




Pharmacokinetics, Pharmacodynamics, and Safety of Entospletinib, a Novel pSYK Inhibitor, Following Single and Multiple Oral Dosing in Healthy Volunteers

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Abstract

Background and Objectives Entospletinib is a selective, reversible, adenosine triphosphate-competitive small-molecule spleen tyrosine kinase (SYK) inhibitor that blocks B cell receptor-mediated signaling and proliferation in B lymphocytes. This study evaluated the safety, pharmacokinetics, and pharmacodynamics of entospletinib in a double-blind, single/multiple ascending dose study in healthy volunteers.

Methods In sequential cohorts, 120 subjects received entospletinib (25–1200 mg; fasted) as single or twice-daily oral doses for 7 days. Along with pharmacokinetics, the study assessed functional inhibition of ex vivo anti-immunoglobulin E-stimulated CD63 expression on basophils and pervanadate-evoked phosphorylated SYK (pSYK) Y525. Safety and tolerability were assessed throughout the study.

Results Entospletinib was generally well-tolerated over a 48-fold dose range. Adverse events (AEs) were generally mild to moderate, with no AE-driven study drug discontinuations noted. Entospletinib displayed a median plasma half-life of 9–15 h; entospletinib exposures reached a plateau at ≥ 600 mg twice daily (likely due to solubility-limited absorption) and provided $>90\%$ CD63 inhibition at peak concentrations and $>60\%$ inhibition at trough

concentrations (corresponding pSYK inhibition of >70 and $>50\%$).

Conclusion The overall safety, pharmacokinetics, and pharmacodynamics profiles of entospletinib support further clinical evaluation.

Key Points

Entospletinib is a selective oral inhibitor of spleen tyrosine kinase (SYK) that has been linked to the pathogenesis of a variety of B cell malignancies, including chronic lymphocytic leukemia and various subtypes of non-Hodgkin's lymphoma.

When administered in single or multiple oral doses in healthy adults, entospletinib monotherapy is generally well-tolerated.

Entospletinib displays a median plasma half-life of 9–15 h; exposures plateau at ≥ 600 mg twice-daily doses, which provides $>50\%$ coverage at trough concentrations (based on CD63/phosphorylated SYK biomarker assessment).

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1 Introduction

Spleen tyrosine kinase (SYK) is a non-receptor, cytoplasmic protein tyrosine kinase that is predominantly expressed in cells of hematopoietic lineage. It is an important mediator of immunoreceptor signaling in macrophages, neutrophils, mast cells, and B cells.

In B lymphocytes, antigen-mediated stimulation of the B cell receptor (BCR) results in the recruitment and phosphorylation of various kinases, including SYK. Phosphorylation of SYK (pSYK) leads to the activation and amplification of downstream receptor signaling and likely plays a role in chemokine signaling and cellular responses such as proliferation, survival, differentiation, and apoptosis [1, 2].

Uncontrolled BCR signaling via SYK has been implicated in the pathogenesis of several common B cell malignancies, including chronic lymphocytic leukemia (CLL), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma, and B-lineage acute lymphoblastic leukemia. Given the vital role of SYK in BCR signaling, inhibitors of SYK activity are an attractive therapeutic option for hematopoietic B cell lymphomas and other non-Hodgkin's lymphoma histologies, where SYK inhibition would prevent BCR-mediated signaling and therefore prevent the uncontrolled growth of lymphoma cells [3, 4].

A number of molecules targeting the SYK pathway for allergic, autoimmune, and neoplastic disorders are currently in various stages of development. These include adenosine triphosphate (ATP)-binding site inhibitors (fostamatinib, CG14979, and P505-15), P-site inhibitors (C-61), antisense oligonucleotides, and small-interfering RNAs [5].

Clinical investigation of fostamatinib, the prodrug of SYK inhibitor R406, in B cell malignancies demonstrated responses in CLL/small lymphocyte leukemia (overall response rate [ORR] 55%), DLBCL (ORR 22%), MCL (ORR 11%), and FL (ORR 10%) following doses of 200 or 250 mg twice daily. Reported toxicities included diarrhea, nausea, hypertension, cytopenias, and fatigue, which limited dosing and have been partially attributed to off-target effects, including the inhibition of multiple kinases in addition to SYK [6].

Entospletinib (GS-9973) is a highly selective and oral SYK inhibitor and is currently undergoing clinical evaluation for autoimmune and oncology indications. Entospletinib is an ATP-competitive inhibitor of SYK that disrupts kinase activity with a half-maximal inhibitory concentration (IC_{50}) of 7.6 nmol/L. A phase II study involving entospletinib 800 mg twice-daily oral administration in subjects with relapsed or refractory CLL ($n = 41$) showed a progression-free survival (PFS) rate of 70.1% (at 24 weeks), median PFS of 13.8 months, and an ORR of 61.0%. The most common treatment-emergent serious adverse events (AEs) included dyspnea, pneumonia, febrile neutropenia, dehydration, and pyrexia. Common Grade 3/4 laboratory abnormalities included neutropenia and reversible alanine aminotransferase/

aspartate aminotransferase elevations. Overall, entospletinib demonstrated clinical activity in subjects with relapsed or refractory CLL with acceptable toxicity [7].

This paper reports the results of the first-in-human (FIH) study conducted to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of entospletinib in healthy volunteers following single- and multiple-dose oral administration. The data from this study provided the basis for subsequent testing of the safety and efficacy of entospletinib in cancer patients.

Further, this paper describes entospletinib pharmacokinetics following multiple-dose administration (doses of 100–1200 mg twice daily) under fed conditions across additional healthy volunteer studies.

