in fasted and fed (with a high-fat meal) conditions in an open-label, two-way crossover design (**Figure S1b**).

MAD study. The participants ($N=40$) were randomized at a 4:1 ratio (eight verum and two placebo in each cohort) into four MAD cohorts to receive either balinatunfib (100–600 mg daily) or placebo (**Figure S1c**). Cohort 1 received a dose of 100 mg balinatunfib or placebo once daily (QD) in the fasted state, and Cohorts 2–4 received a dose of 100, 200, or 300 mg balinatunfib or placebo twice daily (BID) in the fed state for 14 days.

All studies included a screening period of 2–28 days and a follow-up period of 6–8 days. The dose escalation process in the SAD and MAD was based on the review of safety and PK data by the investigator.

Assessments

Safety and tolerability. The safety and tolerability of treatments were assessed throughout the studies by physical examination, clinical laboratory evaluation, vital signs, ECGs, and the assessment of adverse events (AEs), treatment-emergent adverse events (TEAEs), including serious adverse events (SAEs), potentially clinically significant abnormalities (PCSAs), and adverse events of special interest (AESIs). The AESIs included alanine aminotransferase (ALT) increased (ALT $\geq 2 \times$ Upper Limit of the Normal [ULN]), QT prolongation (QTcF ≥ 500 ms), photosensitivity (a Grade 2 and above AE due to sun exposure), pregnancy in the female partner, and symptomatic overdose. The AEs were coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) at the time of database lock (version 24.0 for the SAD study and version 24.1 for the MAD study).

Pharmacokinetics. During the SAD study, plasma samples were collected at pre-dose and 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 60, 72, and 96 hours post-dose (plasma sampling for PK at 96 hours was performed only for dose levels 4–6) for the quantification of balinatunfib in plasma. The same sampling scheme was applied to the food effect study. During the MAD study, plasma samples were collected at pre-dose and 8 hours post-dose time points on Day 1; at pre-dose and 3 hours post-dose on Days 4, 7, and 12; at pre-dose and up to 120 hours post-dose for the last dose in the morning of Day 14. Urine samples were collected over a 24-hour interval at steady state to assess the elimination of balinatunfib in urine. The plasma and urine samples were assayed using a validated and qualified liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method, respectively, with a lower limit of quantification (LLOQ) of 2 ng/mL in both matrices.

The PK parameters (Tables 3 and 4) were calculated by a non-compartmental analysis using Phoenix WinNonlin version 8.1.

Pharmacodynamics. *sTNF α target occupancy* was assessed in the 400 and 600 mg dose cohorts in the SAD study and in all dose cohorts in the MAD study using an *ex vivo* stimulation method as a translational PD marker as described for anti-TNF biologics.¹⁷ For this purpose, blood samples were collected at pre-dose and 3 hours post-dose ($\sim t_{\max}$) in the SAD study, as well as at pre-dose on Day 1 and pre-dose and 3 hours post-dose ($\sim t_{\max}$) on Day 14 in the MAD study. Stimulation was conducted with zymosan, and the levels of total TNF α and balinatunfib-occupied TNF α were determined by Enzyme Linked Immunosorbent Assay to derive the percentage of occupied TNF α , using capture and detection antibodies as reported earlier.^{18,19} The percent change in sTNF α target occupancy from baseline was evaluated for respective time points during the treatment period.

Statistical analysis

The safety analyses were performed using the safety population that included all participants who were exposed to balinatunfib. The TEAEs were summarized by primary system organ class (SOC), preferred term (PT), and dose levels with descriptive statistics.

The PK and PD analyses were performed using the PK and PD populations, respectively, which included all participants with no major or critical deviations related to balinatunfib administration or PK and PD measurement, and for whom the PK and PD data were sufficient and interpretable. In cases where the percentage of AUC determined by extrapolation exceeded 30%, the PK parameters AUC, CL/F, and V_z/F were excluded from descriptive and inferential statistics.

In both SAD and MAD studies, a dose proportionality assessment on $C_{\max}$ and the respective AUC parameter was conducted across the investigated dose range using an empirical power model fitted on the log-transformed scale. The individual time to steady state values following the QD and BID dose regimens of balinatunfib were determined by a nonlinear mixed effects model.

The differences in the PK characteristics between prandial conditions (fed vs. fasted) were assessed using a linear model on log-transformed $C_{\max}$, AUC_{last} , and AUC.

sTNF α occupancy, as raw data and percent change from baseline, was summarized by descriptive statistics by treatment group and scheduled time of measurement.

Ethics statement

The study protocol was reviewed and approved by the Institutional Ethics Committee (State Office for Health and Social Affairs, Office of the Ethics Committee, State of Berlin - Germany) before the study was initiated. This study was conducted in accordance with the protocol and consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for

Human Use (ICH) guidelines for Good Clinical Practice (GCP), and all applicable laws, rules, and regulations. All participants signed an informed consent form.

RESULTS

Participants

All 48 participants who were exposed to balinatunfib/placebo in the SAD study completed the study. Six of the eight participants in the food effect study completed the study, while two participants discontinued the study following Period 1 (one participant discontinued because of an AE (neutropenia) and the other because of a personal reason).

Out of the 40 participants who were exposed to balinatunfib/placebo in the MAD study, 39 completed the study, and one participant from the 100 mg BID cohort discontinued the study on Day 1 after the morning dose because of a personal reason.

In the SAD and MAD studies, the demographic and participant characteristics at baseline were similar between each balinatunfib dose cohort and placebo group, as well as compared to the eight white male participants in the food effect study (Table S1).

Safety and tolerability

SAD study. The incidence of TEAEs was comparable between different dose groups. Overall, five of 36 (13.9%) participants in the balinatunfib groups and two of 12 (16.7%) participants in the placebo group experienced at least one TEAE during the study. No participants experienced any Grade ≥ 3 TEAE, treatment-emergent SAEs, TEAEs leading to permanent study discontinuation, or treatment-emergent AESIs (Table 1). No QT interval corrected by Fridericia formula (QTcF)-related PCSAs were observed in any of the tested cohorts in the SAD study (Table S3).

Food effect study. The number of participants with at least one TEAE was comparable between fasted and fed conditions (five of eight [62.5%] vs. four of six [66.7%] participants) in this cohort. One out of the eight participants reported a TEAE (neutropenia, a neutrophil count of $1.42 \times 10^9/L$ [normal range: $1.46\text{--}5.85 \times 10^9/L$], Grade 2; related to balinatunfib; no clinical symptoms or any other laboratory changes indicative of an infection; recovered), leading to permanent intervention discontinuation and permanent study discontinuation during Period 1 (fasted condition). No participants experienced any Grade ≥ 3 TEAE, treatment-emergent SAEs, or treatment-emergent AESIs (Table 1).

