GenFleet Therapeutics

Clinical Trial Protocol

A first-in-human, randomized, double-blinded, placebo-controlled, two-part study to assess safety/tolerability and pharmacokinetics of single- and multiple-ascending doses and food effect of GFH312 in healthy subjects

Compound number	GFH312
Document type:	Clinical Trial Protocol
Development phase:	Phase I
Version number:	V1.4
Release date:	24-Feb-2022

Sponsor Signatory

As GenFleet Therapeutics ('Sponsor') representative, I confirm that the study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product (IP), as well as with the moral, ethical, and scientific principles governing clinical research as set out in the current Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects) and the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Title: Senior Medical Director

Print Name: Alan Zhu

Signature Alan Zhu Date 26-Feb. 2022

Investigator Protocol Agreement Page

I have read and agree to the protocol GFH312X3101, entitled A first-in-human, randomized, double-blinded, placebo-controlled, two-part study to assess safety/tolerability and pharmacokinetics of single- and multiple-ascending doses and food effect of GFH312 in healthy subjects.

All documentation for this study that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure(s) (IB), electronic Case Report Forms (eCRFs), and other scientific data.

The study will not be commenced without the prior written approval of a properly constituted Human Research Ethics Committee (HREC). No changes will be made to the study protocol without the prior written approval of the Sponsor and the Ethics Committee, except where necessary to avert an immediate hazard to the participants.

I am aware of my responsibilities as an Investigator under the guidelines of ICH GCP, Declaration of Helsinki, National Health and Medical Research Council (NHMRC) National Statement on Ethical Conduct in Human Research 2007 (updated 2018). The conduct of the study will be in accordance with the Integrated Addendum to ICH E6 (R1): Guideline for GCP ICH E6 (R2), annotated with comments by the Australian Therapeutic Goods Administration (TGA; 2018) and the study protocol.

I agree to conduct the study according to these responsibilities and to appropriately direct and assist the staff under my control, who will be involved in the study.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study at my site are informed about their obligations. Mechanisms are in place to ensure that site staff receive the appropriate information throughout the study

Clinical Site:

Investigator:

Print Name	Title
_____	_____
Signature	Date
_____	_____

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AD	Alzheimer disease
AE	Adverse event
ALS	Amyotrophic lateral sclerosis
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
BMI	Body mass index
CNS	Central nervous system
CRF	Case report/record form
CSF	Cerebrospinal fluid
C-SSRS	Columbia suicide severity rating scale
CTC	Common toxicity criteria
CV	Coefficient of variation
CYP	Cytochrome P450
ECG	Electrocardiogram
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
h	Hour
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IC_{50}	Half maximal inhibitory concentration
ICF	Informed consent form
ICH	International conference on harmonization of technical requirements for registration of pharmaceuticals for human use
IEC	Independent ethics committee
INR	International normalized ratio
IRB	Institutional review board
LLOQ	Lower limit of quantification

LLN	Lower limit of normal
MedDRA	Medical dictionary for regulatory activities
mg	Milligram(s)
mL	Milliliter(s)
MLKL	Mixed-lineage kinase domain-like
MS	Multiple sclerosis
NOAEL	No observed adverse effect level
PBMC	Peripheral blood mononuclear cell
PD	Pharmacodynamic(s)
pRIPK	phospho-RIPK
PK	Pharmacokinetic(s)
PT	Prothrombin time
RBC	Red blood cell(s)
RIPK	Receptor-interacting serine/threonine protein kinase
SAE	Serious adverse event
sCR	Serum creatinine
SD	Standard deviation
SUSAR	Suspected unexpected serious adverse reactions
TBL	Total bilirubin
TNF	Tumor necrosis factor
ULN	Upper limit of normal
WBC	White blood cell(s)
WOCBP	Women of child-bearing potential

Pharmacokinetic definitions and symbols

Ae_{0-t}	Amount of drug (or defined metabolite) excreted into the urine from time zero to time 't' where t is a defined time point after administration [mass units or % of dose]
AUC_{0-t}	The area under the plasma (or serum or blood) concentration-time curve from time zero to time 't' where t is a defined time point after administration [mass x time / volume]
AUC_{0-inf}	The area under the plasma (or serum or blood) concentration-time curve from time zero to infinity [mass x time / volume]
AUC_{0-last}	The area under the plasma (or serum or blood) concentration-time curve from time zero to the time of the last quantifiable concentration [mass x time / volume]
AUC_{0-tau}	The area under the plasma (or serum or blood) concentration-time curve from time zero to the end of the dosing interval tau [mass x time / volume]
$C_{av,ss}$	The average steady state plasma (or serum or blood) concentration during multiple dosing
C_{max}	The observed maximum plasma (or serum or blood) concentration following drug administration [mass / volume]
$C_{max,ss}$	The observed maximum plasma (or serum or blood) concentration following drug administration at steady state [mass / volume]
$C_{min,ss}$	The lowest plasma (or serum or blood) concentration observed during a dosing interval at steady state [mass / volume]
CL	The systemic (or total body) clearance from plasma (or serum or blood) following intravenous administration [volume / time]
CL/F	The apparent systemic (or total body) clearance from plasma (or serum or blood) following extravascular administration [volume / time]
CL_r	The renal clearance [volume / time]
F	Bioavailability of a compound.
MRT	Mean residence time determined as $AUMC_{0-inf}/AUC_{0-inf}$ following intravenous administration [time].
R_{acc}	The accumulation ratio
$T_{1/2}$	The terminal elimination half-life [time]
T_{max}	The time to reach the maximum concentration after drug administration [time]
V_d	The volume of distribution during the terminal elimination phase following intravenous administration [volume]

V_d/F	The apparent volume of distribution during the terminal elimination phase following extravascular administration [volume]

Summary of Changes Protocol Amendment

Protocol title: A first-in-human, randomized, double-blinded, placebo- controlled, two-part study to assess safety/tolerability and pharmacokinetics of single- and multiple-ascending doses and food effect of GFH312 in healthy subjects

Project number: GFH312X3101

Amendment Date: 25 March 2021(V1.0 to V1.1)

The main changes and rationales for protocol amendment from version 1.0 to version1.1:

1) The protocol is amended to allow remaining plasma and CSF samples from the study to be used for future exploration of GFH312 metabolites and other potential biomarkers. Descriptions were added to Section Sampling Plan for PK/PD, Section 8.5 and Section 8.6.
2) Inclusion criteria 4 and Exclusion criteria 16, 20 and 24 were further clarified in Section 4.1 and Section 4.2.
3) Clarification regarding subject replacement was confirmed in Section 3.1 and Section 6.6.1.

In addition, administration, typographical and formatting changes were made throughout the protocol.

Amendment Date: 21 Oct 2021 (V1.1 to V1.2)

The main changes and rationales for protocol amendment from version 1.1 to version1.2:

1) Exclusion criterion 24 in Section 4.2 was modified to relax the COVID-19 vaccination restriction during the study period on the premise of ensuring the scientific study design and safety of subjects.
2) The timings of the following assessments were clarified in Assessment Schedule and Section 8.6.2:
   a) ANA test prior to first dosing,
   b) vital sign on Day 1 in Part Ib,
   c) pre-dose CSF sampling for pharmacodynamics in Part II,
   d) and the time window for Study Completion.
3) The order of operations was clarified in Section 8.1 for concurrent vital signs assessment, ECG, and blood sample collection per assessment schedule. The time window for ECG and vital signs assessments were clarified in Section 8.1.
4) Unscheduled PK blood sampling was clarified in Section 8.5.1.

Amendment Date: 28 Oct 2021 (V1.2 to V1.3)

The main changes and rationales for protocol amendment from version 1.2 to version1.3:

1) To avoid the superimposition of any acute toxicities arising from either the vaccine or the study drug, exclusion criterion 24 in Section 4.2 was modified to restrict COVID-19 vaccination within 1 week of the last dosing.

Amendment Date: 24 Feb 2022 (V1.3 to V1.4)

The main changes and rationales for protocol amendment from version 1.3 to version1.4:

1) Exclusion criterion 4 in Section 4.2 was modified to relax the exclusion range of ANA to above the 1:160 titer, considering a positive result of ANA test in 1:160 titer without clinical significant symptoms is not likely to increase the potential risk of immune disorder for such subjects in this study.
2) Exclusion criterion 3 in Section 4.2 was modified to allow the enrolment of healthy subjects with CRP below 10 mg/L without clinical relevance. This change of criterion is not expected to introduce additional safety risk to such healthy subjects or contaminate the safety evaluation of the investigational product.

Protocol Synopsis

<table>
<tr><td>Protocol number</td><td colspan="2">GFH312X3101</td></tr>
<tr><td>Study Title</td><td colspan="2">A first-in-human, randomized, double-blinded, placebo-controlled, two-part study to assess safety/tolerability and pharmacokinetics of single- and multiple-ascending doses and food effect of GFH312 in healthy subjects</td></tr>
<tr><td>Brief title</td><td colspan="2">First-in-human, safety/tolerability and pharmacokinetics study of GFH312 in healthy subjects</td></tr>
<tr><td>Development Phase</td><td colspan="2">Phase I</td></tr>
<tr><td>Rationale</td><td colspan="2">GFH312 is a small molecule inhibitor of receptor-interacting serine/threonine protein-1(RIP1) kinase, a key regulator of the TNF-αdownstream. RIPK1 can regulate the NF- κB signaling and necroptosis, a type of cell death which can trigger immune response and enhance inflammation. As such, GFH312 represents a novel, selective mechanism for the treatment of inflammatory conditions.