2 Methods

2.1 Ethics

All study protocols were reviewed and approved by the local institutional review boards (all studies were conducted at Algorithme Pharma, Inc., Laval, QC, Canada). The studies were conducted in accordance with recognized international scientific and ethical standards, including but not limited to the International Conference on Harmonization guideline for Good Clinical Practice and the original principles embodied in the Declaration of Helsinki. These standards are consistent with the requirements of the US Code of Federal Regulations Title 21, Part 312 (21CFR312) and the European Community Directive 2001/20/EC. Written informed consent was obtained from all participants.

2.2 Study Design

The FIH study was a phase I, randomized, double-blind, placebo-controlled, single and multiple dose-escalation study, involving an oral tablet formulation of the monomesylate salt of entospletinib. Subjects received either single or multiple doses of entospletinib (or placebo) under fasted conditions (Table 1). The study cohorts were enrolled in a staggered fashion; single-dose cohorts were escalated after review of the safety/pharmacokinetic data, allowing at least 1 week between cohorts, and multiple-dose cohorts were initiated/escalated after review of safety/pharmacokinetics from the single-dose cohorts (up to 200 mg) (Electronic Supplementary Material Fig. S1).

Additionally, open-label, multiple-cohort, multiple-dose studies were conducted that evaluated the pharmacokinetics of entospletinib under fed conditions, i.e., co-administration with a standard meal of ~400–500 calories with ~10–13 g of fat (Table 1).

Table 1 Study design and baseline characteristics of phase I clinical studies included in the analyses

Clinical characteristic	FIH study (fasted)		Fed studies
Dose	Single (day 1): ^a 25, 75, 200, 600, 1200 mg	Multiple (day 1 to day 7, twice daily): ^b 25, 75, 200, 600, 900, 1200 mg Multiple (day 1 to day 7, once daily): 900 mg	Multiple (day 1 to day 6, twice daily): ^c 100, 200, 600, 900, 1200 mg
Population	Healthy subjects	Healthy subjects	Healthy subjects
Subjects (<i>n</i>)	50	70	64
Body weight [median (range); kg]	73 (52–94)	74 (46–101)	70 (52–96)
Age [median (range); years]	36 (19–45)	32 (18–45)	33 (18–57)
Sex [<i>n</i> (%); male]	28 (56)	60 (86)	34 (56)
Race [<i>n</i> (%); white]	41 (82)	62 (89)	54 (84)

FIH first-in-human

^a Pharmacokinetic sampling times were conducted at 0, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 18, 24, 28, 36, and 48 h post-dose. Pharmacodynamic sampling times were conducted at 0, 1, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, 24, 28, and 48 h post-dose

^b Pharmacokinetic sampling times for day 1 were conducted at 0, 2, 4, 6, and 12 h post-dose. Pharmacodynamic sampling times for day 1 were conducted at 0, 2, 4, 6, and 12 h post-dose. Pharmacokinetic sampling times for day 7 were conducted at 0, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 18, 20, 24, 28, 36, and 48 h post-dose. Pharmacodynamic sampling times for day 7 were conducted at 0, 1, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, 24, 28, and 48 h post-dose

^c Pharmacodynamic sampling times for day 1 were conducted at 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 5, 8, 10, and 12 h post-dose. Pharmacodynamic sampling times for day 6 were conducted at 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 (pre-afternoon dose), 14, 16, 20, and 24 h post-dose

2.3 Subjects

Healthy male and non-pregnant, non-lactating female subjects aged 18–45 years (inclusive) who were ≥ 45 kg in weight and had a body mass index between 19 and 30 kg/m² were eligible to participate in the FIH study. Similar inclusion criteria were used for the fed studies, except that the eligible enrollment age was increased to 60 years.

In all studies, subjects were required to be free of clinically significant disease or any condition that could jeopardize subject safety or study validity as determined through the subjects' medical history, physical examination, 12-lead electrocardiogram (ECG), and results from clinical laboratory tests that were performed within 2 weeks prior to the baseline visit (day 0). Prior to enrollment in the study, all subjects provided informed written consent. Subjects were ineligible for the studies if they had used any investigational drug and/or participated in any clinical trial within 1 month of the first entospletinib administration. Use of medications, including over-the-counter medications or herbal products, was not allowed within 1 week of commencing study drug dosing. Vitamins, acetaminophen, hormone replacement therapy, and/or oral contraceptives were allowed.

2.4 Safety Assessments

Safety was evaluated by assessment of clinical laboratory tests (hematology profile, chemistry profile, and urinalysis), physical examinations, vital signs, serum pregnancy

tests (for female subjects), 12-lead safety ECGs, and review of concomitant medications performed at screening, at baseline (the day before the first study dose), on days before pharmacokinetic blood sampling, and at various times during the study. Subjects were monitored for AEs from baseline to the follow-up visit. AEs were rated as Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, and Grade 4 = life-threatening, according to the Gilead Sciences' Modified National Institute of Allergy and Infectious Diseases (NIAID) Common Toxicity Grading Scale. Clinical and laboratory AEs were coded (system organ class, high-level group term, high-level term, preferred term, and lower-level term) using the *Medical Dictionary for Regulatory Activities* (MedDRA[®]) Version 15.0.

2.5 Pharmacokinetic Sampling

Serial blood samples were collected pre-dose and over a 12-, 24-, or 48-h period following single or multiple dosing for assessing the pharmacokinetics of entospletinib across all studies (Table 1).

2.6 Pharmacodynamic Sampling

Serial blood samples for pharmacodynamics assessment were collected pre-dose and over a 48-h period following single or multiple dosing in the FIH study. Whole-blood samples were utilized for the analysis. The samples were refrigerated (2–8 °C) immediately post collection and

stored for up to 24 h. Samples were analyzed within 12 h of blood collection.