MAD study. Overall, 12/32 (37.5%) participants in the balinatunfib groups and one (12.5%) participant in the placebo group experienced at least one TEAE during the study. No participants experienced any Grade ≥ 3 TEAE, treatment-emergent SAEs, or TEAEs leading to permanent study intervention discontinuation or study discontinuation. AESIs (ALT increase) were reported in four participants in the balinatunfib groups (three [37.5%] participants in the 100 mg QD cohort with an ALT > 2 ULN

Table 1 The number (%) of participants with TEAE(s) by primary SOC and PT (safety population) in the SAD and food effect studies**SAD study**

Primary SOC PT, n (%)	Balinatunfib dose cohorts													
	Placebo (N=12)		5 mg (N=6)		20 mg (N=6)		60 mg (N=6)		180 mg (N=6)		400 mg (N=6)		600 mg (N=6)	
	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E
Any event	2 (16.7)	2	0	0	0	0	1 (16.7)	1	0	0	2 (33.3)	4	2 (33.3)	3
Infections and infestations	0	0	0	0	0	0	1 (16.7)	1	0	0	0	0	1 (16.7)	1
Nasopharyngitis	0	0	0	0	0	0	0	0	0	0	0	0	1 (16.7)	1
Tooth infection	0	0	0	0	0	0	1 (16.7)	1	0	0	0	0	0	0
Psychiatric disorders	0	0	0	0	0	0	0	0	0	0	0	0	1 (16.7)	1
Insomnia	0	0	0	0	0	0	0	0	0	0	0	0	1 (16.7)	1
Nervous system disorders	0	0	0	0	0	0	0	0	0	0	2 (33.3)	3	0	0
Dysgeusia	0	0	0	0	0	0	0	0	0	0	2 (33.3)	2	0	0
Headache	0	0	0	0	0	0	0	0	0	0	1 (16.7)	1	0	0
Gastrointestinal disorders	1 (8.3)	1	0	0	0	0	0	0	0	0	1 (16.7)	1	1 (16.7)	1
Hypoaesthesia oral	0	0	0	0	0	0	0	0	0	0	0	0	1 (16.7)	1
Abdominal pain	0	0	0	0	0	0	0	0	0	0	1 (16.7)	1	0	0
Diarrhea	1 (8.3)	1	0	0	0	0	0	0	0	0	0	0	0	0
Skin and subcutaneous tissue disorders	1 (8.3)	1	0	0	0	0	0	0	0	0	0	0	0	0
Rash	1 (8.3)	1	0	0	0	0	0	0	0	0	0	0	0	0

Food effect study

Primary SOC, PT, n (%)	Balinatunfib			
	200 mg fed (N=6)		200 mg fasted (N=8)	
	n (%)	E	n (%)	E
Any event	4 (66.7)	6	5 (62.5)	7
Blood and lymphatic system disorders	0	0	1 (12.5)	1
Neutropenia	0	0	1 (12.5)	1
Nervous system disorders	1 (16.7)	1	2 (25.0)	2
Headache	1 (16.7)	1	1 (12.5)	1
Loss of consciousness	0	0	1 (12.5)	1
Gastrointestinal disorders	1 (16.7)	1	2 (25.0)	2
Diarrhea	1 (16.7)	1	1 (12.5)	1
Flatulence	0	0	1 (12.5)	1
Skin and subcutaneous tissue disorders	0	0	1 (12.5)	1
Dry skin	0	0	1 (12.5)	1
Musculoskeletal and connective tissue disorders	2 (33.3)	2	0	0
Muscle tightness	1 (16.7)	1	0	0
Tendonitis	1 (16.7)	1	0	0
General disorders and administration site conditions	1 (16.7)	1	0	0
Fatigue	1 (16.7)	1	0	0
Investigations	1 (16.7)	1	1 (12.5)	1
Blood pressure orthostatic decreased	1 (16.7)	1	1 (12.5)	1

E, events; PT, preferred term; SAD, single ascending dose; SOC, system organ class; TEAE, treatment-emergent adverse event.

and one [12.5%] participant in the 300 mg BID cohort); the event in the 300 mg cohort group was reported as an ALT > 3 ULN (181 IU/L on Day 18, 4 days after the last dose). This event, though asymptomatic and recovered on Day 21, was considered related to balinatunfib by the investigator (Table 2).

A few PCSAs were observed in clinical laboratory parameters, with no notable differences across the balinatunfib and placebo groups. However, none of the PCSAs were considered clinically relevant by the investigator, except for the event of ALT increase reported in the 300 mg BID cohort. No clinically meaningful findings were reported in the vital signs' measurements or ECG assessments (no PCSAs for QTcF > 450 ms and/or change from baseline QTc > 60 ms) related to safety in this study. QTcF-related PCSA's with an increase from baseline of 30–60 msec were observed in four participants with the highest dose tested (300 mg BID cohort) in the MAD study (Table S3).

Overall, all single and repeated oral doses of balinatunfib were well tolerated by all participants. No safety issues were found throughout the studies.

Pharmacokinetics

The PK results were consistent across the SAD, MAD, and food effect studies, supporting both QD and BID dosing.

SAD and food effect study. Balinatunfib concentrations at the 5 mg dose level were below the LLOQ (2 ng/mL). In the food effect study, two participants withdrew from the study following Period 1 (fasted condition) and had no PK samples collected in Period 2 (fed condition).

The mean (+SD) plasma concentration–time profiles of balinatunfib following a single oral administration at different dose levels are depicted in Figure 1a. A summary of the PK parameters for the SAD and food (high-fat meal) effect studies is provided in Table 3.

Over the dose range of 20–600 mg balinatunfib, AUCs and $C_{\max}$ increased supra-proportionally, with the slopes (90% CIs) for $C_{\max}$, AUC_{last} , and AUC being 1.34 (1.25–1.43), 1.43 (1.32–1.53), and 1.29 (1.15–1.43), respectively. This effect was predominant for low doses and less pronounced for high doses ≥ 180 mg (Table S2). Dose-normalized geometric means with 90% CIs for $C_{\max}$ and AUC_{last} are shown in Figure 2.

In the fed condition, the maximum plasma concentrations were obtained 1 hour later compared to the fasted state. No lag time was observed for both dietary conditions. $C_{\max}$, AUC_{last} , and AUC were similar between the fasted and fed states; i.e., only 14%, 16%, and 18% higher, respectively, in the fed state than in the fasted state (Table 3).

MAD study. The mean (+SD) plasma concentration–time profiles of balinatunfib following the first administration on Day 1 and multiple oral administrations for 14 days are shown in Figure 1b,c, respectively. The PK parameters of balinatunfib following the first dose (Day 1) and repeated doses (Day 14) are summarized in Table 4.

Following both first and multiple administrations over the dose range of 100–300 mg BID balinatunfib in the fed state, $C_{\max}$ and AUC_{0-12h} increased supra-proportionally with slopes

(90% CIs) of 1.78 (1.48–2.07) and 1.74 (1.44–2.03) for Day 1, and 1.55 (1.24–1.86) and 1.55 (1.23–1.87) for Day 14. Dose-normalized geometric means with 90% CIs for $C_{\max}$ and AUC_{0-12h} are depicted in Figure S2 for Day 1 and in Figure S3 for Day 14.

The time to steady state (90% CI) was 5.82 (5.61–5.82) days following QD dosing in the fasted condition and 5.45 (5.36–5.92) days following BID dosing in the fed condition.

The fraction of the dose excreted in the urine as unchanged drug over a 24-hour interval on Day 13 was low, ranging from 9.07% to 15.6% across the investigated range of 100–400 mg total daily dose.