This study is the first administration of GFH312 to humans. The purpose of the study is to evaluate the safety/tolerability and pharmacokinetics in healthy subjects. The intention of this study is to provide confidence in the safety of the molecule to inform progression to further proof-of-concept studies. The dose range proposed in this study is based on a low starting dose escalating to supra-therapeutic doses.</td></tr>
<tr><td>Objectives and endpoints</td><td>Objectives</td><td>Endpoints</td></tr>
<tr><td></td><td colspan="2">Primary</td></tr>
<tr><td></td><td>• To assess the safety/ tolerability of single (fed and fasted) and repeat doses of GFH312 in healthy subjects</td><td>• Incidence of adverse events and serious adverse events, change in laboratory values, ECG, vital signs, physical examinations</td></tr>
<tr><td></td><td colspan="2">Secondary</td></tr>
<tr><td></td><td>• To characterize the pharmacokinetic (PK) profile of single doses of</td><td>• Plasma concentration of GFH312 and derived PK parameters, including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, CL/F, CLr, V_d/F and $t_{1/2}$ following single (fed and fasted)</td></tr>
</table>

	GFH312 in healthy subjects	doses, where data allow • Cerebrospinal fluid (CSF) concentration and CSF/plasma ratio of GFH312 in one cohort
	• To characterize the PK profile of repeat doses of GFH312 in healthy subjects	• Plasma concentration of GFH312 and derived PK parameters, including AUC_{0-tau}, $C_{max,ss}$, $C_{min,ss}$ T_{max}, CL/F, V_d/F, $t_{1/2}$ and R_{acc} following repeat doses, where data allow • CSF concentration and CSF/plasma ratio of GFH312 following repeat doses in one cohort.
	Exploratory	
	• To explore the pharmacodynamic (PD) effect of single and repeat doses of GFH312 in healthy subjects and explore neurodegenerative diseases relative inflammatory biomarkers.	• Changes of PD biomarker, pRIPK1 (S166) expression levels in PBMC. • Expression changes of cytokine panel including MIP-1α/β, IL-35, IL-23, IL-6, IL-2, IL-33, IL-1b, etc in CSF samples.
Study Design	The study is planned to include approximately 88 healthy subjects and consists of two parts. Additional subjects/cohorts may be enrolled to allow for evaluation of additional dose levels, or further evaluation of a dose level already studied, or a different regimen. Part I: Eight healthy subjects will be enrolled in each of the 7 sequential dose cohorts with a sentinel dosing approach for part Ia. In each dose cohort of Part Ia, two sentinel participants (one active and one placebo) will be enrolled and dosed, and remainder six participants will be dosed 48 hours later upon safety evaluation of the first two sentinel participants by site Investigator. In each of the cohort, eight subjects will be randomized to receive GFH312 or placebo in a 3:1 ratio to examine the safety/tolerability and PK/PD. One of the 7 cohorts will be selected as a food effect cohort (Part Ib) which is two-treatment (i.e. fed vs. fasted), two-period design with a ≥ 7 days washout period. The dose for this food effect cohort will be selected based on attainment of adequate exposure, safety	

	and an adequate safety margin. After washout period, all subjects of food effect cohort will receive a single dose of GFH312 in the fed state. Part II: Eight healthy subjects will be enrolled in each of 4 planned cohorts to examine the safety/tolerability and PK/PD of a repeated dose of GFH312 over 14 days. Subjects will be randomized to receive GFH312 or placebo in a 3:1 ratio. The selection of appropriate doses for Part II will be performed upon consideration of available safety and tolerability and PK data from Part I and/or any preceding repeat dose cohort in Part II. Additional cohorts may be added in Part I or Part II if necessary. The decision to proceed to the next dose level in each cohort will be made jointly by the site investigator and the sponsor. The review data set will, at minimum, consist of: any adverse events, lab test results, flagged vital signs, ECG, and available PK results in a blinded manner. Enrollment of cohorts at the next higher dose level will begin once the preceding dose is deemed safe. The dosing will be halted and progression to the next higher level will be stopped if: • Two or more subjects experience a similar moderate severity (≥CTCAE Grade 2) or clinically significant non-serious adverse event (based on investigator judgement) which has a reasonable possibility of relation to study drug. • One or more subjects experience a severe (≥CTCAE Grade 3) non-serious adverse event (based on investigator judgement) which has a reasonable possibility of relation to study drug. • Two or more subjects with an increase in aminotransferase enzymes (ALT or AST) of greater than 3× ULN or elevation of serum TBL to >2 × ULN. • The Principal Investigator and the Sponsor consider that the number and/or severity and/or system organ class of adverse events justify discontinuation of drug administration within the cohort. • The Sponsor unilaterally requests it. The study will be placed on hold and may be stopped if: • Two or more subjects experience a similar AE which was assessed as severe in intensity (≥CTCAE Grade 3), and are considered as potentially related to the study drug.

	• One subject experiences any SAE suspected to be related to the study drug. • The Principal Investigator and the Sponsor consider that the number and/or severity of adverse events justify discontinuation of the study. • The Sponsor considers that the number and/or severity of AEs, abnormal safety monitoring tests or abnormal laboratory findings justify putting the study on hold. The protocol allows some alteration from the current outlined dosing schedule, but the maximum total daily dose will not exceed the exposure of no observed adverse effect level (NOAEL) in the pre-clinical studies. Any special case should be consciously made by the joint committee based on all available safety and PK data.
Study Population	Healthy subjects.
Number of Subjects (planned)	Part I: 7 cohorts of 8 subjects each (N=56) Part II: 4 cohorts of 8 subjects each (N=32)
Main Inclusion Criteria	• Healthy male and female subjects age 18 to 55 years of age included. • Subjects must weigh at least 50 kg to participate in the study, and must have a body mass index (BMI) within the range of 18-32 kg/m^2 inclusive. BMI = Body weight (kg) / [Height (m)]^2. • At screening, vital signs (systolic and diastolic blood pressure, pulse rate and respiratory rate) will be assessed in the sitting position after the subject has rested for at least three minutes, and again (when required) after three minutes in the standing position. Sitting vital signs should be within the defined ranges. • Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant must use highly effective methods of contraception during intercourse while taking drug and for 30 days after stopping study medication; and sexually active males must use a condom, during intercourse while taking drug and for 30 days after stopping study medication. For WOCBP, the investigator should evaluate the influence of menstrual period on the safety assessments and increasing risks to the subjects.
Main Exclusion	• Abnormal lab test results including: ALT, AST, bilirubin, total cholesterol or triglyceride ≥1.5×ULN; GFR ≤60(mL/min/1.73m^2) estimated by the CKD-

<table>
<tr><td>Criteria</td><td>EPI equation.
• Significant abnormal ECG: Average QTcF > 450 msec, PR > 220 msec, QRS complex > 120 msec.
• Presence of hepatitis B surface antigen (HBsAg) or core antibody (HBcAb), positive hepatitis C antibody with positive HCV RNA, positive human immunodeficiency virus (HIV) antibody, positive tuberculosis test result.
• Significant illness which has not resolved within two (2) weeks prior to initial dosing.
• Recent (within the last three years) and/or recurrent history of autonomic dysfunction (e.g., recurrent episodes of fainting, palpitations, etc.).
• History of significant cardiovascular, respiratory, or neurological disease.
• Use of other investigational drugs at the time of enrollment, or within 5 half-lives after last dosing before enrollment, or within 30 days, or twice the duration of the biological effect of the investigational product, whichever is longer; or longer if required by local regulations.
• Exposure to more than four new chemical entities within 12 months prior to the first dosing day.
• (Part II only) Score ‘yes’ on item 4 or item 5 of the Suicidal Ideation section of the C-SSRS, if this ideation occurred in the past 6 months, or ‘yes’ on any item of the Suicidal Behavior section, except for the ‘Non-Suicidal Self-Injurious Behavior’ (item also included in the Suicidal Behavior section), if this behavior occurred in the past 2 years. Or history of drug abuse or mental disease.</td></tr>
<tr><td>Study Treatment</td><td>GFH312 tablet and matched placebo tablet.
Planned GFH312 doses:
<table><tr><th>Dose</th><th>Planned Dose(mg)</th><th>Increased</th></tr><tr><td>1</td><td>5</td><td>NA</td></tr><tr><td>2</td><td>15</td><td>3×</td></tr><tr><td>3</td><td>45</td><td>3×</td></tr><tr><td>4</td><td>120</td><td>2.7×</td></tr><tr><td>5</td><td>300</td><td>2.5×</td></tr><tr><td>6</td><td>500</td><td>1.7×</td></tr><tr><td>7</td><td>720</td><td>1.4 ×</td></tr></table></td></tr>
</table>

Key Safety Assessments	• Vital signs, Weight, Physical Examination • ECG • Hematology/Chemistry/Coagulation/Urine tests/ Serum troponin, CRP, ANA • Columbia Suicide Severity Rating Scale (Part II) • Adverse event and serious adverse event
Key Pharmacokinetic Assessments	• Plasma, urine and CSF concentration of GFH312 • Derived PK parameters (as seen in secondary endpoints)
Key Pharmacodynamic Assessments	• pRIPK1 S166 • Cytokine panel including MIP-1α/β, IL-35, IL-23, IL-6, IL-2, IL-33, IL-1b, etc
Sample Size Calculation	Eight subjects per each cohort in Part I and Part II. This sample size is typical in first-in-human studies and is deemed sufficient to meet the objective of safety and tolerability assessment. No formal sample size calculation has been performed, and also no power evaluation is provided. At a sample size of 6 subjects per each cohort who will receive GFH312, an adverse event of an underlying occurrence rate of 30% or higher would have ≥88% probability that at least one subject will report an adverse event.

Statistical Analysis	Analysis sets • Full analysis set (FAS) will include all subjects that receive any study drug which includes both GFH312 and placebo. • Safety set (SS) will include all subjects from FAS who have received any study drug (GFH312 or placebo) and had at least one valid post-baseline safety assessment. • The PK analysis set (PKAS) consists of all subjects with at least one sample providing evaluable PK data after drug administration and no protocol deviations with relevant impact on PK data. The number, percentage, and severity of adverse events will be summarized to assess safety and tolerability per treatment group. The number and percentage of subjects with adverse events will be tabulated by system organ class and preferred term. The results of vital sign, ECG, and physical examination will be listed for each subject and summarized by descriptive statistics per treatment group. GFH312 plasma concentration data will be summarized by treatment and scheduled sampling time point. Descriptive statistics of PK parameters will include mean (arithmetic and geometric), standard deviation (SD), coefficient of variation (CV), median, minimum and maximum (except T_{max} for which maximum and median will be presented).
Interim analysis	No formal interim analysis will be performed.

Assessment Schedule

Table 1 Assessment Schedule: Part Ia

Study Phase	Screening	Baseline[1]	Treatment Period				Study Completion	Safety follow-up
Visit Numbers	V1	V2	V3	V4	V5	V6	V7	V8
Study Days	*-28 to -2*	*-1*	*1*	*2*	*3*	*4*	*8±1*	*31±3*
Inform consent	×							
Eligibility criteria	×	×[2]						
Medical history	×	×						
Concomitant medications/therapies			×					
Demography	×							
Physical examination	×[3]	×[3]	×	×	×	×	×	
Alcohol/Drug/Cotinine test	×	×						
Pregnancy test[4]	×	×					×	
Hepatitis/HIV screen	×							
Tuberculosis test[5]	×							
Body height	×							
Body weight	×	×					×	
Body temperature	×	×	×[7]	×	×	×	×	
Blood pressure, pulse rate and respiratory rate [6]	×	×	×[7]	×	×	×	×	
Blood chemistry[12]	×	×	×[7]	×	×	×	×	
Hematology	×	×	×[7]	×	×	×	×	
Coagulation panel	×	×	×[7]	×	×	×	×	
Serum Troponin	×	×	×[7]	×	×	×	×	
ECG and telemetry[8]	×	×	×	×	×	×	×	
Urinalysis	×	×	×[7]	×	×	×	×	

Study Phase	Screening	Baseline[1]	Treatment Period				Study Completion	Safety follow-up
Visit Numbers	V1	V2	V3	V4	V5	V6	V7	V8
Study Days	*-28 to -2*	*-1*	*1*	*2*	*3*	*4*	*8±1*	*31±3*
Drug administration			×					
PK sample collection			See section 8					
PK urine collection			See section 8					
Biomarker blood			See section 8					
Adverse events[9]	×							
Serious adverse events[10]	×							
Safety follow-up call[11]								×

1. Bioanalytical results within 3 days before baseline are acceptable.
2. Eligibility must be confirmed prior to assigning randomization numbers.
3. A complete physical examination will be conducted.
4. For WOCBP only. Serum pregnancy test at screening, and urine pregnancy test at Day-1 and Day 8. A positive urine test needs to be confirmed with a serum test.
5. Conducted at standard practice of the site.
6. BP and pulse should be taken after the subject has been sitting for 3 minutes, as well as after standing for 3 minutes at screening and baseline.
7. This assessment should occur pre-dose. Vital signs assessment should also occur at 2, 6 and 12 hours post-dose.
8. ECG should occur pre-dose and approximately 2 hours post-dose. Additional time points may be added at investigator discretion. Continuous telemetry will be implemented up to six hours post-dose.
9. Adverse Events are to be collected from time of signing informed consent until 30 calendar days after the last administration of GFH312. Details see section 9.
10. Serious Adverse Events are to be collected from time of signing informed consent until 30 calendar days after the last administration of GFH312. Details see section 9.
11. Post study safety contact is via phone or email; information collected will be kept as source document only; Exception: in the case of a reported SAE, follow instructions for SAE reporting outlined in the protocol.
12. It is acceptable to use the result of anti-nuclear antibody (ANA) at the screening visit for eligibility review, and the Day -1 ANA result will be collected as baseline data.