2.7 Bioanalytical Procedures

Entospletinib plasma concentrations were determined with a validated high-performance liquid chromatography/tandem mass spectrometry (MS/MS) bioanalytical assay (Covance[®], Madison, WI, USA) [8]. A 50 μ L aliquot of plasma sample was mixed with internal standard and reconstituted with water/acetonitrile at 50:50 (v:v) followed by a liquid/liquid extraction procedure using methyl tert-butyl ether. Chromatography was performed using a Synergi[™] Polar-RP column (Phenomenex, Torrance, CA, USA), with mobile phases consisting of formic acid in water and formic acid in acetonitrile at a flow rate of 0.6 mL/min. The MS/MS precursor ion \rightarrow product ion transition was m/z 412.2 \rightarrow m/z 105.1 for entospletinib. The assay calibration curves were linear, from 1 to 1000 ng/mL ($R^2 = 0.9070$, intercept = 0.000038). Samples were diluted and re-analyzed if the concentrations exceeded the curve range.

Dilution quality control (QC) samples were included, at least in duplicate, for each analytical batch containing samples requiring dilution. These QC samples were diluted with blank matrix to the same extent as the most highly diluted sample. At least one-half of the dilution QC samples had to be within the range of 85.0–115.0% of theoretical for the results of diluted samples to be accepted. The inter-assay precision range (% relative standard deviation) was 2.5–5.6, and the inter-assay accuracy range (% relative error) was –3.2 to 4.3. The stability in frozen matrix was 91 days at –10 to –30 $^{\circ}$ C and 343 days at –60 to –80 $^{\circ}$ C.

2.8 Pharmacodynamic Procedures

2.8.1 CD63 Assay

CD63 surface expression on human whole-blood basophils was measured using a BasoTest[™] Kit (Orpegen Pharma, Heidelberg, Germany). Briefly, heparinized blood was incubated at 37 $^{\circ}$ C. Following the incubation, 20 μ L of potentiation buffer B from the BasoTest[™] Kit was added to each well and incubated for an additional 10 min at 37 $^{\circ}$ C. Subsequently, the Fc ϵ receptor (Fc ϵ R) was cross-linked by the addition of 100 μ L of anti-immunoglobulin E (anti-IgE) to a final concentration of 10 μ g/mL and incubated for 20 min at 37 $^{\circ}$ C. Degranulation was arrested by placing the plate on ice for 5 min. Cells were stained with 20 μ L of a staining cocktail, containing CD123-PE/CD63-FITC/HLA-DR-PerCP and incubated for 20 min on ice. A total of 2 mL of red cell lysis buffer (Buffer G from the BasoTest[™] Kit) was added to each well, mixed

thoroughly, and incubated at room temperature for 10 min. The plate was centrifuged and the supernatant discarded. The cell pellet was washed with 2 mL of washing solution (Buffer A from the BasoTest[™] Kit), and the centrifugation was repeated. The cells were re-suspended in buffer A and kept on ice in a 250 μ L 96-well U-bottom plate until read by multicolor flow cytometry on a BD[™] FACS LSR II (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Fluorescence-activated cell sorter (FACS) sampling of 100,000 total events was collected using a high-throughput screen autosampler. FACS data were collected and CD123+/human leukocyte antigen–antigen D related negative (HLA-DR–) basophils were gated. The percentages of CD63+ cells in this population were recorded for each well. Electronic Supplementary Material Fig. S2 provides a representative scatter plot of the CD63+ assay and gate strategy.

2.8.2 Phosphorylated Spleen Tyrosine Kinase (Y525) Assay

Heparinized blood was incubated in a 96-deep well V-bottom plate for 1 h at 37 $^{\circ}$ C. Following the incubation, 10 μ L of 6 mmol/L pervanadate was added to each well and incubated for an additional hour at 37 $^{\circ}$ C. Subsequently, 750 μ L of phosphate-buffered saline was added to each well and immediately centrifuged at 3300 rpm for 3 min at room temperature. The supernatant was removed with a 96-well block aspirator (V&P Scientific, Inc., San Diego, CA, USA). The wash step was repeated and supernatant was discarded. The cell pellet was placed on ice and 75 μ L of ice-cold 2X cell lysis buffer was added, mixed immediately, and kept on ice for 30 min prior to being transferred to 96-well polymerase chain reaction plates and stored frozen until use. Mesoscale (MSD) chemiluminescent capture plates were prepared by adding 5 μ L of 2 ng/ μ L N-19 total SYK antibody diluted in Tris-buffered saline (TBS) to a 96-well goat anti-rabbit small spot plate. The plate was incubated for 1 h at room temperature. Each well was blocked with 25 μ L of blocker buffer containing 5% rabbit serum for 1 h with shaking on an orbital shaker. Prior to use, the blocker buffer was decanted and the wells were washed with 200 μ L of TBS + 0.2% Tween-20 (TBST). The wash was decanted, and 25 μ L of the whole-blood lysate was added to each well. The plate was placed on an orbital shaker for 1 h at room temperature; the supernatant was then decanted into a 10% bleach solution and the plate was washed twice with 200 μ L TBST. An antibody cocktail was prepared with anti-pSYK (Y525) biotinylated antibody (1:1000) plus streptavidin sulfo-tagged reagent (1:1000) in blocker buffer containing 5% rabbit serum. A total of 25 μ L was added to

each well and incubated on an orbital shaker for 1 h at room temperature. The wells were decanted and washed once with 200 μ L TBST. Again, the wells were decanted, and 150 μ L of read buffer was added and read immediately on the MSD instrument. MSD data were collected, and electrochemiluminescence readings were recorded for each well.