Pharmacodynamics

sTNF α target occupancy was determined as a translational PD marker using an ex vivo stimulation method¹⁸ and evaluated based on the available information from anti-TNF antibodies¹⁷. In the SAD study, complete occupancy (~100%) was observed in the 400 mg and 600 mg dose cohorts at 3 hours after dosing. In the placebo group, no difference was seen in the level of sTNF α target occupancy between baseline (4.9%) and 3 hours (5.2%) post-dose (Table 5). In the MAD study also, very high occupancy (95%–100%) was observed in all cohorts measured on Day 14 at pre-dose and 3 hours post-dose. In the placebo group, no difference was observed in the levels of sTNF α target occupancy between baseline and Day 14 (Table 5).

DISCUSSION

Balinatunfib is an orally administered, small molecule targeting the TNF pathway by inhibiting TNFR1 signaling. This FIH study evaluated the safety, tolerability, and PK and PD of single and multiple ascending doses of balinatunfib in healthy adult male participants. Balinatunfib was well tolerated in healthy participants up to a 600 mg single and total daily dose for up to 14 days.

A starting dose of 5 mg was selected in the SAD study based on balinatunfib plasma exposures observed pre-clinically at the no-observed-adverse-effect level in the most sensitive species in a 3-month general toxicity study and the lowest predicted human clearance using allometric scaling (data not shown). The dose escalation in the SAD study was based on the review of safety and PK data from previous doses and stopped at 600 mg, a dose level that was anticipated and confirmed to have reached a full biological effect without any safety concerns.

The food effect was investigated at a 200 mg single dose, half of the clinically well-tolerated dose determined in the fasted condition at that stage. The dose levels in the MAD study were selected based on the available safety, tolerability, and PK data from the SAD and food effect studies.

No serious or severe TEAEs or AESIs were reported in the SAD or the food effect study, except for one participant who reported a Grade 2 event of neutropenia that led to treatment discontinuation in the food effect study as per the protocol-defined criteria and was not associated with any clinical symptoms. No other findings based on clinical chemistry, urinalysis, vital signs' parameters, or ECG assessments were clinically meaningful in both studies. In the MAD study, no events leading to treatment discontinuation

Table 2 The number (%) of participants with TEAE(s) by primary SOC and PT (safety population) in the MAD study

MAD study

Primary SOC, PT, n (%)	Balinatunfib dose cohorts									
	Placebo (N=8)		100 mg QD (N=8)		100 mg BID (N=8)		200 mg BID (N=8)		300 mg BID (N=8)	
	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E
Any event	1 (12.5)	1	4 (50.0)	9	2 (25.0)	3	4 (50.0)	7	2 (25.0)	3
Nervous system disorders	1 (12.5)	1	1 (12.5)	1	0	0	1 (12.5)	1	0	0
Dysgeusia	0	0	0	0	0	0	1 (12.5)	1	0	0
Headache	1 (12.5)	1	1 (12.5)	1	0	0	0	0	0	0
Respiratory, thoracic, and mediastinal disorders	0	0	0	0	2 (25.0)	2	2 (25.0)	2	0	0
Nasal dryness	0	0	0	0	0	0	2 (25.0)	2	0	0
Oropharyngeal pain	0	0	0	0	1 (12.5)	1	0	0	0	0
Sneezing	0	0	0	0	1 (12.5)	1	0	0	0	0
Gastrointestinal disorders	0	0	2 (25.0)	3	0	0	1 (12.5)	1	1 (12.5)	2
Abdominal pain	0	0	1 (12.5)	1	0	0	0	0	1 (12.5)	1
Constipation	0	0	0	0	0	0	0	0	1 (12.5)	1
Diarrhea	0	0	2 (25.0)	2	0	0	1 (12.5)	1	0	0
Skin and subcutaneous tissue disorders	0	0	0	0	0	0	2 (25.0)	2	0	0
Dry skin	0	0	0	0	0	0	2 (25.0)	2	0	0
Musculoskeletal and connective tissue disorders	0	0	1 (12.5)	1	0	0	0	0	0	0
Neck pain	0	0	1 (12.5)	1	0	0	0	0	0	0
Reproductive system and breast disorders	0	0	0	0	1 (12.5)	1	0	0	0	0
Testicular mass	0	0	0	0	1 (12.5)	1	0	0	0	0
General disorders and administration site conditions	0	0	0	0	0	0	1 (12.5)	1	0	0
Medical device site reaction	0	0	0	0	0	0	1 (12.5)	1	0	0
Investigations	0	0	3 (37.5)	3	0	0	0	0	1 (12.5)	1
Alanine aminotransferase increased	0	0	3 (37.5)	3	0	0	0	0	1 (12.5)	1
Injury, poisoning, and procedural complications	0	0	1 (12.5)	1	0	0	0	0	0	0
Wound	0	0	1 (12.5)	1	0	0	0	0	0	0

BID, twice daily; MAD, multiple ascending dose; QD, once daily; PT, preferred term; SOC, system organ class; TEAE, treatment-emergent adverse event.

and no serious or severe TEAEs were observed. AESIs (ALT increase) were observed in four participants in the MAD cohorts; the ALT increase was reversible without any dose dependency, and examinations (hepatobiliary ultrasonography and recommended laboratory parameters per the protocol) revealed no abnormalities. QTcF-related PCSA observed with the highest dose cohort in the MAD study suggested dose-dependent effects that require further investigation according to regulatory guidelines.²⁰ Overall, balinatunfib was well tolerated in all three studies. None of the major AEs (e.g., severe infections) associated with anti-TNF biologics (infliximab, etanercept, adalimumab, certolizumab, and golimumab)¹⁰ were observed, suggesting a favorable safety profile of balinatunfib in this FIH study.

Following oral administration in the healthy participants, balinatunfib was steadily absorbed with a median $t_{\max}$ of 2.5–5.0 hours. The apparent volume of distribution was high (> 1,000 L), and a minor fraction of the administered dose was found as unchanged drug excreted in urine (9.1%–15.6% within 24 hours at steady state). This is in line with the reported preclinical PK data of balinatunfib,¹⁵ suggesting that, in contrast to anti-TNF biologics, the compound can bind TNF intracellularly and is likely mainly excreted via feces.

The mean terminal half-life ($t_{1/2z}$) was comparable across the investigated dose range following single and repeated oral administration of balinatunfib, ranging from about 22 to 30 hours. Based on % coefficient of variation (CV), low-to-moderate total