Table 2 Assessment Schedule: Part Ib

Study Phase	Washout	Baseline[1]	Treatment Period				Study Completion	Safety follow-up
Visit Numbers	V1	V2	V3	V4	V5	V6	V7	V8
Study Days	*≥ 7 days*	*-1*	*1*	*2*	*3*	*4*	*8±1*	*31±3*
Eligibility criteria		×[2]						
Medical history		×						
Concomitant medications/therapies			×					
Physical examination		×[3]	×	×	×	×	×	
Alcohol/Drug/Cotinine test		×						
Pregnancy test[4]		×					×	
Body height		×						
Body weight		×					×	
Body temperature		×	×[6]	×	×	×	×	
Blood pressure, pulse rate and respiratory rate[5]		×	×[6]	×	×	×	×	
Blood chemistry[12]		×	×[6]	×	×	×	×	
Hematology		×	×[6]	×	×	×	×	
Coagulation panel		×	×[6]	×	×	×	×	
Serum Troponin		×	×[6]	×	×	×	×	
ECG and telemetry[7]		×	×[6]	×	×	×	×	
Urinalysis		×	×[6]	×	×	×	×	
Meal[8]			×					
Drug administration			×					
PK sample collection			See section 8					
Adverse events[9]	×							
Serious adverse events[10]	×							
Safety follow-up call[11]								×

1. Bioanalytical results within 3 days are not required to repeat and acceptable as baseline data.

2. Eligibility must be confirmed again for the food effect part.
3. A complete physical examination will be conducted.
4. For WOCBP only. Urine pregnancy test at Day-1 and Day 8. A positive urine test needs to be confirmed with a serum test.
5. BP and pulse should be taken after the subject has been sitting for 3 minutes, as well as after standing for 3 minutes at screening and baseline.
6. This assessment should occur pre-dose. Vital signs assessment should also occur at 2, 6 and 12 hours post-dose.
7. ECG should occur pre-dose and approximately 2 hours post-dose. Additional time points may be added. Continuous telemetry will be implemented up to six hours post-dose.
8. Meal should be provided pre-dose following an overnight fast of at least 10 hours.
9. Adverse Events are to be collected from time of signing informed consent until 30 calendar days after the last administration of GFH312.
10. Serious Adverse Events are to be collected from time of signing informed consent until 30 calendar days after the last administration of GFH312.
11. Post study safety contact is via phone or email; information collected will be kept as source document only; Exception: in the case of a reported SAE, follow instructions for SAE reporting outlined in the protocol.
12. It is acceptable to use the result of anti-nuclear antibody (ANA) at the screening visit for eligibility review, and the Day -1 ANA result will be collected as baseline data.

Table 3 Assessment Schedule: Part II

Study Phase	Screening	Baseline[1]	Treatment Period															Study Completion	Safety follow-up
Visit Numbers	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19
Study Days	*-28 to -2*	*-1*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*21±1*	*Last treatment +30 ±3*
Inform consent	×																		
Eligibility criteria	×	×[2]																	
Medical history	×	×																	
Concomitant medications/therapies			×																
Demography	×																		
Physical examination	×[3]	×[3]	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	
Alcohol/Drug/Cotinine test	×	×																	
Pregnancy test[4]	×	×																×	
Hepatitis/HIV screen	×																		
Tuberculosis test[5]	×																		
Body height	×																		
Body weight	×	×							×							×		×	
C-SSRS	×								×							×			
Body temperature[7]	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	
Blood pressure, pulse rate and respiratory rate [6,7]	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	
Blood chemistry[7,12]	×	×	×	×		×			×			×			×	×	×	×	
Hematology[7]	×	×	×	×		×			×			×			×	×	×	×	
Coagulation panel[7]	×	×	×	×		×			×			×			×	×	×	×	
Serum Troponin[7]	×	×	×	×		×			×			×			×	×	×	×	
ECG and telemetry[8]	×	×	×	×		×			×			×			×	×	×	×	
Urinalysis[7]	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	
Drug administration			×	×	×	×	×	×	×	×	×	×	×	×	×	×			

Study Phase	Screening	Baseline[1]	Treatment Period															Study Completion	Safety follow-up
Visit Numbers	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19
Study Days	***-28 to -2***	***-1***	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***21±1***	***Last treatment +30 ±3***
PK sample collection			See section 8																
Biomarker blood			See section 8																
Biomarker CSF		See section 8																	
Adverse events[9]	×																		
Serious adverse events[10]	×																		
Safety follow-up call[11]																			×

1. Bioanalytical results within 3 days before baseline are acceptable.
2. Eligibility must be confirmed prior to assigning randomization numbers.
3. A complete physical examination will be conducted.
4. For WOCBP only. Serum pregnancy test at screening, and urine pregnancy test at Day-1 and Day 21. A positive urine test needs to be confirmed with a serum test.
5. Conducted at standard practice of the site.
6. BP and pulse should be taken after the subject has been sitting for 3 minutes, as well as after standing for 3 minutes at screening and baseline.
7. This assessment should occur pre-dose. Vital signs should also occur at 2, 6 and 12 hours after the first dose.
8. ECG should occur pre-dose and approximately 2 hours post-dose. Additional time points may be added. Continuous telemetry will be implemented up to six hours post-dose.
9. Adverse Events are to be collected from time of signing informed consent until 30 calendar days after the last administration of GFH312.
10. Serious Adverse Events are to be collected from time of signing informed consent until 30 calendar days after the last administration of GFH312.
11. Post study safety contact is via phone or email; information collected will be kept as source documentation only; Exception: in the case of a reported SAE, follow instructions for SAE reporting outlined in the protocol.
12. It is acceptable to use the result of anti-nuclear antibody (ANA) at the screening visit for eligibility review, and the Day -1 ANA result will be collected as baseline data.

Sampling Plan for PK/PD

Part I [1]							
Day	Whole blood			CSF [2]		Urine [4]	
	Time points (hours post-dose)	PK [3]	PD	**Time points** (hours post-dose)	PK [5]	**Time points** (hours post-dose)	PK
1	Pre-dose	x	x			Pre-dose	x
	0.5 ± *10 min*	x				0-12h	x
	1 ± *10 min*	x					
	2 ± *10 min*	x	x				
	4 ± *15 min*	x	x	4 ± *30 min*	x (first 4 subjects)		
	6 ± *15 min*	x					
	8 ± *30 min*	x	x	8 ± *30 min*	x (second 4 subjects)		
	12 ± *30 min*	x	x				
2	24 ± *1 h*	x	x			12-24h	x
3	48 ± *1 h*	x	x			24-48h	x
4	72 ± *1 h*	x				48-72h	x

[1] For food effect period (PartIb), only whole blood for PK is applicable.

[2] Only one cohort will be selected to collect CSF samples. If necessary, the timepoint of CSF sampling will be adjusted according to the plasma data from Part I.

[3] The remaining samples can be used to evaluate the metabolites of GFH312 and to explore potential biomarkers in plasma.

[4] Urine samples collection will start from the third cohort.

[5] The remaining samples can be used to explore potential biomarkers in CSF.

Part II						
Day	**Whole blood**			**CSF** [1]		
	Time points (hours post-dose)	**PK** [2]	**PD**	**Time points** (hours post-dose)	**PK**[3]	**PD**[3]
1	Pre-dose	x	x	Pre-dose		x
	0.5 ± *10 min*	x				
	1 ± *10 min*	x				
	2 ± *10 min*	x	x			
	4 ± *15 min*	x	x			
	8 ± *30 min*	x	x			
	12 ± *30 min*	x	x			
2	24 ± *1 h* (pre-dose)	x	x			
7	Pre-dose	x	x			
13	Pre-dose	x				
14	Pre-dose	x	x			
	0.5 ± *10 min*	x				
	1 ± *10 min*	x				
	2 ± *10 min*	x	x			
	4 ± *15 min*	x	x	4 ± *30min*	x	
	8 ± *30 min*	x	x			
	12 ± *30 min*	x	x			
15	24 ± *1 h*	x	x			

[1] Only one cohort will be selected to collect CSF samples. If necessary, the timepoint of CSF sampling will be adjusted according to the data from Part I.

[2] The remaining samples can be used to evaluate the metabolites of GFH312 and to explore potential biomarkers in plasma.

[3] The remaining samples can be used to explore potential biomarkers in CSF. It is acceptable to collect pre-dose CSF samples on Day -1.

1. Introduction

Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is a master regulator of the cellular decision between pro- survival nuclear factor kappa B (NF-κB) signaling and death in response to a broad set of inflammatory, apoptosis and necroptosis in human diseases[1]. RIPK1 kinase activation has been demonstrated in human pathological samples of autoimmune, inflammatory and neurodegenerative conditions, meanwhile the monogenic and polygenic variants of known RIPK1 regulators also contributed to the inflammatory and neurodegenerative diseases. Inhibition of RIPK1 kinase activity has shown efficacy in a wide range of animal models of human diseases.

Activation of RIPK1 kinase promotes a number of human diseases, such as inflammatory diseases, neurodegenerative diseases, and ischemic diseases etc. In the cleavage-resistant RIPK1-Induced autoinflammatory (CRIA) syndrome, RIPK1 D324 mutation resistant to the caspase 8 cleavage leads to over activation of RIPK1 and is also associated with systemic hyper inflammation[2,3]. In human multiple sclerosis (MS) disease, TNF and TRAIL are elevated in the active brain lesions. The TNF levels in the serum and cerebrospinal fluid (CSF) are correlated with the severity of the lesions and MS disease progression. MS patients' brain has been shown to have defective caspase 8 activation, increased activation and expression of RIPK1, and presence of insoluble aggregates of RIPK1 and RIPK3, which altogether contribute to RIPK1 mediated necroptosis and disease progression in MS patients. Meanwhile, necroptosis markers were observed in human Alzheimer disease (AD) brains and correlated positively with Braak stage and negatively with brain mass and cognition. Moreover, with the increased CSF levels of TNF, active RIPK1 was also found in human AD pathological samples. In human amyotrophic lateral sclerosis (ALS) pathological samples, hallmarks of necroptosis including increased levels of RIPK1, RIPK3 and mixed-lineage kinase domain-like (MLKL), and increased active p-RIPK1 and p-MLKL in both microglia and oligodendrocytes were observed. By inducing necroptosis and inflammation, RIPK1 may be a common mediator of axonal pathology in ALS[4,5].