2.9 Pharmacokinetic Analysis

The pharmacokinetic parameters of entospletinib were assessed by non-compartmental methods using a non-linear curve-fitting software package (Phoenix[®] WinNonlin[®] Professional, Pharsight[®] Corporation, Mountain View, CA, USA). The following pharmacokinetic parameters were evaluated for entospletinib: C_{\max} (the maximum observed concentration of the drug), t_{\max} (the time [observed time-point] of C_{\max}), $t_{1/2}$ (the estimate of the terminal elimination half-life of the drug; calculated by dividing the natural log of 2 by the terminal elimination rate constant [λ_z ; which is estimated by linear regression of the terminal elimination phase of the concentration of drug versus time curve]), AUC_{last} (area under the plasma concentration–time curve [AUC] from time zero to the time of the last quantifiable concentration), AUC_{∞} (AUC extrapolated to infinity; calculated as $AUC_{\text{last}} + C_{\text{last}}/\lambda_z$ [the last observed quantifiable concentration of the drug in plasma divided by λ_z]) (single dose), AUC_{τ} (AUC over the dosing interval [τ]), and C_{trough} (concentration at the end of the dosing interval) (multiple dose).

2.10 Pharmacodynamic Analysis

The blood pharmacodynamic measurements conducted for entospletinib in the FIH study included the number of CD63+ basophils and pSYK (Y525). The changes in CD63+ and pSYK (Y525) expression versus time profiles were assessed by calculating the following pharmacodynamic parameters: percentage CD63+ basophils and percentage pSYK (Y525), reported as normalized to baseline/pre-dose values.

2.11 Statistical Methods

An evaluable pharmacokinetics population (subjects who completed at least one pharmacokinetics sampling session and for whom pharmacokinetic parameters could be calculated), safety population (subjects who received at least one dose of entospletinib), and pharmacodynamics population (subjects who received at least one dose of entospletinib and had specific laboratory data available for calculating estimates of pharmacodynamic parameters) were identified for this study. Pharmacokinetics,

pharmacodynamics, and safety analyses were conducted based on the corresponding evaluable population.

3 Results

3.1 Subject Demographics

A total of 184 healthy subjects were enrolled and analyzed for this report. The majority of healthy subjects were male, white, and of non-Hispanic/Latino ethnicity. The demographics and other baseline characteristics are shown in Table 1.

3.2 Safety and Tolerability

Safety results are presented for both the FIH and fed studies (Table 2). Overall, entospletinib monotherapy was generally well-tolerated when administered as a single dose or in multiple doses. No discontinuations due to treatment-related AEs were reported for the FIH study, but two subjects in the fed studies (Grade 3 urticaria and moderate urinary tract infection) discontinued. The majority of AEs were mild or moderate in severity, with no Grade 4 severity events. No apparent dose response was observed with any AE in either the single- or multiple-dose cohorts. The majority of graded laboratory abnormalities were Grade 1 severity. No subjects experienced serious AEs attributed to the study drug. No deaths were reported.

3.3 Pharmacokinetics of Entospletinib

3.3.1 Single Dose

The plasma concentration–time profiles of single oral doses of entospletinib 25, 75, 200, 600, 900, and 1200 mg under fasted conditions are illustrated in Fig. 1. A summary of the pharmacokinetic parameters following single-dose administration is presented in Table 3. Entospletinib was rapidly absorbed, generally achieving C_{\max} within 2–3 h after administration for all doses. After attaining C_{\max} , entospletinib plasma concentrations declined with a $t_{1/2}$ of approximately 9 h at doses ≥ 200 mg. The exposures following entospletinib 200 mg increased in a greater-than-dose-proportional manner compared with those following 75 mg. The exposure increase from 600 to 1200 mg was less than dose proportional.

3.3.2 Multiple Doses

The plasma concentration–time profiles of entospletinib following administration of multiple (twice-daily) oral doses of 25, 75, 200, 600, 900, and 1200 mg in healthy

Table 2 Summary of treatment-related adverse events occurring in $\geq 10\%$ of patients and Grade ≥ 3 laboratory abnormalities

AEs	FIH study (fasted) [<i>n</i> (%)]		Fed studies [<i>n</i> (%)]
	Single dose	Multiple dose	
<i>n</i> ^a	40	56	40
AEs (preferred term) ^b			
Headache	5 (12.5)	18 (32.1)	10 (25.0)
Nausea	6 (10.7)		
Rhinorrhea		7 (12.5)	
Oropharyngeal pain		6 (10.7)	5 (12.5)
Urinary tract infection			1 (2.5)
Laboratory parameter (Grade ≥ 3)			
ALT		1 (1.8)	
AST		1 (1.8)	
Creatinine kinase	1 (2.5)		
Total bilirubin		1 (1.8)	

AEs adverse events, ALT alanine aminotransferase, AST aspartate aminotransferase, FIH first-in-human

^a Data from placebo cohorts are excluded

^b AEs were mapped (e.g., to system organ class) using the *Medical Dictionary for Regulatory Activities* (MedDRA[®]) Version 15.0. AEs/laboratory abnormalities were rated as Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, and Grade 4 = life-threatening, according to the Gilead Sciences' Modified National Institute of Allergy and Infectious Diseases (NIAID) Common Toxicity Grading Scale