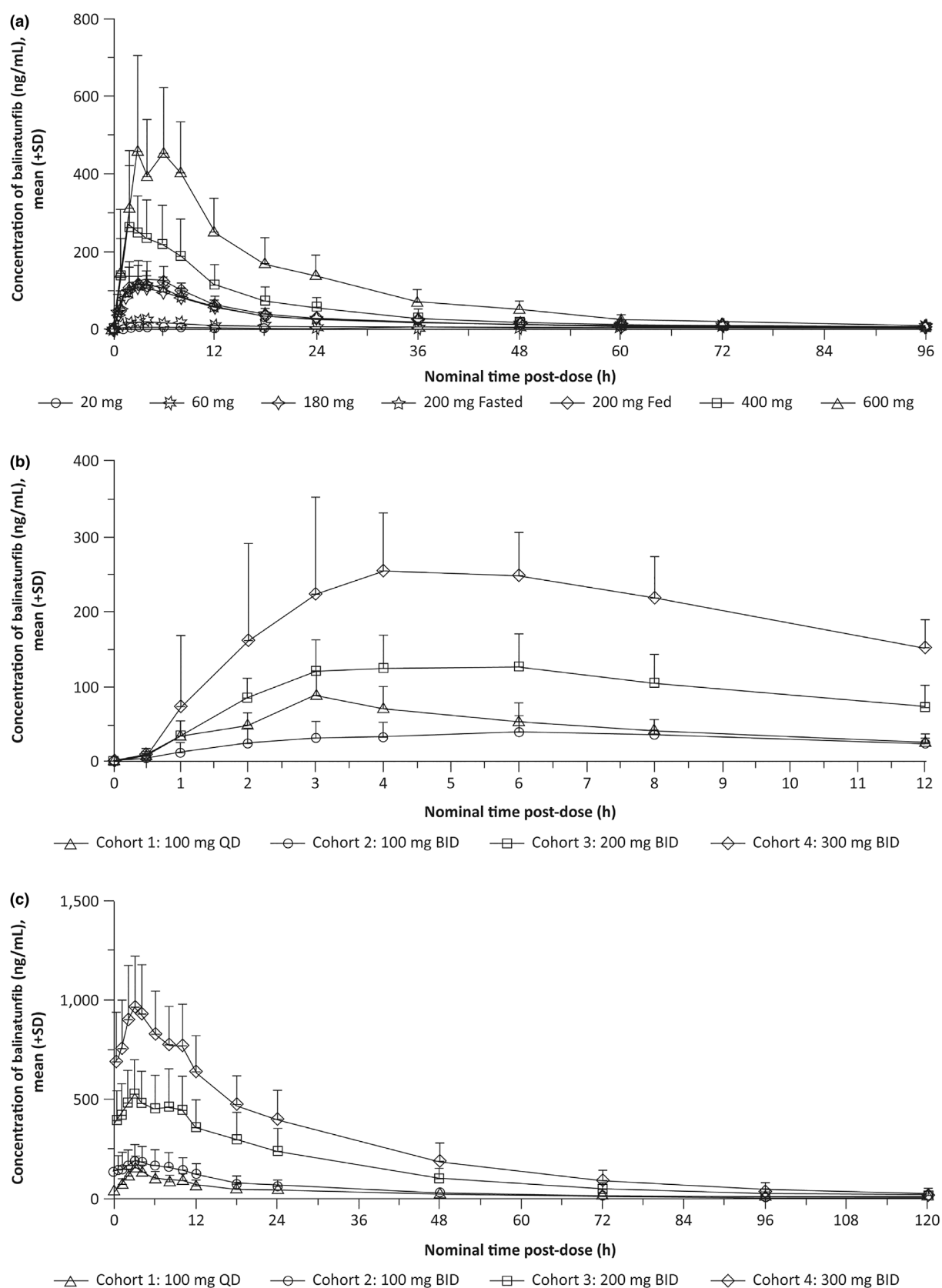


Figure 1 Mean (+SD) plasma concentrations of balinatumfib following (a) single ascending doses of balinatumfib in the SAD study, and (b) multiple ascending doses of balinatumfib on Day 1 (first dose) and (c) on Day 14 in the MAD study. Balinatumfib was administered in the fasted state for Cohort 1 and in the fed state for Cohorts 2 through 4 in the MAD study (panels b and c). MAD, multiple ascending dose; SAD, single ascending dose; SD, standard deviation.

Table 3 Pharmacokinetic parameters of balinatunfib in the SAD and food effect studies and statistical assessment of the food effect

SAD study—summary of PK parameters: mean $\pm$ SD (geometric mean) [CV%]					
PK parameters	20 mg Balinatunfib	60 mg Balinatunfib	180 mg Balinatunfib	400 mg Balinatunfib	600 mg Balinatunfib
N	6	6	6	6	6
$C_{\max}$ (ng/mL)	5.25 $\pm$ 1.24 (5.12) [24]	21.0 $\pm$ 5.08 (20.5) [24]	122 $\pm$ 43.7 (117) [36]	285 $\pm$ 139 (261) [49]	525 $\pm$ 206 (483) [39]
$t_{\max}^a$ (h)	5.00 (3.00, 8.00)	2.50 (2.00, 6.00)	2.50 (1.00, 4.00)	3.00 (2.00, 4.00)	3.00 (3.00, 6.00)
t_{last}^a (h)	18.05 (12.18, 36.00)	48.00 (36.00, 72.72)	96.21 (72.02, 96.98)	96.16 (72.25, 96.43)	96.01 (96.00, 97.73)
AUC_{last} (ng·h/mL)	70.4 $\pm$ 35.9 (62.8) [51]	344 $\pm$ 84.9 (335) [25]	2,270 $\pm$ 720 (2,170) [32]	4,400 $\pm$ 2,210 (4,050) [50]	9,290 $\pm$ 3,440 (8,570) [37]
AUC (ng·h/mL)	101 ^b $\pm$ NC (NC) [NC]	420 $\pm$ 103 (409) [25]	2,450 $\pm$ 782 (2,330) [32]	4,580 $\pm$ 2,240 (4,220) [49]	9,640 $\pm$ 3,540 (8,900) [37]
$t_{1/2z}$ (h)	15.0 ^c $\pm$ 7.21 (13.6) [48]	21.6 $\pm$ 6.14 (20.9) [28]	27.4 $\pm$ 4.74 (27.1) [17]	24.7 $\pm$ 6.47 (24.0) [26]	24.4 $\pm$ 5.43 (23.9) [22]

Food effect study—summary of PK parameters: mean $\pm$ SD (geometric mean) [CV%]

PK parameters	200 mg Balinatunfib, Fasted	200 mg Balinatunfib, Fed
N	8	6 ^d
$C_{\max}$ (ng/mL)	123 $\pm$ 39.5 (119) [32]	138 $\pm$ 48.1 (132) [35]
$t_{\max}^a$ (h)	3.00 (3.00, 6.00)	4.00 (2.00, 6.00)
t_{lag}^a (h)	0.00 (0.00, 0.00)	0.00 (0.00, 1.00)
AUC_{last} (ng·h/mL)	1,980 $\pm$ 527 (1,930) [27]	2,310 $\pm$ 804 (2,210) [35]
AUC (ng·h/mL)	2,060 $\pm$ 539 (2,010) [26]	2,430 $\pm$ 815 (2,340) [33]
$t_{1/2z}$ (h)	22.5 $\pm$ 5.23 (21.9) [23]	27.6 $\pm$ 4.84 (27.3) [18]

Assessment of the food effect on $C_{\max}$, AUC_{last} , and AUC—point estimates and 90% CI for the geometric mean ratio of food condition (fed vs. fasted)

Comparison	Parameter	Point estimate	90% CI
Fed vs. fasted	$C_{\max}$	1.14	(1.07–1.22)
	AUC_{last}	1.16	(1.07–1.26)
	AUC	1.18	(1.09–1.27)