RIPK1 is an important drug target not only due to its key role in tumor necrosis factor (TNF) signaling responses, but also because of the kinase structure of RIPK1 which is highly amenable for developing small molecule inhibitors. To date, more than six RIPK1 kinase inhibitors have progressed into human clinical trials for the treatment of inflammatory diseases, central nervous system (CNS) indications and infectious disease.

GFH312 is a small molecule targeting RIPK1 being developed by GenFleet for the treatment of inflammatory and neurological indications.

1.1 Background

Detailed background information on the chemistry, pharmacology, toxicology and pharmacokinetics of GFH312 are given in the Investigator's Brochure. The most relevant data for the present study are summarized in the sections below.

1.2 Nonclinical data

1.2.1 In vitro

GFH312 showed the inhibition of human RIPK1 kinase activity with biochemical Half maximal inhibitory concentration (IC50) at 40nM. As widely reported cellular function of RIPK1 kinase inhibitor, GFH312 showed anti-necroptosis effect on human monocyte cell line U937 with IC50 at 12nM, associating with the dose dependent downregulation of pS166 RIPK1 which indicating the RIPK1 kinase activity. Regarding to the cross-species activities, GFH312 showed anti-necroptosis effect on mouse fibroblast cell line L929 and rat bone marrow derived macrophage (BMDM) with IC50 at 4nM and 67 nM respectively.

GFH312 demonstrated good selectivity when tested against a focused kinase panel based on kinase diversity, sequence similarity and target related pathway (N=117). GFH312 at 10μM only exhibited inhibitory effects on 3 kinases, Aurora-B (IC50=967nM), CK1γ1 (IC50=1218nM) and Tie2 (IC50=871nM). GFH312 was also profiled by Eurofins SafetyScreen44™ panel in the in vitro competition binding and uptake assays to assess potential off-target pharmacological activities, across the 44 tests, GFH312 at 10μM exhibited binding inhibition on CCK1 (agonist, 57.4%) and 5-HT2B (agonist, 51.1%).

1.2.2 In vivo

In TNF-α and caspase inhibitor induced systemic inflammatory response syndrome (SIRS) mouse model, the animals treated with single GFH312 oral dosing were protected from hypothermia, and all survived the TNF-α challenge in dose dependent pattern, at 4 hours post model induction, the protection ratios of the body temperature change of the 0.01mg/kg, 0.1mg/kg and 1mg/kg treatment group were 30%, 92% and 95% respectively, and the survival rate of 0.1mg/kg and 1mg/kg treatment GFH312 treatment groups was 100% within 48 hours in this TNF-α induced sepsis shock model. Aligned with these observation, IL-6 in the serum as clinic SIRS prognostic marker, was also down regulated with GFH312 dosing, at 4 hours post model induction, the protection ratios of the IL-6 secretion of the 0.01mg/kg, 0.1mg/kg and 1mg/kg treatment group were 63%, 93% and 95% respectively. In the myelin oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE) mouse model, symptoms of the animals showed good similarities of human multiple sclerosis, including the inflammatory in CNS, demyelination of neurons and motor function impairment. Continuously 26 days oral dosing 1mg/kg GFH312 (twice daily) could ameliorate the model symptoms like body weight loss and paralysis score (Day15, Day18,

D19), meanwhile regulated the immune cell infiltration in spinal cord and cerebellum (Day18); continuum oral dosing 3mg/kg GFH312 (twice daily) could also rescue the model symptoms like body weight loss and paralysis score (Day14-D19) with the significant protection effect on immune cell infiltration and axon demyelination in spinal cord and cerebellum (Day18).

1.2.3 **Safety Pharmacology**

GFH312 inhibited human ether-à-go-go related gene (hERG) channel activity with an IC_{50} of > 2.588 μM (the highest tested concentration in test system), suggesting low potential for prolonging the QT interval. GFH312 had no effects on the central nervous, respiratory, or cardiovascular systems following a single dose of GFH312 up to 300 mg/kg in rats or monkeys. Additionally, GFH312 had no effects on electrocardiography (ECG) in the 28-day good laboratory practice (GLP)-compliant toxicology study in monkeys following once daily oral gavage with up to 300 mg/kg of GFH312 for 28 days.

1.2.4 **Pharmacokinetics**

GFH312 had an acceptable pharmacokinetic profile. GFH312 demonstrated high permeability across the Caco-2 cell monolayer and was a poor or non-substrate for efflux transporters. Following a single intravenous administration of GFH312 (2 mg/kg for rats and 1 mg/kg for monkeys), the mean plasma clearance (CL) was low in rats (4.39 mL/min/kg) and monkeys (3.91 mL/min/kg), the mean apparent volume of distribution (Vd) was 0.842 L/kg for rats and 1.20 L/kg for monkeys. The terminal half-life ($T_{1/2}$) was between 4.55 (monkeys) to 5.39 (rats) hours (h). Oral bioavailability of GFH312 was 48.7% in rats with a single dose of 10 mg/kg and 30.9% in monkeys with a single dose of 5 mg/kg. Toxicokinetics (TK) assessment was performed within the 28-day GLP-compliance toxicology studies in rats and monkeys. After repeated dose of GFH312, T_{max} values were observed between 2.0 and 4.0 hours post-dose in rats and 2.0 and 8.0 hours post-dose in monkeys. The systemic exposure (C_{max} and $AUC_{0\text{-}24h}$) increased less than dose-proportionally in both species. After repeated doses, no significant accumulation was observed at any dose levels in both species, based on $AUC_{0\text{-}24h}$. No significant gender differences of systemic exposure ($AUC_{0\text{-}24h}$) were noted in monkeys, but in rats, females had a higher systemic exposure than males (3.3-4.8 folds).

GFH312 exhibited a high (≥95.0%) binding to plasma proteins and a low partitioning into erythrocytes in the mouse, rat, dog, monkey, and human. GFH312 rapidly distributed throughout the body in rats and was mainly excreted via feces.

Overall, the *in vitro* metabolic profiles of GFH312 in rat, monkey, and human hepatocytes were qualitatively similar, with no unique metabolites in human. GFH312 was moderately metabolized in the mouse, rat, monkey, and human liver microsomes and slowly metabolized in the dog liver microsomes. Cytochrome P450 (CYP450) isoform of CYP3A was the major

metabolic enzyme responsible for formation of its main metabolites. GFH312 showed no obvious inhibition for all seven CYP450 isoforms at tested concentrations, but GFH312 was a potential inducer of CYP1A2 and CYP3A4 at 10 μM.

1.2.5 Non-clinical Safety

Sprague Dawley (SD) rats and cynomolgus monkeys were chosen as the nonclinical test species to assess safety of GFH312 via oral gavage, the intended clinical route of administration. The nonclinical toxicology evaluation included single and repeated dose toxicity and genotoxicity studies.

The single maximum tolerated dose (MTD) was 1000 mg/kg (the highest dose level) in rats and monkeys.

Two pivotal 28-day repeated dose toxicity studies were conducted in rats and monkeys. GFH312 was well tolerated at 300 mg/kg/day, the highest dose level in both species. In rats, no test article-related adverse effects were noted in any dose levels. Therefore, the no observed adverse effect level (NOAEL) of GFH312 in rats was 300 mg/kg/day, the corresponding exposure (expressed by C_{max} and $AUC_{0\text{-}24h}$) following the last dose was 5210 ng/mL and 50300 h*ng/mL for male rats and 13700 ng/mL and 184000 h*ng/mL for female rats. In monkeys, test article-related adverse effects limited to microscopic findings of arteritis/periarteritis in multiple organs and were observed in male monkeys at 300 mg/kg/day. Target organs of toxicity in monkeys included coronary artery, colon adventitia, epididymis, and seminal vesicle. All changes resolved at the end of recovery phase. Consequently, the NOAEL of GFH312 in monkeys was 75 mg/kg/day, the corresponding exposure (expressed by C_{max} and $AUC_{0\text{-}24h}$) following the last dose was 1770 ng/mL and 27400 h*ng/mL for male monkeys, 2910 ng/mL and 45100 h*ng/mL for female monkeys.

GFH312 was non-mutagenic and non-clastogenic as assessed in *in vitro* bacterial reverse mutation (AMES) and chromosomal aberration assays and an *in vivo* rat peripheral blood micronucleus assay.

GFH312 has not been tested for reproductive or developmental toxicity in this stage. However, in the 28-day repeated dose toxicity study in monkeys, arteritis/periarteritis in epididymis and seminal vesicle were observed at 300 mg/kg/day.

1.3 Clinical data

This is the first-in-human study of GFH312 and clinical data is not available yet.

2. Study objectives and endpoints

This study will be a first-in-human assessment of safety/tolerability and pharmacokinetics of GFH312 in healthy subjects after single dose or multiple dose administration in a dose

escalating manner. This study will also explore the effect of food on pharmacokinetics of single dose of GFH312.

Objectives	Endpoints
Primary	
• To assess the safety/tolerability of single (fed and fasted) and repeat doses of GFH312 in healthy subjects	• Incidence of adverse events and serious adverse events, change in laboratory values, ECG, vital signs, physical examinations
Secondary	
• To characterize the PK profile of single doses of GFH312 in healthy subjects	• Plasma concentration of GFH312 and derived PK parameters, including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, CL/F, CL_r, V_d/F and $t_{1/2}$ following single (fed and fasted) doses, where data allow • Cerebrospinal fluid (CSF) concentration and CSF/plasma ratio of GFH312 in one cohort
• To characterize the PK profile of repeat doses of GFH312 in healthy subjects	• Plasma concentration of GFH 312 and derived PK parameters, including AUC_{0-tau}, $C_{max,ss}$, $C_{min,ss}$ T_{max}, CL/F, V_d/F, $t_{1/2}$ and R_{acc} following repeat doses, where data allow • CSF concentration and CSF/plasma ratio of GSH312 following repeat doses in one cohort.
Exploratory	
• To explore the PD effect of single and repeat doses of GFH312 in healthy subjects and explore neurodegenerative diseases relative inflammatory biomarkers.	• Changes of PD biomarker, pRIPK1 (S166) expression levels in PBMC. • Expression changes of cytokine panel including MIP-1α/β, IL-35, IL-23, IL-6, IL-2, IL-33, IL-1b, etc in CSF samples.