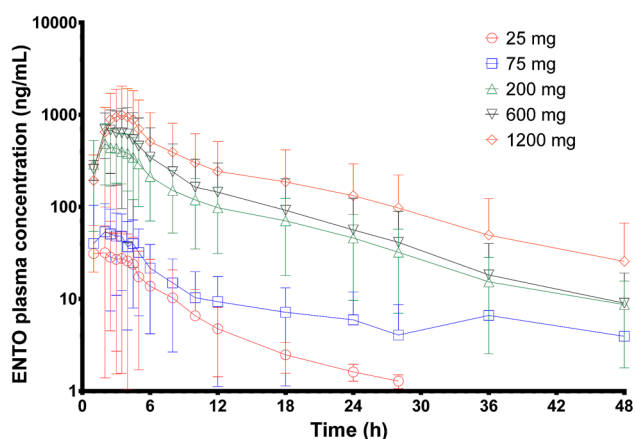


Fig. 1 Mean (\pm standard deviation) plasma concentrations of entospletinib following a single oral dose of entospletinib under fasted conditions. *ENTO* entospletinib

volunteers under fasted conditions are presented in Fig. 2. A summary of the corresponding steady-state (day 7) pharmacokinetic parameters is presented in Table 4. Following 7 days of multiple dosing, there was an approximate 2- to 4-fold increase in entospletinib exposure (C_{\max} and AUC) on day 7 compared with day 1 (Table 4). Following C_{\max} (~ 2 – 3 h post-dose), plasma concentrations declined with a $t_{1/2}$ of approximately 9 h at doses ≤ 200 mg twice daily and approximately 14 h at doses > 200 mg twice daily. Steady-state concentrations were achieved after 3–4 days with twice-daily dosing, which is consistent with the estimated $t_{1/2}$. Entospletinib steady-state exposures

increased with increasing doses, ranging from 25 to 600 mg twice daily. Similar to single-dose data, at doses greater than 600 mg the entospletinib exposure increases were less than dose proportional, suggesting that exposure was approaching a plateau.

3.4 Effect of Food

Entospletinib pharmacokinetics were also evaluated following multiple (twice-daily) dosing of entospletinib 100, 200, 600, 900, and 1200 mg under fed conditions. A summary of the corresponding steady-state pharmacokinetic parameters under fed conditions is presented in Table 5. Absorption of entospletinib under fed conditions was slower than in the fasted state, with C_{\max} attained within 4 h after administration (vs. 2 h under fasted conditions). Entospletinib exposures increased with increasing doses without any apparent exposure plateau, unlike exposures in fasted conditions. At a given dose level, entospletinib exposure was generally lower following fed versus fasted administration.

3.5 Pharmacodynamics of Entospletinib

The effect of entospletinib on inhibition of ex vivo anti-IgE-stimulated cell-surface CD63 expression in basophils after single dosing is illustrated in Electronic Supplementary Material Fig. S3 and multiple dosing (fasted) is illustrated in Fig. 3. Overall, inhibition of cell surface

Table 3 Mean (% coefficient of variation) pharmacokinetic parameters following single oral administration of entospletinib under fasted conditions according to dose cohort/group

Parameter	25 mg	75 mg	200 mg	600 mg	1200 mg
<i>n</i>	8	8	8	8	8
C_{max} (ng/mL)	42.3 (75.4)	67.8 (83.9)	534.5 (55.6)	778.9 (69.6)	1059.8 (100.7)
t_{max} (h) ^a	2.00 (1.00–3.26)	2.75 (2.00–3.01)	2.00 (2.00–3.50)	2.00 (2.00–3.00)	3.25 (2.75–3.76)
$t_{1/2}$ (h) ^a	6.77 (5.28–7.25)	5.31 (3.97–8.65)	9.51 (7.41–10.79)	7.72 (6.78–9.55)	9.59 (7.47–12.13)
AUC_{last} (ng·h/mL)	218.3 (85.2)	392.5 (97.8)	4136.3 (64.2)	5834.2 (90.5)	9376.2 (109.1)
AUC_{∞} (ng·h/mL)	231.1 (80.1)	413.1 (97.6)	4248.7 (64.5)	5957.7 (90.5)	9744.4 (110.6)

AUC_{∞} area under the plasma concentration–time curve extrapolated to infinity, calculated as $AUC_{last} + (C_{last}/\lambda_z)$ [the last observed quantifiable concentration of the drug in plasma divided by λ_z], AUC_{last} area under the plasma concentration–time curve from time zero to the time of the last quantifiable concentration, C_{max} maximum observed concentration of drug in plasma, $t_{1/2}$ half-life, t_{max} time (observed timepoint) of C_{max}

^a Median value (interquartile range) are reported

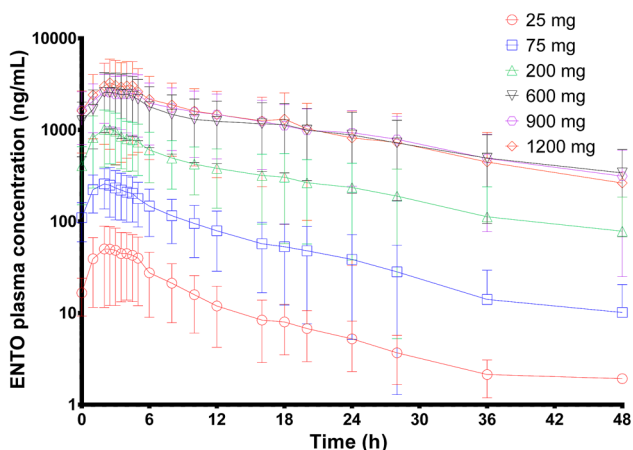


Fig. 2 Mean (\pm standard deviation) steady-state plasma concentrations of entospletinib following multiple oral twice-daily doses of entospletinib under fasted conditions. *ENTO* entospletinib

CD63 expression (expressed as percentage of baseline) increased with doses of entospletinib from 25 to 600 mg following single- or multiple-dose administration. For doses ≥ 600 mg twice daily, the maximal mean inhibition of CD63 expression peaked at 2–4 h post-dose at $\geq 86\%$ inhibition, with $\geq 63\%$ inhibition being maintained at C_{trough} (i.e., 12 h post-dose). The maximal mean inhibition of CD63 expression was comparable between entospletinib 600, 900, and 1200 mg twice daily.