In the SAD study, all doses were administered under fasted conditions. The PK parameters were not calculable for any participant receiving a single dose of 5 mg balinatunfib, as all concentrations were below the LLOQ (2 ng/mL). AUC, area under the plasma concentration–time curve from time 0 to infinity; AUC_{last} , AUC from time 0 to the last measurable non-zero concentration; CI, confidence interval; $C_{\max}$, maximum observed concentration in plasma; CV, coefficient of variation; LLOQ, lower limit of quantification; NC, not calculated; PK, pharmacokinetic; SAD, single ascending dose; SD, standard deviation; t_{lag} , time delay between drug administration and the first quantifiable concentration; t_{last} , time of the last measurable concentration; $t_{\max}$, time to the maximum observed concentration; $t_{1/2z}$, terminal half-life. ^aMedian (min, max). ^b $n=1$, the percentage of AUC determined by extrapolation exceeded 30% for four participants; therefore, their AUC values were excluded from statistics. Additionally, AUC was not calculable for one participant because there were <3 quantifiable concentrations occurring after $C_{\max}$ in the PK profile. ^c $n=5$; $t_{1/2z}$ and AUC were not calculable for one participant because there were <3 quantifiable concentrations occurring after $C_{\max}$ in the PK profile. ^dTwo participants were withdrawn from the study following Period 1 (fasted) and had no PK samples collected in Period 2 (fed).

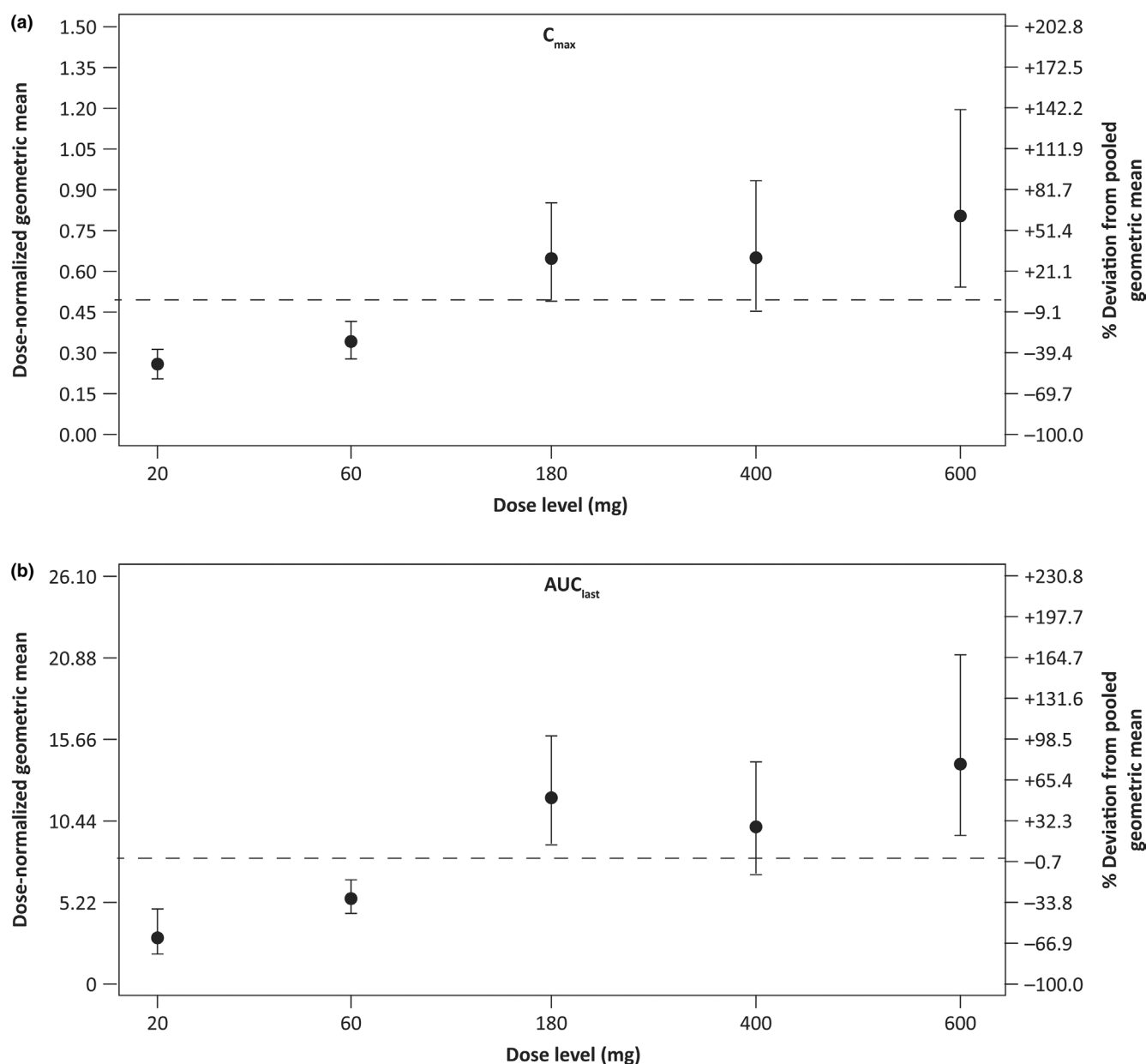


Figure 2 Dose proportionality assessment in the SAD study. Dose-normalized geometric means with 90% CIs for (a) C_{max} and (b) AUC_{last} . AUC, area under the plasma concentration–time curve; AUC_{last} , AUC from time 0 to the last measurable non-zero concentration; CI, confidence interval; C_{max} , maximum observed concentration in plasma; SAD, single ascending dose.

variability was noted for AUC_{last} , AUC, and C_{max} , with values ranging from 25% to 51%.

The time to steady state of approximately 5 days and the observed accumulation of 2.52-fold after QD dosing and 4.40- to 5.46-fold after BID dosing are in line with the expectations based on the single-dose data.

The increases in the systemic exposure (C_{max} , AUC, and $AUC_{0-\tau}$) were greater than dose proportional following single (20–180 mg, fasted) and repeated oral administration (100–300 mg BID, fed state). Between the 180 and 600 mg single doses in the fasted condition, there appeared to be little deviation from dose

proportionality. According to *in vitro* studies, balinatunfib is a substrate of active transport by P-glycoprotein (P-gp).¹³ Contribution and saturation of P-gp-mediated intestinal efflux might be a potential reason for the supra-proportional exposure increase observed in healthy participants. Taking the totality of preclinical¹⁵ and clinical data into account, it seems unlikely that P-gp mediated efflux might markedly restrict bioavailability at the therapeutically relevant doses.