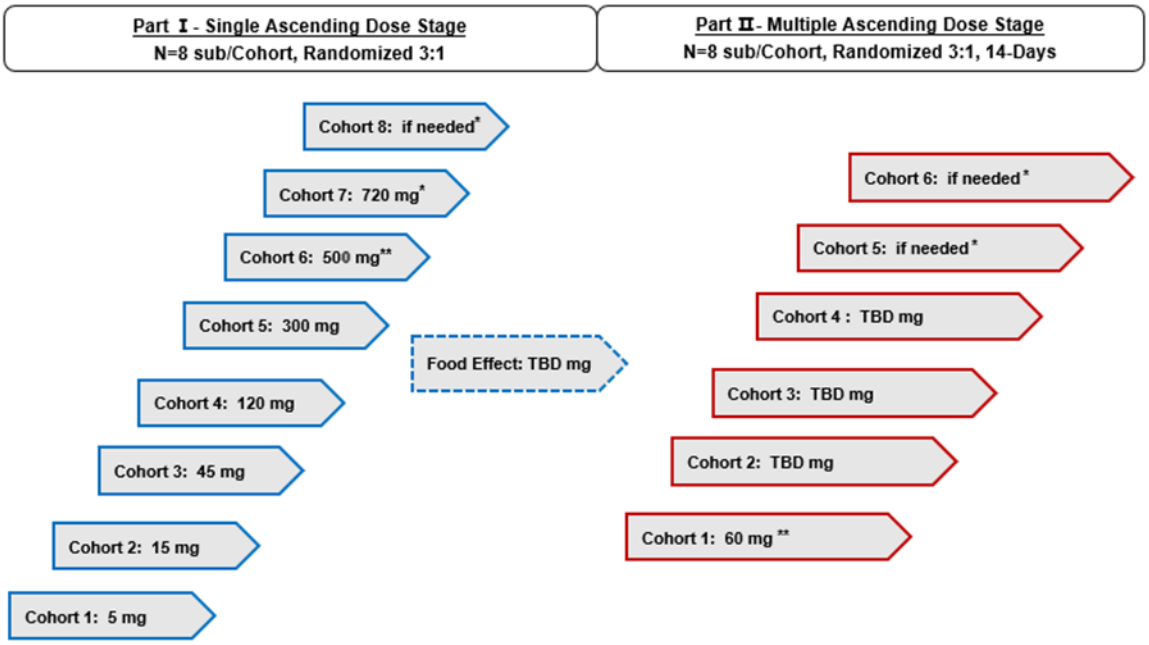
3. Investigational plan

This is a first in human study of single- and multiple-ascending doses and food effect of GFH312.

3.1 Study design

The study will be divided into two parts: Part I and Part II. Part I uses a randomized, double-blinded, placebo-controlled single-ascending dose design (Part Ia) with an additional food effect cohort (Part Ib) in healthy subjects. Part II uses a randomized, double-blinded, placebo-controlled multiple-ascending dose design in healthy subjects.

Figure 1 Study Design



*Higher, intermediate, lower or repeat dose, if needed
** indicated planned dose; doses may be changed based on emerging data

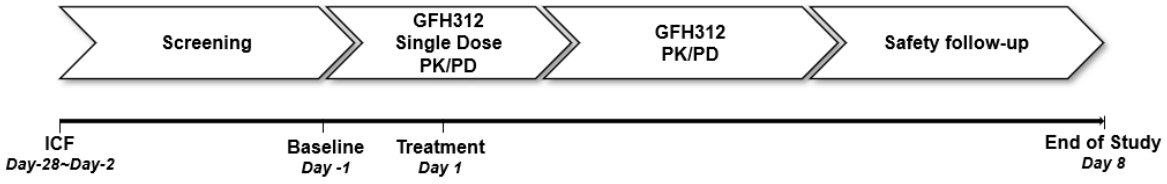
Part Ia: Single-Ascending Dose Stage

Principal Investigator (PI) will need to review all the available safety data to evaluate the starting dose. A sentinel dosing approach will be used in Part Ia cohorts. Two sentinel participants (one active and one placebo) will be enrolled and dosed, and remainder six participants will be dosed around 48 hours later upon safety evaluation of the first two sentinel participants by site Investigator for each single-ascending dose (SAD) cohort.

Subjects who meet eligibility criteria will be admitted for baseline evaluations. All baseline safety evaluation results must be available prior to dosing. Approximately eight subjects per cohort will be randomized to receive a single dose of GFH312 or a matching placebo in a 3:1 ratio. Part Ia is an ascending dose design with 7 planned cohorts (up to 8 cohorts, if needed), using up to 56 subjects. One cohort will receive an additional second dose to study food effect. Doses of 5,15, 45, 120, 300, 500 and 720 mg are envisioned, depending on safety and tolerability finding. Changes in the provisional dose may be considered in any cohort, except cohort 1, based on the available emerging data.

Part Ia of this study is comprised of a 28-day screening period, a baseline evaluation, a treatment period (Day 1 through Day 4) with a single dose of GFH312 on Day 1, PK/PD sampling on Day 1~4, and an end of study (EOS) (Day 8).

Figure 2 Study Scheme: Part Ia Single-Ascending Dose



For each cohort, subjects will be admitted to the study site on Day -1. At this time baseline assessments will be made to confirm eligibility. Subjects will be fasted ≥10 hours overnight between Day -1 and Day 1 and remain fasted 4 hours post-dose on Day 1. On Day 1 subjects will be randomized to receive either the study drug or matching placebo depending on the randomization assignment. Subjects will remain domiciled for 72 hours following dose administration unless PK or safety evaluations suggest a longer period is needed. If at 72 hours post-dose there are any clinically significant abnormalities or safety concerns, the subject will remain domiciled until the investigator deems them fit to be discharged. PK/PD samples will be collected pre-dose and at different time points post-dose, as indicated in the Part Ia Assessment Schedules and Sampling plan for PK/PD. The timing of PK or safety evaluations may be adjusted based on PK assessments from earlier dosed cohorts.

Safety assessments during treatment periods will include ECGs (telemetry), vital signs, hematology, blood chemistry, urinalysis, adverse events and serious adverse events.

Once all Day 4 assessments are completed, subjects may be discharged from the study site. Subjects should return to the site on Day 8 for study completion evaluations. If a subject withdraws consent, the study completion evaluations (including an unscheduled PK blood sample if the subject withdraws after dosing) should be conducted at that time.

Dose escalation

To minimize risk during dose escalation in Part I of the study, all blinded safety assessments and tolerability data up to 72 hours post dose for at least six subjects who received GFH312 or placebo from the preceding cohort must be reviewed and assessed as acceptable. The decision to escalate to the next dose will be made jointly by the Investigator and the Sponsor. The review data set will, at minimum, consist of: any adverse events, lab test results, flagged vital signs, ECG, and available PK results in a blinded manner. Safety will be the primary endpoint determining dose escalation. Enrollment of cohorts at the next higher dose level will begin once the preceding dose is deemed safe. If notable adverse events or safety

concerns are observed at one of the planned doses, a change in the next planned dose level will be considered.

Dose escalation, stopping criteria and/or study termination criteria are presented in Section 7. Possible changes in dose administration include, but are not limited to: administration of an intermediate dose between the current and preceding dose, administration of an intermediate dose between the current and next planned dose, repeated administration of the current dose, or termination of any further escalation of study drug.

Exploratory analysis of interim safety, PK, PD or biomarker data may be conducted to help with refining PK/PD sampling scheme or to guide decision on dosing regimen selection for the additional dose cohorts. For these analyses, the investigator and GenFleet study team will jointly perform a blinded review of safety and tolerability (vital signs, ECGs, adverse events, and laboratory safety parameters).

Additional subjects will be enrolled to replace subjects who are randomized but not receive the study drugs. The randomization number assignment for replacement subject please see section 6.6.

Part Ib: Food Effect

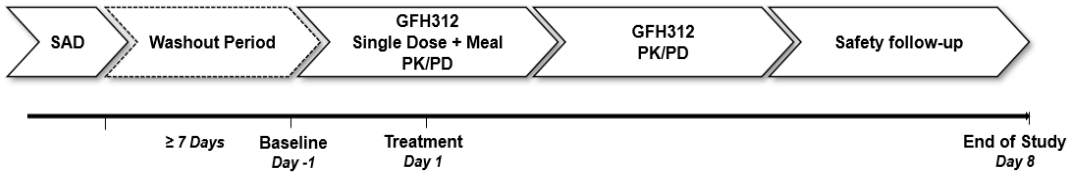
One dosing cohort will undergo a second dosing period to explore the food effect on GFH312 pharmacokinetics.

After a ≥ 7 days washout period, all 8 subjects in this cohort will receive a same dose of GFH312 (no placebo) as that in the first dosing period, and start the High Fat meal about 30 minutes prior to administration of GFH312, consume the meal within 30 minutes

The cohort chosen to participate in Part Ib will be determined by the emerging PK data and safety from previous 2 or 3 cohorts. Subjects will be informed prior to the start of Part Ia that they will likely participate in Part Ib. Upon return to the clinical site for Part Ib, subjects will be re-baselined to determine that they are still eligible to participate in this study.

Part Ib of this study is comprised of a baseline evaluation (Day-1), a treatment period (Day 1 through Day 4) with a single dose of GFH312 on Day 1, PK/PD sampling on Day 1~4 and an EOS (Day 8). Subjects will be admitted to the study site on Day -1. At this time baseline assessments will be made to reconfirm eligibility. Subjects will be fasted ≥10 hours overnight.

Figure 3 Study Scheme: Part Ib Food Effect



On Day 1, a high-fat meal will be administered followed by a single dose of study drug, and assessments will be performed over the following 72 hours. Subjects will remain domiciled for 72 hours following dose administration unless PK or safety evaluations suggest a longer period of time is needed. If at 72 hours post-dose there are any clinically significant abnormalities or safety concerns, the subject will remain domiciled until the investigator deems him fit to be discharged. PK/PD samples will be collected pre-dose and at different time points post-dose, as indicated in the Part Ib Assessment Schedule and Sampling plan for PK/PD. The timing of PK or safety evaluations may be adjusted based on PK assessments from earlier dosed cohorts.

Safety assessments during treatment periods will include ECGs(telemetry), vital signs, hematology, blood chemistry, urinalysis, adverse events and serious adverse events.

Once all Day 4 assessments are completed, subjects may be discharged from the study site. Subjects should return to the site on Day 8 for study completion evaluations. If a subject withdraws consent or is discontinued, the study completion evaluations (including an unscheduled PK blood sample if the subject withdraws after dosing) should be conducted at that time.

No replacement subject is required for part Ib.

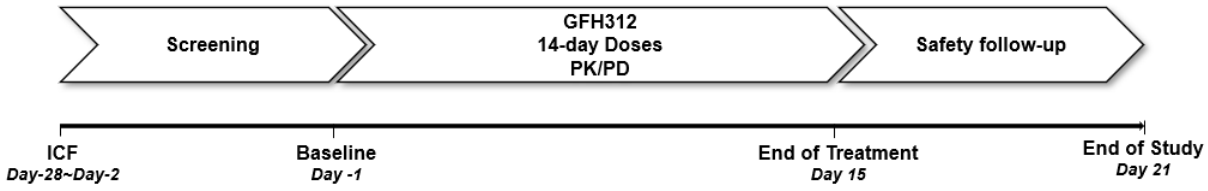
No more than 600ml of blood will be collected from the participant during their study participation in study part Ia and Ib.

Part II: Multiple-Ascending Dose Study

Part II of the study may begin prior to completion of Part I provided the safety of the starting dose has been demonstrated. Subjects who meet eligibility criteria will be admitted for baseline evaluations. All baseline safety evaluation results must be available prior to dosing. Approximately eight subjects per cohort will be randomized to receive GFH312 or matching placebos in a 3:1 ratio for 14 days. This Part is an ascending dose design with 4 planned cohorts (up to 6 cohorts, if needed), using up to approximately 32 subjects. Changes in the planned dose may be considered in any cohort.