Electronic Supplementary Material Fig. S4 illustrates the effect of entospletinib on inhibition of pervanadate-evoked pSYK-Y525 after single dosing and Fig. 4 illustrates multiple dosing (fasted). The baseline levels of pSYK are shown in Electronic Supplementary Material Fig. S5. Overall, there was increasing inhibition of pSYK expression (expressed as percentage of baseline) with entospletinib doses of 25–600 mg following single- or multiple-day (twice-daily) administration. For doses ≥ 600 mg twice daily, the maximal mean inhibition of pSYK expression peaked at 4–5 h post-dose at $\geq 72\%$

inhibition, with $\geq 54\%$ inhibition being maintained at C_{trough} (i.e., 12 h post-dose). The maximal mean inhibition of pSYK expression was comparable between entospletinib 600, 900, and 1200 mg twice daily.

Figure 5 illustrates the exposure–response relationship between entospletinib concentrations and inhibition of CD63 expression and pSYK (represented as percentage of baseline). Entospletinib inhibited both of these parameters in a concentration-dependent manner with a median effective concentration (EC_{50}) of 429 and 110 ng/mL for inhibition of CD63 and pSYK, respectively.

4 Discussion

Entospletinib is a potent, highly selective, oral, small-molecule inhibitor of SYK that is being developed for the treatment of hematological malignancies. Entospletinib has greater than 14-fold selectivity for SYK versus other kinase targets [8], and hence has the potential for an improved efficacy and safety profile in patients versus other SYK inhibitors [5]. This paper reports the results of the FIH study conducted to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of entospletinib in healthy volunteers following single- and multiple-dose oral administration under fasted conditions. Additionally, safety, tolerability, and pharmacokinetics following entospletinib co-administration with food are presented.

Following single and multiple oral administration of entospletinib under fasted conditions, entospletinib concentrations showed a bi-exponential decline. Based on a terminal $t_{1/2}$ of ~ 9 h, entospletinib is suitable for a twice-daily dosing regimen. Entospletinib exposures increased in a less-than-dose-proportional manner at doses >200 mg, with exposures reaching a plateau at >600 mg twice daily. The solubility of entospletinib administered as a monomesylate salt is highly pH-dependent: its aqueous solubility is 3 mg/mL in pH 2 buffer but <0.001 mg/mL at pH ≥ 3.0 .

Table 4 Mean (% coefficient of variation) steady-state pharmacokinetic parameters following multiple (twice-daily) oral administration of entospletinib under fasted conditions according to dose cohort/group

Parameter	25 mg	75 mg	200 mg	600 mg	900 mg	1200 mg
<i>n</i>	8	8	8	8	8	8
Day 1						
C_{\max} (ng/mL)	23.6 (99.8)	99.8 (74.6)	475.8 (80.3)	1006.6 (77.4)	1033.8 (95.5)	828.2 (109.7)
t_{\max} (h) ^a	2.00 (2.00–4.00)	2.00 (2.00–3.00)	2.00 (2.00–2.00)	2.00 (2.00–2.00)	2.00 (2.00–4.00)	2.00 (2.00–3.00)
AUC _{last} (ng·h/mL)	115.6 (94.2)	505.3 (79.6)	2254.0 (80.3)	5455.3 (99.7)	5862.5 (100.1)	4185.6 (116.3)
Day 7						
C_{\max} (ng/mL)	57.8 (68.3)	273 (52.1)	1052 (58.6)	2687 (61.1)	2814 (58.3)	3427 (77.1)
t_{\max} (h) ^a	2.26 (2.00–2.75)	2.00 (2.00–2.01)	2.27 (2.00–2.50)	2.25 (2.00–3.25)	2.26 (2.00–4.00)	2.75 (2.25–3.50)
C_{trough} (ng/mL)	11.9 (64.5)	79.2 (64.0)	380 (63.3)	1242 (65.6)	1473 (67.2)	1471 (79.6)
$t_{1/2}$ (h) ^a	8.66 (7.72–9.65)	8.91 (6.72–10.9)	10.0 (8.24–15.7)	15.4 (11.4–20.5)	13.6 (11.6–17.0)	13.9 (9.65–17.0)
AUC _τ (ng·h/mL)	356 (64.8)	1878 (48.2)	7655 (57.4)	21,484 (63.8)	23,895 (61.9)	26,775 (76.8)

AUC_τ area under the plasma concentration–time curve over the dosing interval, AUC_{last} area under the plasma concentration–time curve from time zero to the time of the last quantifiable concentration, C_{\max} maximum observed concentration of drug in plasma, C_{trough} observed drug concentration at the end of the dosing interval, $t_{1/2}$ half-life, t_{\max} time (observed timepoint) of C_{\max}

^a Median value (interquartile range) are reported

Table 5 Mean (% coefficient of variation) steady-state pharmacokinetic parameters following multiple (twice-daily) oral administration of entospletinib under fed conditions according to dose cohort/group

Parameter	100 mg	200 mg	600 mg	900 mg	1200 mg
<i>n</i>	16	8	16	16	7
C_{\max} (ng/mL)	268 (42.8)	618 (39.6)	1573 (59.8)	2321 (66.0)	2799 (35.9)
t_{\max} (h) ^a	4.00 (3.00–4.00)	4.00 (4.00–4.00)	4.00 (3.0–4.0)	4.00 (3.00–4.00)	4.00 (2.50–4.00)
C_{trough} (ng/mL)	92.5 (61.8)	201 (66.4)	832 (82.4)	1211 (77.0)	1376 (40.2)
$t_{1/2}$ (h) ^a	5.31 (4.27–8.89)	7.74 (5.23–13.0)	12.9 (8.90–14.8)	10.1 (7.46–18.4)	8.95 (7.58–17.7)
AUC _τ (ng·h/mL)	1872 (49.4)	4146 (48.1)	12,689 (69.1)	19,085 (71.0)	22,788 (35.5)

AUC_τ area under the plasma concentration–time curve over the dosing interval, C_{\max} maximum observed concentration of drug in plasma, C_{trough} observed drug concentration at the end of the dosing interval, t_{\max} time (observed timepoint) of C_{\max} , $t_{1/2}$ half-life

A standard meal (~400–500 calories with ~10–13 g fat) was used to simulate the fed condition

^a Median value (interquartile range) are reported

As such, the absorption of entospletinib may be limited, particularly at high-dose levels, due to the pH-dependent solubility as it transits through the intestine during absorption and thus may explain the plateau in entospletinib exposures.