Following a single 200 mg oral dose, exposure to SAR441566 was slightly increased in the presence of food (Table 3), which is considered not clinically relevant given the narrow confidence

Table 4 Pharmacokinetic parameters of balinatunfib in the MAD studyMean $\pm$ SD (geometric mean) [CV%] pharmacokinetic parameters for balinatunfib on Day 1 following first dosing

PK parameters	Cohort 1: 100 mg QD (fasted)	Cohort 2: 100 mg BID (fed)	Cohort 3: 200 mg BID (fed)	Cohort 4: 300 mg BID (fed)
N	8	8	8	8
$C_{\max}$ (ng/mL)	88.6 $\pm$ 34.0 (83.1) [38]	42.6 $\pm$ 21.2 (38.1) [50]	139 $\pm$ 43.9 (132) [32]	278 $\pm$ 85.4 (268) [31]
$t_{\max}^a$ (h)	3.00 (3.00, 3.00)	6.00 (2.00, 8.00)	5.00 (2.00, 6.00)	4.00 (3.00, 6.00)
C_{av} (ng/mL)	29.5 $\pm$ 9.33 (28.1) [32]	30.4 $\pm$ 16.2 (27.0) [53]	94.5 $\pm$ 29.5 (90.3) [31]	190 $\pm$ 60.4 (182) [32]
AUC ₀₋₁₂ (ng·h/mL)	559 $\pm$ 194 (527) [35]	364 $\pm$ 194 (324) [53]	1,130 $\pm$ 354 (1,080) [31]	2,280 $\pm$ 725 (2,180) [32]
AUC ₀₋₂₄ ^b (ng·h/mL)	708 $\pm$ 224 (674) [32]	NA	NA	NA

Mean $\pm$ SD (geometric mean) [CV%] pharmacokinetic parameters for balinatunfib on Day 14 following multiple oral administration

PK parameters	Cohort 1: 100 mg QD (fasted)	Cohort 2: 100 mg BID (fed)	Cohort 3: 200 mg BID (fed)	Cohort 4: 300 mg BID (fed)
N	8	7^f	8	8
$C_{\max}$ (ng/mL)	161 $\pm$ 49.2 (153) [31]	189 $\pm$ 86.3 (173) [46]	547 $\pm$ 193 (511) [35]	982 $\pm$ 261 (947) [27]
$t_{\max}^c$ (h)	3.00 (2.00, 4.00)	3.00 (1.00, 4.00)	3.00 (2.00, 8.00)	3.00 (2.00, 6.00)
C_{trough} (ng/mL)	37.2 $\pm$ 11.5 (35.2) [31]	136 $\pm$ 70.8 (122) [52]	383 $\pm$ 163 (349) [43]	676 $\pm$ 215 (639) [32]
C_{av} (ng/mL)	72.9 $\pm$ 19.6 (70.0) [27]	155 $\pm$ 74.4 (141) [48]	448 $\pm$ 167 (416) [37]	806 $\pm$ 221 (774) [27]
FL _{rel} (%)	171 $\pm$ 34.1 (168) [20]	46.9 $\pm$ 9.16 (46.1) [20]	46.2 $\pm$ 12.3 (44.7) [27]	48.0 $\pm$ 13.7 (46.1) [29]
AUC _{0-tau} (ng·h/mL)	1,750 $\pm$ 471 (1,680) [27]	1,860 $\pm$ 892 (1,690) [48]	5,370 $\pm$ 2,010 (4,990) [37]	9,670 $\pm$ 2,650 (9,290) [27]
$t_{1/2z}$ (h)	30.3 $\pm$ 8.43 (29.5) [28]	23.8 $\pm$ 3.18 ^g (23.6) [13]	24.1 $\pm$ 3.28 (23.9) [14]	21.7 $\pm$ 4.92 (21.3) [23]
CL _{ss} /F (L/h)	62.5 $\pm$ 23.4 (59.5) [37]	64.6 $\pm$ 28.2 (59.1) [44]	43.7 $\pm$ 20.6 (40.1) [47]	33.9 $\pm$ 12.7 (32.3) [37]
V_z/F (L)	2,700 $\pm$ 1,020 (2,530) [38]	2,500 $\pm$ 1,270 ^f (2,250) [51]	1,590 $\pm$ 983 (1,380) [62]	1,030 $\pm$ 299 (991) [29]
$R_{\text{ac}} \cdot C_{\max}$	1.87 $\pm$ 0.400 (1.84) [21]	4.77 $\pm$ 0.730 (4.72) [15]	3.91 $\pm$ 0.612 (3.87) [16]	3.62 $\pm$ 0.861 (3.53) [24]
R_{ac}	2.52 $\pm$ 0.431 (2.50) [17]	5.46 $\pm$ 0.951 (5.38) [17]	4.65 $\pm$ 0.654 (4.60) [14]	4.40 $\pm$ 1.22 (4.25) [28]
Fe ₀₋₂₄ ^{d,e} (%)	9.07 $\pm$ 1.85 (8.91) [20]	9.41 $\pm$ 4.66 (8.18) [50]	11.9 $\pm$ 3.54 (11.4) [30]	15.6 $\pm$ 4.40 (15.0) [28]
CL _R ^{d,e} (L/h)	5.53 $\pm$ 1.67 (5.30) [30]	5.16 $\pm$ 2.10 (4.83) [41]	4.62 $\pm$ 0.862 (4.55) [19]	4.92 $\pm$ 0.929 (4.85) [19]

AUC_{0-tau}, AUC to the end of the dosing period; AUC₀₋₁₂, area under the plasmaconcentration-time curve from time 0 to 12 hours post-dose; AUC₀₋₂₄, AUC from time 0 to 24 hours post-dose; BID, twice daily; C_{av} , average plasma concentration; CL_{ss}/F, apparent total body clearance after oral administration at steady state; CL_R, renal clearance; C_{trough} , concentration observed at the last planned time point prior to dosing; CV, coefficient of variation; Fe₀₋₂₄, cumulative fraction of the dose excreted as unchanged parent in urine over the 24-hour collection interval; FL_{rel}, relative fluctuation; NA, not applicable; MAD, multiple ascending dose; PK, pharmacokinetic; QD, once daily; R_{ac} , accumulation ratio; $R_{\text{ac}} \cdot C_{\max}$, accumulation ratio based on $C_{\max}$; SD, standard deviation; V_z/F , apparent volume of distribution. ^aMedian (min, max). ^bCalculated by extrapolation. ^cMedian (min, max). ^d24-hour urine PK collection occurred on Day 13. ^eCL_R was calculated using Day 14 AUC₀₋₂₄; for the BID cohorts, AUC₀₋₂₄ was calculated as AUC_{0-tau} * 2. ^fOne participant withdrew and did not have PK samples collected after Day 1. ^gn = 6; $t_{1/2z}$ and V_z/F for one participant were excluded from statistics, as the terminal elimination phase could not be adequately characterized; this participant's Day 14 concentration data starting at 18 hours post-dose and onward were excluded because the participant received a second daily dose on Day 14 in error.

Table 5 sTNF α occupancy (%) in the SAD and MAD studies

	N	Mean	SD	SEM	Median	Min	Max
SAD study							
Placebo							
Baseline	4	4.9	2.5	1.2	4.4	3.0	8.0
D1 3 h	4	5.2	2.2	1.1	4.6	3.0	8.0
Balinatunfib 400mg							
Baseline	6	6.3	0.5	0.2	6.3	5.0	7.0
D1 3 h	6	102.2	7.5	3.0	105.3	89.0	109.0
Balinatunfib 600mg							
Baseline	6	3.3	1.1	0.5	3.4	1.0	4.0
D1 3 h	6	109.3	8.1	3.3	109.2	97.0	119.0
MAD study							
Placebo							
Baseline	8	2.8	0.8	0.3	2.7	2.0	4.0
D14 0h	8	2.5	0.4	0.1	2.6	2.0	3.0
D14 3h	8	2.6	0.5	0.2	2.5	2.0	3.0
Balinatunfib 100mg QD							
Baseline	8	3.8	2.7	1.0	2.8	2.0	9.0
D14 0h	8	95.0	9.4	3.3	96.8	83.0	109.0
D14 3h	8	97.2	6.2	2.2	98.4	85.0	105.0
Balinatunfib 100mg BID							
Baseline	7	2.6	0.6	0.2	2.9	2.0	3.0
D14 0h	7	103.1	9.2	3.5	104.0	85.0	116.0
D14 3h	7	104.9	5.0	1.9	105.3	97.0	111.0
Balinatunfib 200mg BID							
Baseline	8	3.9	1.0	0.34	3.8	3.0	5.0
D14 0h	8	101.1	1.8	0.62	101.8	97.0	103.0
D14 3h	8	105.2	6.9	2.43	106.6	91.0	114.0
Balinatunfib 300mg BID							
Baseline	8	2.4	0.5	0.16	2.2	2.0	3.0
D14 0h	8	101.3	7.0	2.48	101.8	87.0	110.0
D14 3h	8	99.8	16.4	5.81	102.8	63.0	117.0