Part II of this study is comprised of a 28-day screening period, a baseline evaluation a treatment period (Day 1 through Day 14) and an EOS (Day 21).

Figure 4 Study Scheme: Part II Multiple-Ascending Dose Study



The starting dose for the first multiple-dose cohort will be initiated when at least the first three dosing levels in Part I have been reviewed for safety, tolerability and PK, and have been determined to be safe and tolerable. The provisional starting dose will be 60 mg. Subsequent doses will be guided by the safety assessments as dose escalation continues. The dosing frequency will be determined based on the data from Part Ia but is likely to be Q.D.

For each cohort, subjects will be admitted to the study site on Day -1 and will remain until Day 15. On Day -1 baseline assessments will be made to confirm eligibility. On the evening prior to the dosing day, subjects will be fasted for ≥10 hours overnight and remain fasted for 2 or 4 hours (referred to Section 5.3) post-dose. On Day 1 subjects will be randomized. On days 1 to 14, subjects will receive either the study drug or matching placebo depending on the randomization assignment. Subjects will remain domiciled for 24 hours following the Day 14 dose administration unless safety evaluations suggest a longer period of time is needed. If at 24 hours post-dose there are any clinically significant abnormalities or safety concerns, the subject will remain domiciled until the investigator deems him fit to be discharged. PK/PD samples will be collected pre-dose and at different time points post-dose, as indicated in the Part II Assessment Schedule and Sampling plan for PK/PD.

Safety assessments during treatment periods will include ECGs, vital signs, hematology, blood chemistry, urinalysis, suicide risk evaluation, adverse events and serious adverse events.

Once all Day 15 assessments are completed, subjects may be discharged from the study site. Subjects should return to the site on Day 21 for study completion evaluations. If a subject withdraws consent or is discontinued, the study completion evaluations (including an unscheduled PK blood sample) should be conducted at that time.

For part II of the study, if subjects prematurely discontinue the study, additional replacement subjects may be enrolled at the discretion of the Sponsor and in consultation with the Investigator, in order to guarantee that sufficient subjects (e.g., four subjects receive GFH312) are treated and evaluated at any given dose before escalating to the following dose.

The randomization number assignment for replacement subject please see section 6.6.

Dose escalation

To minimize risk during dose escalation in Part II of the study, all safety assessments and tolerability data up to 24 hours post last dose (Day 14) for at least 6 subjects (GFH312 or placebo) from the preceding cohort must be reviewed and assessed as acceptable. Any dose administered in Part II will require safety data from at least one dosing level higher in Part Ia. The decision to escalate to the next dose will be made jointly by the Investigator and the Sponsor. Safety will be the primary endpoint analyzed to determine dose escalation. If notable adverse events or safety concerns are found at one of the planned doses a change in the next planned dose level will be considered.

Dose escalation, stopping criteria and/or study termination criteria are presented in Section 7.

Possible changes in dose administration include, but are not limited to: administration of an intermediate dose between the current and preceding dose, administration of an intermediate dose between the current and next planned dose, repeated administration of the current dose, or termination of any further escalation of study drug.

Exploratory analysis of interim safety, PK, PD or biomarker data may be conducted to help with refining PK/PD sampling scheme or to guide decision on dosing regimen selection for the additional dose cohorts. For these analyses, the investigator and GenFleet team/CRO presentative will jointly perform a blinded review of safety and tolerability (vital signs, ECGs, adverse events, and laboratory safety parameters).

No more than 500ml of blood will be collected from the participant during their study participation in study part II.

3.2 Rationale of study design

This is a first-in-human, randomized, double-blinded, placebo-controlled, two-part study to assess safety, tolerability, pharmacokinetics, and pharmacodynamics of single- and multiple-ascending doses and food effect of GFH312 in healthy subjects. The study design is typical for FIH studies.

- Healthy subjects: The absence of co-morbid disease in healthy subjects allows for an unbiased assessment of the safety and tolerability of GFH312.
- Randomization: This decreases the chance of an imbalance in subject characteristics between groups, thereby facilitating an unbiased assessment of safety and tolerability.
- Double-blinded: Blinding of subjects, investigators and sponsor allows for an unbiased assessment of subjective readouts such as adverse events. Unblinded information will be limited to the unblinded teams of the site, sponsor and lab staff.
- Placebo comparator: This is a first in human study of a non-cytotoxic drug. The use of a double-blinded placebo arm as a comparator is to provide a comparison group for an unbiased collection of safety and tolerability data.
- Sequential cohorts: This allows for an adequate safety and tolerability assessment prior to the initiation of each ascending dose.
- A pilot study of food effect on GFH312 is included in the single-ascending part to gain early understanding of this effect.

3.3 Rationale of dose/regimen and duration of treatment

3.3.1 Starting dose

GFH312 has exhibited a favorable toxicology and safety pharmacology profile in preclinical studies. The first-in-human (FIH) clinical trial of GFH312 will be conducted in healthy subjects. The selection of starting dose for this trial is largely based on preclinical efficacy and safety considerations.

The relevant species for preclinical safety evaluation were SD rats and cynomolgus monkeys. Based on the 28-day GLP-compliance repeat-dose toxicity studies, the monkey was the more sensitive species for GFH312, thus the starting dose will be calculated based on the monkey toxicology data. The NOAEL in monkeys was 75 mg/kg/day, the corresponding human equivalent dose (HED) was 24.3 mg/kg/day, based on body surface area. Using a safety factor of 10, the proposed FIH starting dose was 145 mg once daily (QD) (based on a body weight of 60 kg/subject).

Meanwhile, the pharmacologic active dose (PAD) in EAE mouse model was 1 mg/kg twice daily (BID), the HED was 9.7 mg QD (total daily dose, based on a body weight of 60 kg/subject). Therefore, the starting dose for GFH312 is selected as 5 mg for the FIH study following FDA and EMA guidelines in adults healthy subjects.

3.3.2 Planned dose levels

Part I

Seven dose levels of GFH312 are planned as indicated in below table:

Table 4 Part I dose levels

Cohort	Dose level of GFH312 (mg)	Increased
1	5	NA
2	15	3×
3	45	3×
4	120	2.7×
5	300	2.5×
6	500	1.7×
7	720	1.4 ×

Part II

Four dose levels of GFH312 will be determined according to available safety and PK data from Part I

3.3.3 Dose regimen

According to the PK and/ or PD data in Part I, a 14-day QD or BID dosing schedule will be determined for Part II. The 14-day regimen is based on similar drug development experience. Considering emerging data, other alternative dosing regimens could be explored[6].

3.4 Purpose and timing of interim analyses/design adaptations

Prior to each step of dose escalation, the Investigator, GenFleet team, and CRO team will jointly perform a review of all safety and tolerability data (vital signs, ECGs, adverse events and laboratory safety parameters) from the preceding dose cohort(s).

The intended dose to evaluate the food effect on the pharmacokinetics of GFH312 will be determined by PK and safety analysis of first 2-3 SAD cohorts.

Exploratory analysis of interim safety, PK, PD or biomarker data may be conducted to help with refining PK/PD sampling scheme or to guide decision on dosing regimen selection for the additional dose cohorts. For these analyses, the investigator and GenFleet will jointly perform a blinded review of safety and tolerability (vital signs, ECGs, adverse events, and laboratory safety parameters).

3.5 Risks and benefits

There is no benefit expected for subjects participating in this study. The risk to subjects in this trial will be minimized by adherence to the inclusion/exclusion criteria, close clinical monitoring and stopping rules.

Non-clinical toxicology data

Test article-related effects that have been identified in non-clinical studies include serum chemistry changes and histopathological changes of arteritis/periarteritis in multiple organs. In all cases these changes were reversible after cessation of the study drug as detailed in the investigator's brochure and summarized below.

Slight serum chemistry changes were observed in 28-day toxicology studies, including slight increases of total bilirubin in rats at 100 and 300 mg/kg/day and slight increases of creatinine in monkeys at ≥ 15 mg/kg/day. These changes were not considered adverse effects as they were small in magnitude, absent of relevant histopathology findings, and reversible at the end of recovery phase. Test article-related adverse effects of arteritis/periarteritis in multiple organs were observed in male monkeys at 300 mg/kg/day. Target organs of toxicity in monkeys included coronary artery, colon adventitia, epididymis, and seminal vesicle. All changes resolved at the end of recovery phase.

Unknown risks

Since this is the FIH study with GFH312 there may be unknown risks which may be serious and unforeseen.

4. Population

The study population will be comprised of male and female healthy subjects. Approximately 88 eligible subjects will be enrolled to participate in the study, and more subjects may be needed for reasons of screening failure or discontinuation.

The investigator must ensure that all subjects being considered for the study meet the following eligibility criteria. No additional criteria should be applied by the investigator, in order that the study population will be representative of all eligible subjects.

Subject selection is to be established by checking through all inclusion/exclusion criteria. A relevant record of the eligibility criteria must be stored with the source documentation at the study site.

Deviation from **any** entry criterion excludes a subject from enrollment into the study.

4.1 Inclusion Criteria

Subjects eligible for inclusion in this study have to fulfill **all** of the following criteria:

1. Written informed consent must be obtained before any assessment is performed.
2. Healthy male and female subjects age 18 to 55 years of age included.
3. Subjects must weigh at least 50 kg to participate in the study, and must have a body mass index (BMI) within the range of 18-32 kg/m^2 inclusive. BMI = Body weight (kg) / $[Height(m)]^2$.
4. At screening, vital signs (systolic and diastolic blood pressure, pulse rate and respiratory rate) will be assessed in the sitting position after the subject has rested for at least three minutes, and again (when required) after three minutes in the standing position. Sitting vital signs should be within the following ranges:
   - oral body temperature between 35.0-37.5 °C or tympanic temperature between 35.5-37.5 °C.
   - systolic blood pressure, 90-140 mm Hg
   - diastolic blood pressure, 40-90 mm Hg
   - pulse rate, 40-90 bpm
   - respiratory rate, 12-25 times per minute

   Subjects should be excluded if their standing vital signs (relative to sitting) show findings which, in the opinion of the Investigator, are associated with clinical manifestation of postural hypotension (i.e., absence of any other cause). The Investigator should carefully consider enrolling subjects with either a > 20 mm Hg decrease in systolic or a >10 mm Hg decrease in diastolic blood pressure, accompanied by a > 20 bpm increase in heart-rate (comparing standing to sitting results).
5. Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant must use highly effective methods of contraception during intercourse while taking drug and for 30 days after stopping study medication; and

sexually active males must use a condom, during intercourse while taking drug and for 30 days after stopping study medication.

For WOCBP, the investigator should evaluate the influence of menstrual period on the safety assessments and increased risks to the subjects.