Further, there was an approximate 3- to 4-fold increase in entospletinib exposures following multiple doses that was greater than expected (~2-fold), given an average 9-h $t_{1/2}$. Given a slight increase in $t_{1/2}$ between single and multiple doses, the accumulation may not be completely attributed to a non-linear systemic clearance mechanism (e.g., hepatic auto-inhibition), but is also likely due to increased extent of entospletinib absorption (e.g., auto-inhibition of intestinal metabolism/transport). Similar non-linear accumulation following multiple doses has been reported for other molecules (e.g., nefazodone; non-linear accumulation attributed to auto-inhibition of pre-systemic

metabolism) [9]. Additional studies to characterize the disposition of entospletinib via metabolic/transporter-mediated pathways, and non-linear mixed-effects modeling-based population pharmacokinetic analyses are ongoing to elucidate the mechanism.

Food and food type can affect the pharmacokinetics of a drug [10]; therefore, entospletinib pharmacokinetics were evaluated following co-administration with a high-fat meal. A dose-dependent reduction in both the absorption rate (20–70% reduction in C_{\max} and t_{\max} prolongation by ~2 h) and extent (20–80% reduction in AUC) of entospletinib exposures was observed under fed conditions with comparable terminal $t_{1/2}$, suggesting a decrease in the total bioavailability of entospletinib without significant effect on total body clearance. The results may be explained by the pH-dependent solubility of the monomesylate salt, since food intake can increase pH in the

Fig. 3 Mean (\pm standard deviation) inhibition of CD63 pharmacodynamics on day 7 (pharmacokinetic steady state) following multiple oral twice-daily doses of entospletinib under fasted conditions. Day 1 pre-dose CD63 pharmacodynamic values are considered as baseline

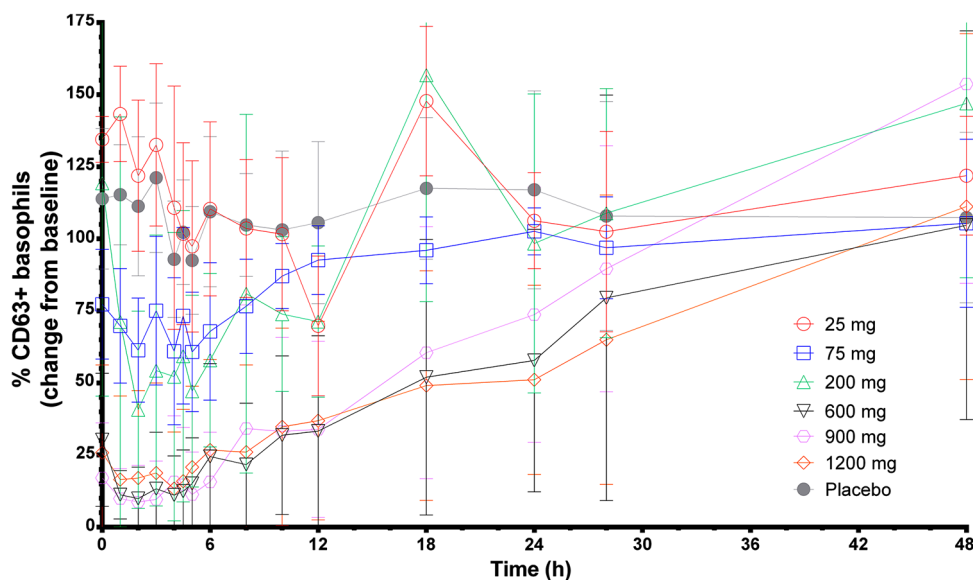
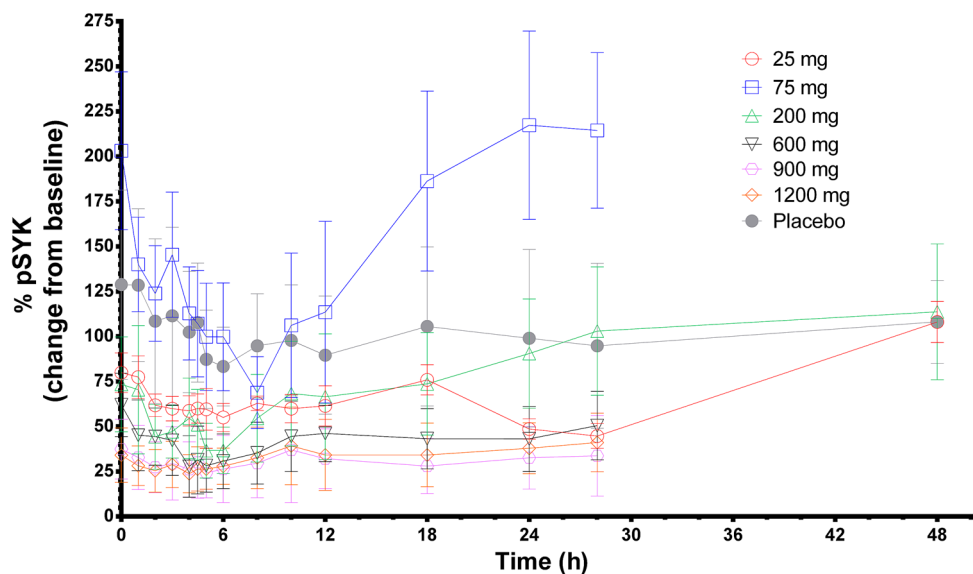


Fig. 4 Mean (\pm standard deviation) inhibition of phosphorylated spleen tyrosine kinase pharmacodynamics on day 7 (pharmacokinetic steady state) following multiple oral doses of entospletinib under fasted conditions. Pre-dose phosphorylated spleen tyrosine kinase pharmacodynamic values are considered as baseline. pSYK phosphorylated spleen tyrosine kinase



gastrointestinal tract [10] (which appears to take precedence over the solubilizing effect of food on entospletinib).