BID, twice daily; D, day; h, hour; MAD, multiple ascending dose; QD, once daily; SAD, single ascending dose; SD, standard deviation; SEM, standard error of the mean; TNF α , tumor necrosis factor alpha; sTNF α , soluble TNF α .

interval of the effect (upper bound < 1.30), the low-to-moderate PK variability, and the good safety profile up to the highest dose tested in the MAD under fed conditions.

The consistent PK characteristics for different dosing regimens and the negligible impact of a high-fat meal on balinatunfib bioavailability suggest that both QD and BID dosing regimens, regardless of food intake, may be adequate for further clinical development.

The evaluation of sTNF α target occupancy indicated an interaction of balinatunfib with sTNF α at the doses tested in the SAD and MAD studies. This was in alignment with the suggested mechanism of action of balinatunfib, which is expected to act via binding to the center of the sTNF α trimer at low nanomolar concentrations.^{13,15} Based on anti-TNF antibody data reported in the literature,¹⁷ the observed TNF occupancy at all doses in the MAD study is expected

to be clinically relevant for efficacy in many immune-mediated diseases. In a previous work, we predicted the TNF α occupancy using a quantitative systems pharmacology model¹⁵ which demonstrated that full TNF α occupancy (achieved by anti-TNF biologics) can be reached with the high doses selected for SAD/MAD, even if TNF levels are higher in patients. While TNF α levels in the circulation are similar in patients and healthy volunteers,²¹ there seem to be higher levels of TNF α in inflamed tissues. Depending on the study and assay used, a meta-analysis showed a two-fold difference based on median TNF α levels in synovial fluid compared to blood in selected arthritic diseased populations (Table S4).^{22–34} Of note, TNF secretion was stimulated ex vivo in the present occupancy assay, mimicking TNF α levels in inflamed tissues. Therefore, similar TNF occupancy results can be expected with the same doses of balinatunfib when administered in patients.

Overall, the PK and PD results were consistent across the studies reported herein and in alignment with the expectation based on *in vitro*, animal, and *in silico* data reported previously.¹⁵

These studies have certain limitations. The cohort size was relatively small in both studies but usual for FIH studies. As these studies were conducted in healthy participants, *ex vivo* stimulated assays were performed to analyze the target occupancy after clinical treatment with balinatunfib. Further, the target occupancy evaluation was performed using only the high doses in the SAD study. Hence, the dose–response relationship could not be fully elucidated from these investigations. The dose–response relationship will be investigated in further studies in patients, including efficacy read-out, since target occupancy may not linearly translate into clinical response. Nevertheless, the very high and comparable target occupancy observed at time points corresponding to C_{trough} and C_{max} of balinatunfib across all doses in the MAD study suggests that target saturation can be achieved at the selected dose regimens throughout the dosing interval.

The advantages of orally bioavailable small molecules such as balinatunfib over protein/peptides^{35,36} combined with favorable clinical pharmacology properties justify the advancement of balinatunfib into further clinical studies. Overall, these studies demonstrated that orally administered balinatunfib was well tolerated and achieved ~100% target occupancy in humans at feasible dose regimens, including both QD and BID dosing. The preclinical-to-clinical translation was proven successful for this first oral small molecule inhibitor of TNFR1 signaling that has entered into clinical development for the treatment of TNF-mediated autoimmune diseases. Currently, balinatunfib is under clinical investigation for the treatment of patients with Crohn's disease³⁷ and Rheumatoid Arthritis, among other inflammatory diseases.³⁸

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

ACKNOWLEDGMENTS

Balinatunfib (SAR441566) was identified in a research collaboration with UCB. The authors acknowledge Agnès Tardat and Sandrine Logerot, from Sanofi, for their operational support during the study conduct. Medical writing support for this manuscript was provided by Sindhu Doppalapudi, PhD, from Sanofi, according to the [Good Publication Practice guidelines](#).

FUNDING

This study was sponsored by Sanofi.

CONFLICTS OF INTEREST

N.N., F.R., D.W., M.R., M.K., F.S., A.L., A.K., L.P., M.W., and M.A.N.: Employees of Sanofi and may hold stocks/stock options in the company. J.G.: Was an employee of Sanofi at the time this study was conducted and may hold stocks/stock options in the company. Currently, he is an employee of Provadis School of International Management and Technology, Frankfurt, Germany. F.D.W.: Declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

N.N. and M.A.N. wrote the manuscript; N.N., M.A.N., F.R., and D.W. designed the research; F.D.W. and N.N. performed the research; N.N., M.A.N., M.K., J.G., L.P., M.R., A.L., F.S., A.K., and M.W. analyzed the data.

DATA AVAILABILITY STATEMENT

Qualified researchers may request access to patient-level data and related study documents, including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Participant-level data will be anonymized, and study documents will be redacted to protect the privacy of the trial participants. Further details on Sanofi's data-sharing criteria, eligible studies, and process for requesting access can be found at: <https://www.vivli.org/>.

© 2025 Sanofi. *Clinical Pharmacology & Therapeutics* published by Wiley Periodicals LLC on behalf of American Society for Clinical Pharmacology and Therapeutics.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](#) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