6. Able to communicate well with the investigator, to understand and comply with the requirements of the study.

4.2 Exclusion Criteria

Healthy subjects fulfilling **any** of the following criteria are not eligible for inclusion in this study:

1. Any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of drugs, or which may jeopardize the subject in case of participation in the study. The Investigator should make this determination in consideration of the subject's medical history and/or clinical or laboratory evidence of any of the following:
    - Inflammatory bowel disease, peptic ulcers, gastrointestinal (including rectal) bleeding, irritable bowel syndrome.
    - Major gastrointestinal tract surgery such as gastrectomy, gastroenterostomy, cholecystectomy or bowel resection.
    - Pancreatic injury or pancreatitis.
    - Liver disease or liver injury as indicated by abnormal liver function tests:
        i. Any single parameter of ALT, AST, γ-GT, alkaline phosphatase or serum bilirubin must not exceed 1.5×ULN.
        ii. Any elevation above ULN of more than one parameter of ALT, AST, alkaline phosphatase or serum bilirubin will exclude a subject from participation in the study.
    - Total cholesterol or triglyceride ≥1.5×ULN.
    - History or presence of impaired renal function as indicated by clinically significantly abnormal creatinine and/or urea values(GFR ≤60(mL/min/1.73m^2, estimated by the CKD-EPI equation), or abnormal urinary constituents.
    - Evidence of urinary obstruction or difficulty in voiding.
2. Hemoglobin levels below the lower limit of normal (LLN) as set by the laboratory.
3. An elevated C-reactive protein (CRP) outside of the normal reference range and has clinical significance, or above 10 mg/L.

4. A positive anti-nuclear antibody (ANA) above 1:160 titer.
5. A positive Tuberculosis test.
6. A history of clinically significant ECG abnormalities, or any of the following ECG abnormalities:
   - PR > 220 msec
   - QRS complex > 120 msec
   - QTcF > 450 msec
7. History of immunodeficiency diseases, including a positive test for HIV antibody.
8. Chronic infection with Hepatitis B (HBV) or Hepatitis C (HCV). A positive HBV surface antigen (HBsAg) test, or if standard local practice, a positive HBV core antibody test, excludes a subject. Subjects with a positive HCV antibody test should have HCV RNA levels measured. Subjects with positive (detectable) HCV RNA should be excluded.
9. Infections requiring parenteral antibiotics within the 6 months prior to Screening.
10. History of any venous thromboembolism, TIA, intracranial hemorrhage, neoplasm, arteriovenous malformation, vasculitis, bleeding disorder, coagulation disorders or screening blood tests that indicate altered coagulability (platelet count, APTT, PT, etc.).
11. History of significant cardiovascular, respiratory, or neurological disease.
12. Significant illness which has not resolved within two (2) weeks prior to initial dosing.
13. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years, regardless of whether there is evidence of local recurrence or metastases.
14. Recent (within the last three years) and/or recurrent history of autonomic dysfunction (e.g., recurrent episodes of fainting, palpitations, etc.).
15. Known family history or known presence of long QT syndrome, or concomitant use of agents known to prolong the QT interval unless they can be permanently discontinued for the duration of study.
16. History of hypersensitivity to any of the study treatments or excipients (e.g., lactose monohydrate) or to drugs of similar chemical classes; and history of anaphylaxis or other significant allergy in the opinion of the Investigator.
17. Donation or loss of 400 ml or more of blood within eight (8) weeks prior to initial dosing, or longer if required by local regulation.
18. Plasma donation (>400 mL) within 14 days prior to first dosing.

19. Use of any prescription drugs, herbal supplements (including *Silybum marianum* and *Valeriana officinalis*), within two weeks prior to initial dosing, and/or over-the- counter (OTC) medication, dietary supplements (vitamins included) within one weeks prior to initial dosing. If needed, (i.e., an incidental and limited need) multivitamins and occasional paracetamol are acceptable at lower dose, but must be documented in the CRF.
20. Smokers of more than two per week. Subjects who were unable to abandon smoking during the study period. Subjects whose test is positive for Urine cotinine during screening will be excluded.
21. History of drug abuse or unhealthy alcohol use[#] within the 12 months prior to dosing, or evidence of such abuse as indicated by the laboratory assays conducted during screening.

    [#]Unhealthy alcohol use is defined as a history of, or current alcohol misuse/abuse, defined as 'Five or more drinks on the same occasion on each of 5 or more days in the past 30 days.
22. History of recreational cannabis use within four weeks prior to dosing, or evidence of such use as indicated by the laboratory assays conducted during screening. This exclusion criterion applies even if cannabis use is legalized where the site is located. Any prescribed, medicinal use of cannabis is to be handled according to the prescription drug usage criteria defined above.
23. Participant is unwilling to refrain from strenuous exercise (including weightlifting) from 7 days prior to admission to the site until completion of the final Follow-up visit.
24. With a plan to receive vaccination with a live vaccine within 4 weeks prior to the first dosing or within 4 weeks of the last dosing; With a plan to receive COVID-19 vaccination within 2 weeks prior to the first dosing or within 1 week of the last dosing.
25. Exposure to any significantly immune suppressing drug (including experimental therapies as part of a clinical trial) within the 4 months prior to screening or 5 half-lives, whichever is longer.
26. Use of other investigational drugs at the time of enrollment, or within 5 half-lives of enrollment, or within 30 days, or twice the duration of the biological effect of the investigational product, whichever is longer; or longer if required by local regulations.
27. Exposure to more than four new chemical entities within 12 months prior to the first dosing day.
28. (Part II only) Score 'yes' on item 4 or item 5 of the Suicidal Ideation section of the C-SSRS, if this ideation occurred in the past 6 months, or 'yes' on any item of the Suicidal Behavior section, except for the 'Non-Suicidal Self-Injurious Behavior' (item also included in the Suicidal Behavior section), if this behavior occurred in the past 2 years. Or history of drug abuse or mental disease.

No additional exclusions may be applied by the investigator, in order to ensure that the study population will be representative of all eligible subjects.

If necessary, laboratory testing may be repeated on one occasion (as soon as possible) prior to randomization, to rule out any laboratory error.

5. Restrictions for study subjects

For the duration of the study, the subjects should be informed and reminded of the restrictions outlined in this section.

5.1 Contraception requirements

Sexually active males should be reminded of the requirement to wear a condom to prevent delivery of investigational drug via seminal fluid to their partner as study treatment may involve unknown risks to the fetus if pregnancy were to occur. Males who are continuously not heterosexually active are exempt from contraceptive requirements.

Women of childbearing potential (WOCBP) should use highly effective contraception method. WOCBP are defined as any female who has experienced menarche and who has not undergone ≥ 1 year of spontaneous amenorrhea, serum FSH >40 mIU/mL, and≥ 6 weeks since surgical bilateral oophorectomy or ≥6 weeks since complete hysterectomy. Menopause is defined as 12 months of amenorrhea in the absence of other biological causes.

Investigators will advise on the use of an adequate methods of contraception, which is defined as use of a condom by the male partner **combined with** use of a highly effective method of contraception by the female partner. A highly effective method of contraception is one that has a failure rate of < 1% when used consistently and correctly. Male participants must inform their female partners who are WOCBP of the contraceptive requirements of the protocol and are expected to adhere to using contraception with their partner.

Highly effective methods of contraception are listed below:

- Hormonal methods of contraception including oral contraceptives containing combined estrogen and progesterone, a vaginal ring, injectable and implantable hormonal contraceptives, intrauterine hormone-releasing system (e.g., Mirena) and progestogen-only hormonal contraception associated with inhibition of ovulation
- Nonhormonal intrauterine device (IUD)
- Bilateral tubal occlusion
- Vasectomized subject/partner with documented azoospermia 90 days after procedure, if that partner is the sole sexual partner.

If there is any question that the subject will not reliably comply, the subject should not be entered or continue in the study. Male subjects should be informed that if a female partner

becomes pregnant while he is enrolled in the study, contact with the female partner will be attempted to request her consent to collect pregnancy outcome information.

5.2 Prohibited treatment

Except for medication which may be required to treat adverse events, no medication or herbalremedies other than study drugs will be allowed from the first dosing until all of the Study Completion evaluations have been conducted.

According to preclinical pharmacokinetic studies, GFH312 is mainly metabolized by CYP3A. In order to avoid potential drug-drug interactions, strong inducers and inhibitors of drug-metabolizing enzyme CYP3A4/5 are prohibited. The typical drugs include but not limited to the ones below.

Table 5 Some typical inducers and inhibitors of CYP3A4/5

Interaction mechanism	Drug
Strong inducer	Carbamazepine, Phenytoin, Rifampicin
Strong inhibitor	Clarithromycin, Ritonavir, Ketoconazole, Itraconazole

Should a subject have an *incidental and limited* need for a medication to be taken within the restricted pre-dose timeframe (e.g., acetaminophen for a headache, antibiotic prophylaxis prior to dental surgery, etc.), the sponsor should be advised, as administration of any concomitant medication *may* require the subject to be replaced. Decisions regarding replacements will be discussed with the sponsor on a case-by-case basis. Use of salicylate or other Nonsteroidal Anti-inflammatory Drugs (NSAIDS) are prohibited. Administration of acetaminophen (no more than 500 mg in a single dose and no more than 1000 mg per day) is acceptable, but must be documented.

5.3 Dietary, fluid and other restrictions

During recruitment, screening/informed consent review, and baseline visit, the subjects will be informed and reminded of the following restrictions:

- Subjects will lie on their back for at least two hours and be monitored for signs of neurological problems after lumbar puncture for CSF sampling.
- No strenuous physical exercise (e.g., weight training, aerobics, football) for 7 days before dosing until after Study Completion evaluation.
- No alcohol for 48 hours before dosing until after Study Completion evaluation.
- No cruciferous vegetables (Brussels sprouts, broccoli, cabbage, cauliflower) may be ingested for 7 days prior to dosing until after Study completion evaluation.

- No grapefruit or grapefruit juice is to be consumed for at least 7 days prior to the first dosing and during the entire study period.
- Intake of xanthine (e.g., caffeine) containing food or beverages must be limited to a total of 340 grams/day 48 hours before dosing. An equivalent caffeine intake may be consumed between foods and beverages (i.e., coffee, tea, soda, chocolate) while the subjects are domiciled. If a deviation occurs, it must be noted in the CRF.
- Only thoroughly cooked meat will be allowed for four days prior to baseline and throughout the study (all beef must be 'well done' so that no red is evident).

All subjects will be fasted (i.e., no food and liquid except water) for at least 10 hours prior to administration of study drug. On the dosing day of Part Ib, a High Fat meal will be provided 0.5 hours prior to administration. In Part Ia, Part Ib and Day 1 and 14 of Part II, subjects will continue to be fasted for at least 4 hours post dosing. On the rest dosing days of Part II, subjects will be fasted for at least 2 hours post dosing and followed with a standard breakfast. No fluid intake apart from the fluid given at the time of drug intake is allowed from 2 h before until 2 h after dosing.

Subjects can drink water ad libitum, however, to ensure adequate hydration for urine collection (if applicable), subjects should have a fluid intake of at least 240 ml every 4 hours during waking hours on Day 1 (Part I) in addition to fluid taken with meals and medication.

A copy of the diet (including high fat tolerance test) with content and nutritional information (amount of protein, carbohydrates, fat and calories for each meal) will be provided to the Sponsor prior to study start upon request.

Smoking is not allowed during any of the domiciled portions of the study.