The potency of entospletinib to inhibit SYK downstream of the Fc ϵ R was determined in basophils. Crosslinking of the Fc ϵ R by anti-IgE induces a phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) repeat in the Fc ϵ R, thereby engaging and activating SYK. The resultant intracellular signaling can be monitored by an increase in the cell-surface expression of the activation marker CD63 in basophils [11]. In addition, direct inhibition of pervanadate-induced SYK phosphorylation (at the Y525/526 autophosphorylation site) by entospletinib was evaluated in whole blood.

A good correlation existed between plasma entospletinib concentrations and the pharmacodynamic measurements; increasing entospletinib plasma concentrations resulted in

greater suppression of CD63 expression/pSYK, with maximal inhibition observed at C_{max} . Furthermore, pharmacodynamic inhibition increased with doses of entospletinib up to 600 mg with comparable maximal inhibition at higher doses.

The estimated EC_{50} values for inhibition of CD63 and pSYK were 429 and 110 ng/mL, respectively. Therefore, at entospletinib doses of 600 mg twice daily or higher, the steady-state C_{trough} levels (approximately >1200 ng/mL) achieved would provide complete coverage of the pharmacodynamics EC_{50} values throughout the 12-h dosing interval.

Based on the results shown previously, and considering the inter-individual variability, entospletinib 800 mg twice daily (fasted) was selected as the phase II dose to provide maximum drug exposure for subjects with malignant disease.

Overall, entospletinib was generally well-tolerated when administered as a single dose or in multiple doses. At

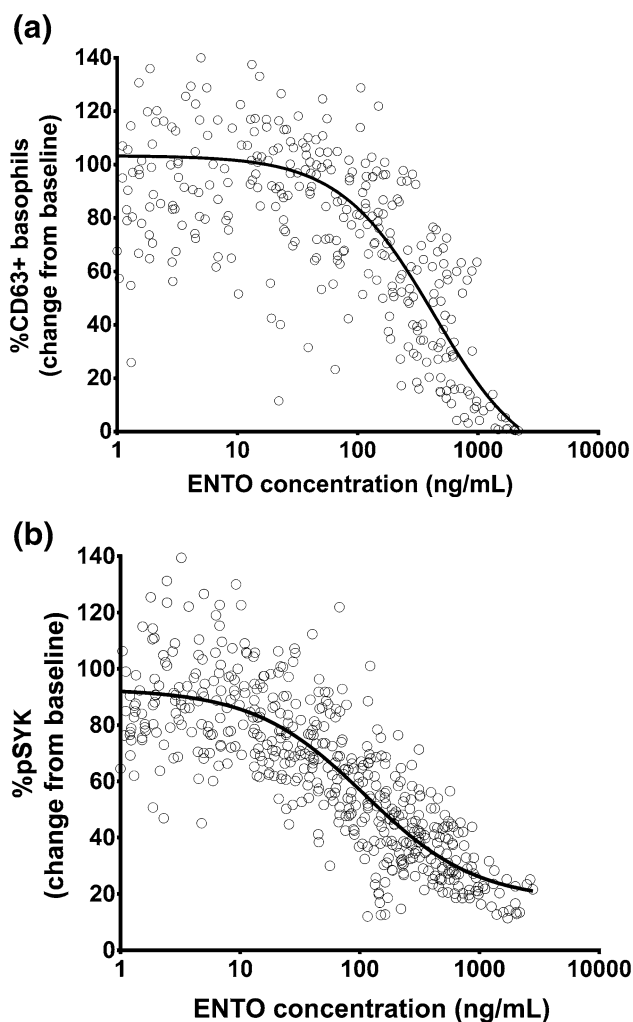


Fig. 5 Pharmacodynamic response, change from baseline in CD63+ basophils (a) and phosphorylated spleen tyrosine kinase (b) versus plasma concentrations. *ENTO* entospletinib, *pSYK* phosphorylated spleen tyrosine kinase

higher doses, some subjects developed modest increases in indirect bilirubin, consistent with uridine 5'-diphosphoglucuronosyltransferase (UGT) 1A1 inhibition (in vitro $IC_{50} \sim 2.5 \mu\text{mol/L}$) [8]. Increases in bilirubin were not associated with other liver function test abnormalities. Increased transaminases were noted in six subjects, including two placebo subjects, and tended to be observed approximately 2 weeks after completion of the study drug. All elevated total bilirubin and transaminase levels returned to normal during follow-up.

5 Conclusions

Entospletinib exposures reached a plateau at 600 mg twice daily under fasted conditions, and provided >50% CD63/*pSYK* inhibition at C_{trough} . Overall, entospletinib displayed

an acceptable safety profile, supporting further clinical evaluation in cancer patients.

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Compliance with Ethical Standards

Ethics This study was performed in compliance with current Good Clinical Practice (GCP) and was conducted under the review of the affiliated Institutional Review Board (IRB), whose operations were in compliance with Section 56 of Title 21 of the Code of Federal Regulations (CFR). This study was sponsored by Gilead Sciences, Inc.

Informed Consent Informed consent was obtained from all subjects.

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Conflict of Interest SR, JAD, FJ, LS, SS, MR, and BPK are employees of Gilead Sciences, Inc. and may own stock or hold stock options in the company.

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