1. Jang, D.I. et al. The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *Int. J. Mol. Sci.* **22**, 2719–2734 (2021).
2. Kalliolias, G.D. & Ivashek, L.B. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat. Rev. Rheumatol.* **12**, 49–62 (2016).
3. Monaco, C., Nanchahal, J., Taylor, P. & Feldmann, M. Anti-TNF therapy: past, present and future. *Int. Immunol.* **27**, 55–62 (2015).
4. Dömling, A. & Li, X. TNF-α: the shape of small molecules to come? *Drug Discov. Today* **27**, 3–7 (2022).
5. Edmunds, J. Untangling perception and reality: small-molecule TNF-α inhibitors. In *2019 medicinal chemistry reviews*, Vol. **54** (ed. Bronson, J.J.) 127–144 (Medicinal Chemistry Division of the American Chemical Society, Boston, MA, 2019).
6. Chédotal, H. et al. Small-molecule modulators of tumor necrosis factor signaling. *Drug Discov. Today* **28**, 103575 (2023).
7. Zhang, H. & Xiao, W. TNFR1 and TNFR2 differentially mediate TNF-α-induced inflammatory responses in rheumatoid arthritis fibroblast-like synoviocytes. *Cell Biol. Int.* **41**, 415–422 (2017).
8. Zhang, N., Wang, Z. & Zhao, Y. Selective inhibition of tumor necrosis factor receptor-1 (TNFR1) for the treatment of autoimmune diseases. *Cytokine Growth Factor Rev.* **55**, 80–85 (2020).
9. Salomon, B.L. Insights into the biology and therapeutic implications of TNF and regulatory T cells. *Nat. Rev. Rheumatol.* **17**, 487–504 (2021).
10. Willrich, M.A., Murray, D.L. & Snyder, M.R. Tumor necrosis factor inhibitors: clinical utility in autoimmune diseases. *Transl. Res.* **165**, 270–282 (2015).
11. Megna, M. et al. Psoriatic disease treatment nowadays: unmet needs among the “jungle of biologic drugs and small molecules”. *Clin. Rheumatol.* **37**, 1739–1741 (2018).
12. Murdaca, G., Colombo, B.M., Cagnati, P., Gulli, R., Spanò, F. & Puppo, F. Update upon efficacy and safety of TNF-α inhibitors. *Expert Opin. Drug Saf.* **11**, 1–5 (2012).
13. O'Connell, J. et al. Small molecules that inhibit TNF signalling by stabilising an asymmetric form of the trimer. *Nat. Commun.* **10**, 5795 (2019).
14. He, M.M. et al. Small-molecule inhibition of TNF-α. *Science* **310**, 1022–1025 (2005).
15. Vugler, A. et al. An orally available small molecule that targets soluble TNF to deliver anti-TNF biologic-like efficacy in rheumatoid arthritis. *Front. Pharmacol.* **13**, 1037983 (2022).
16. Kalden, J.R. & Schulze-Koops, H. Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment. *Nat. Rev. Rheumatol.* **13**, 707–718 (2017).
17. Tani, K., Takayanagi, R., Yokoyama, H. & Yamada, Y. Theoretical analysis of efficacy of biological agent for rheumatoid arthritis based on target molecular binding occupancy. *Rheumatol. Int.* **33**, 1791–1795 (2013).

18. Hickford, E.S. *et al.* Target occupancy biomarker assay development using a conformation-selective antibody against small-molecule-bound TNF. *Bioanalysis* **15**, 343–359 (2023).
19. Lightwood, D.J. *et al.* A conformation-selective monoclonal antibody against a small molecule-stabilised signalling-deficient form of TNF. *Nat. Commun.* **12**, 583 (2021).
20. ICH E14 clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs - scientific guideline. [ICH E14 Clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs - Scientific guideline](#) | European Medicines Agency (EMA). Accessed 14 February 14, 2025.
21. Berkhout, L.C. *et al.* Dynamics of circulating TNF during adalimumab treatment using a drug-tolerant TNF assay. *Sci. Transl. Med.* **11**, eaat3356 (2019).
22. Wright, H.L., Bucknall, R.C., Moots, R.J. & Edwards, S.W. Analysis of SF and plasma cytokines provides insights into the mechanisms of inflammatory arthritis and may predict response to therapy. *Rheumatology (Oxford)* **51**, 451–459 (2012).
23. Perry, M.G., Jessop, D.S., Hunt, L.P., Sharif, M. & Kirwan, J.R. Overnight variations in IL-6 in synovial fluid and plasma in patients with active rheumatoid arthritis. *Int. J. Clin. Rheumatol.* **5**, 593–600 (2010).
24. Andrés Cerezo, L. *et al.* Calgizzarin (S100A11): a novel inflammatory mediator associated with disease activity of rheumatoid arthritis. *Arthritis Res. Ther.* **19**, 79 (2017).
25. Ng, C.T. *et al.* Synovial tissue hypoxia and inflammation in vivo. *Ann. Rheum. Dis.* **69**, 1389–1395 (2010).
26. Cañete, J.D. *et al.* Differential Th1/Th2 cytokine patterns in chronic arthritis: interferon gamma is highly expressed in synovium of rheumatoid arthritis compared with seronegative spondyloarthropathies. *Ann. Rheum. Dis.* **59**, 263–268 (2000).
27. Horiuchi, T. *et al.* The increase of parathyroid hormone-related peptide and cytokine levels in synovial fluid of elderly rheumatoid arthritis and osteoarthritis. *Endocr. J.* **46**, 643–649 (1999).
28. Ribbens, C. *et al.* Increased synovial fluid levels of interleukin-12, sCD25 and sTNF-RII/sTNF-RI ratio delineate a cytokine pattern characteristic of immune arthropathies. *Eur. Cytokine Netw.* **11**, 669–676 (2000).
29. Lettesjö, H. *et al.* Synovial fluid cytokines in patients with rheumatoid arthritis or other arthritic lesions. *Scand. J. Immunol.* **48**, 286–292 (1998).
30. Westacott, C.I., Whicher, J.T., Barnes, I.C., Thompson, D., Swan, A.J. & Dieppe, P.A. Synovial fluid concentration of five different cytokines in rheumatic diseases. *Ann. Rheum. Dis.* **49**, 676–681 (1990).
31. Avrănescu, C., Vere, C.C., Mărgăritescu, C., Turculeanu, A., Bălăsoiu, M. & Rogoz, S. Cytokine panel in rheumatoid arthritis and correlation with histological patterns of synovitis – active type of disease. *Romanian J. Morphol. Embryol.* **46**, 87–92 (2005).
32. Manicourt, D.H., Triki, R., Fukuda, K., Devogelaer, J.P., Nagant de Deuxchaisnes, C. & Thonar, E.J. Levels of circulating tumor necrosis factor alpha and interleukin-6 in patients with rheumatoid arthritis. Relationship to serum levels of hyaluronan and antigenic keratan sulfate. *Arthritis Rheum.* **36**, 490–499 (1993).
33. Chen, D.Y., Chen, Y.M., Chen, H.H., Hsieh, C.W., Lin, C.C. & Lan, J.L. Increasing levels of circulating Th17 cells and interleukin-17 in rheumatoid arthritis patients with an inadequate response to anti-TNF- α therapy. *Arthritis Res. Ther.* **13**, R126 (2011).
34. Barrera, P., Joosten, L.A., den Broeder, A.A., van de Putte, L.B., van Riel, P.L. & van den Berg, W.B. Effects of treatment with a fully human anti-tumour necrosis factor alpha monoclonal antibody on the local and systemic homeostasis of interleukin 1 and TNFalpha in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **60**, 660–669 (2001).
35. Barclay, N., Tarallo, M., Hendrikx, T. & Maret, S. PMS78 - patient preference for oral versus injectable and intravenous methods of treatment for rheumatoid arthritis. *Value Health* **16**, A568 (2013).
36. Hsiao, B. & Fraenkel, L. Patient preferences for rheumatoid arthritis treatment. *Curr. Opin. Rheumatol.* **31**, 256–263 (2019).
37. A study to investigate efficacy and safety of SAR441566 in patients with Crohn's disease. <<https://clinicaltrials.gov/study/NCT06637631>>. Accessed February 14, 2025.
38. A study to evaluate SAR441566 efficacy and safety in adults with rheumatoid arthritis. <<https://clinicaltrials.gov/study/NCT06073093>>. Accessed February 14, 2025.