6. Treatment

6.1 Investigational drug

- Name: GFH312 tablets
- Strength: 5 mg, 20 mg, 100 mg
- Dosage form/Route: Tablets; Oral
- Excipients: lactose monohydrate, microcrystalline cellulose, low-substituted hydroxypropyl cellulose, colloidal silicon dioxide, magnesium stearate
- Packaging: High-density polyethylene (HDPE) capped with child resistant polypropylene (PP) caps, and 1g silica gel desiccant. Each bottle contains 30 units of GFH312 tablets.
- Storage condition: 20 - 25°C (68 - 77°F); excursions permitted to 15° - 30°C (59 - 86°F)
- Shelf-life: Tentative 24 months

6.2 Protocol requested treatment

The investigational drug, GFH312, 5 mg, 20 mg, 100 mg, and matching placebo tablets will be prepared by GenFleet and supplied to an unblinded pharmacist at the site as open labeled bulk medication.

6.3 Treatment arms

Part Ia Single Ascending Dose

Subjects will be assigned to one of the following treatment arms:

- Single doses of GFH312 which are estimated to be: 5 mg, 15 mg, 45 mg, 120 mg, 300 mg, 500 mg or 720 mg.
- Single dose of placebo

Part Ib Food Effect

One cohort from Part Ia will be asked to return to participate in an additional food effect study. Subjects will receive a single dose of GFH312 that will be determined during Part Ia along with a high fat meal.

Part II Multiple Ascending Dose

- Fourteen daily doses of GFH312 which are estimated to be 60 mg or a higher dose. The dosing levels for each cohort for Part II are to be determined (TBD) based on the PK, PD, and safety/tolerability data collected during Part I. Q.D. dosing is planned, but BID may be considered based upon the available data.
- Fourteen daily doses of placebo

6.4 Treatment assignment

Randomization numbers will be assigned in ascending, sequential order to eligible subjects. The investigator will enter the randomization number on the CRF.

The randomization numbers will be generated using the following procedure to ensure that treatment assignment is unbiased and concealed from subjects and investigator staff. A randomization list will be produced by an independent (unblinded) statistician who is independent from the blinded study team and will be provided to the unblinded pharmacist at the site according to usual practice.

6.5 Treatment blinding and emergency breaking of assigned treatment code

This is a double-blinded study.

The placebo will be supplied by GenFleet. The identity of the treatments will be concealed because the placebo and investigational medicinal products are identical in appearance, odor, packaging, labeling and schedule of administration. Drug product will be supplied in bulk,

so an unblinded pharmacist who is independent of the blinded study team will be required in order to maintain the blind. This unblinded pharmacist will receive a randomization list with the appropriate treatment allocation numbers. Appropriate measures must be taken by the unblinded pharmacist to ensure that the study team remains blinded throughout the course of dose administration and the remainder of the study.

Randomization data are strictly confidential and should be accessible only to authorized personnel.

The bioanalyst will receive a copy of the randomization list to facilitate analysis of the samples. The bioanalyst will provide the sample data to the team under blinded conditions. Both the pharmacist and bioanalyst will keep this information confidential until clinical database lock. The study pharmacokineticist may evaluate PK parameters from this blinded concentration data at any time if needed.

Unblinding a single subject at site for safety reasons (necessary for subject management) will occur via an emergency system in place at the site. Emergency unblinding must only be undertaken when it is essential to treat the subject safely and efficaciously. Most often, study treatment discontinuation and knowledge of the possible treatment assignments are sufficient to treat a study subject who presents with an emergency condition. A set of sealed emergency code break envelopes will be provided by Novotech and will be available in a secure storage location. Pharmacy staff are responsible for the receipt and return of the code break envelopes and for accountability. All code break envelopes must be retained until the end of the study and returned to sponsor. They must be stored in a secure place but be accessible to the investigator 24 hours per day in case of emergency. In the event that the Investigator considers an AE/SAE to be of such severity as to require specific knowledge of the identity and dose of the relevant product, the Investigator may break the study code for that participant only. Unless the participant is in immediate danger, the Investigator should discuss unblinding with the Sponsor or appropriate study personnel before breaking the study code. A record, including date, time, name and signature of the person opening the envelope and reason, must be made both on the opened envelope and in the participant's medical record. The opened annotated code break envelope and completed Study Unblinding Report Form must be retained. The sponsor or the designee should be informed promptly of all unblinding events.

6.6 Treating the subject

6.6.1 Subject numbering

Screening number

Each subject screened is assigned a unique screening number. Screening numbers will be assigned sequentially in the format 'SNNN' (i.e., S001, S002, S003, etc.). If the subject is a screen fail, then the screening number may not be re-used.

Randomization

If the subject is deemed eligible for the study and will commence dosing, a randomization number will be assigned in format 'RPCrNN' with:

- P: Study part with '1' for SAD and '2' for MAD
- C: Cohort (i.e., 1-6)
- r: Replacement (e.g., 1st replacement for R11001 would be R11101, 2nd replacement would be R11201)
- NN: Subject number assigned sequentially from 01 to 08 per cohort.

Once assigned to a subject, a randomization number will not be reused.

The randomization number becomes the definitive subject number as soon as a subject receives the first dose of the respective study treatment. There should be a source document maintained at the site which links the screening number to the randomization number (once assigned).

Subjects will be assigned randomization numbers according to Table 6.

For part Ia of the study, additional subjects will be enrolled to replace subjects who are randomized but not receive the study drugs. For part II of the study, if subjects prematurely discontinue the study, additional replacement subjects may be enrolled at the discretion of the Sponsor and in consultation with the Investigator, in order to guarantee that sufficient subjects (e.g., four subjects receive GFH312) are treated and evaluated at any given dose before escalating to the following dose.

. It is allowed up to two replacements for the same subject. Once a subject has been confirmed as eligible, a prescription will be signed by the Medical Team and supplied to the Pharmacist. The Pharmacist will assign IP for that subject based on the cohort being dosed and dispense medication in accordance with the randomization list. Replacement subjects will be assigned randomization numbers according to Table 6.

Table 6 Treatment Assignment Numbering

	Cohort	Randomization Numbers	Replacement Randomization Numbers
Part I	1	Sentinels: R11001-R11002 Remainder: R11003-R11008	1st Replacement: Sentinels: R11101-R11102 Remainder: R11103-R11108 2nd Replacement: Sentinels: R11201-R11202 Remainder: R11203-R11208
	2	Sentinels: R12001-R12002 Remainder: R12003-R12008	1st Replacement: Sentinels: R12101-R12102 Remainder: R12103-R12108 2nd Replacement: Sentinels: R12201-R12202 Remainder: R12203-R12208
	3	Sentinels: R13001-R13002 Remainder: R13003-R13008	1st Replacement: Sentinels: R13101-R13102 Remainder: R13103-R13108 2nd Replacement: Sentinels: R13201-R13202 Remainder: R13203-R13208
	4	Sentinels: R14001-R14002 Remainder: R14003-R14008	1st Replacement: Sentinels: R14101-R14102 Remainder: R14103-R14108 2nd Replacement: Sentinels: R14201-R14202 Remainder: R14203-R14208
	5	Sentinels: R15001-R15002 Remainder: R15003-R15008	1st Replacement: Sentinels: R15101-R15102 Remainder: R15103-R15108 2nd Replacement: Sentinels: R15201-R15202 Remainder: R15203-R15208
	6	Sentinels: R16001-R16002 Remainder: R16003-R16008	1st Replacement: Sentinels: R16101-R16102 Remainder: R16103-R16108 2nd Replacement: Sentinels: R16201-R16202 Remainder: R16203-R16208
	7	Sentinels: R17001-R17002 Remainder: R17003-R17008	1st Replacement: Sentinels: R17101-R17102 Remainder: R17103-R17108 2nd Replacement: Sentinels: R17201-R17202 Remainder: R17203-R17208
Part II	1	R21001-R21008	1st Replacement: R21101-R21108 2nd Replacement: R21201-R21208
	2	R22001-R22008	1st Replacement: R22101-R22108 2nd Replacement: R22201-R22208
	3	R23001-R23008	1st Replacement: R23101-R23108 2nd Replacement: R23201-R23208

4	R24001-R24008	1st Replacement: R24101-R24108 2nd Replacement: R24201-R24208

6.6.2 Dispensing the study treatment

The investigational medicinal products, GFH312 tablets and matching placebo will be provided by GenFleet and supplied to the Investigator as open labeled bulk medication.

For preparation of the study medication, a copy of the randomization list will be sent to the pharmacist / technician.

Appropriate documentation of the subject specific dispensing process must be maintained.

Medication labels will comply with legal requirements of the country where the study is performed and be printed in the local language. Storage conditions for the study drug will be included on the medication label. More details about investigational drug handling please refer to separate document.

6.6.3 Handling of study treatment

Investigational treatment must be received at the study site by a designated person, handled and stored safely and properly, and kept in a secured location to which only the Investigator and designated staff have access. Upon receipt, the study drugs should be stored according to the instructions specified on the label.

Storage conditions must be adequately monitored, and appropriate temperature/humidity logs maintained as Source data. The Investigator must maintain an accurate record of the shipment and dispensing of study drug in a drug accountability ledger.

All drug supplies are to be used only for this protocol and not for any other purpose. Unless specifically instructed by GenFleet, the Investigator must not destroy any drug labels, or any partly used or unused drug supply.

Only after receiving a written authorization by GenFleet, the Investigator/designee will send all the unused and partly used drug supplies as well as the empty containers to the address provided at the time of authorization for destruction.

6.6.4 Instructions for prescribing and taking study treatment

Study medication will be administered by the study center personnel with 240 ml of water.

Each subject's mouth must be checked to ensure that the medication was swallowed. Subjects must be instructed not to chew the medication, but to swallow it whole.

All dosages prescribed and dispensed to the subject and all dose changes during the study must be recorded on the Dosage Administration Record CRF.

6.6.5 Permitted dose adjustments and interruptions of study treatment

Any changes or deviation from the dosing instructions that occur must be recorded on the Dosage Administration Record CRF page.

Dose adjustments of any kind are not permitted in Part II. If a subject in Part II misses two or more than two doses, the investigators will inform the sponsor to confirm if the subject should be discontinued.

6.6.6 Rescue medication

Not applicable.

6.6.7 Concomitant treatment

All prescription medications, over-the-counter drugs and significant non-drug therapies (including physical therapy) administered or taken within the timeframe defined in the entry criteria prior to the start of the study and during the study, must be recorded on the Concomitant medications/ Significant non-drug therapies section of the CRF.

Medication entries should be specific to trade name, the single dose and unit, the frequency and route of administration, the start and discontinuation date and the reason for therapy.

7. Study completion and discontinuation

7.1 Study completion

Study completion is defined as when the last subject completes their Study Completion visit, and any repeat assessments associated with this visit have been documented and followed-up appropriately by the Investigator, or in the event of an early study termination decision, the date of that decision.

All randomized and/or treated subjects should have a safety follow-up call conducted 30 days after last administration of study treatment. The information collected is kept as source documentation. All SAEs reported during this time period must be reported as described in Section 9. Documentation of attempts to contact the subject should be recorded in the source documentation.

7.2 Discontinuation of study treatment

Discontinuation of study treatment for a subject occurs when study treatment is stopped earlier than the protocol planned duration. Discontinuation of study treatment can be decided by either the subject or the investigator.

The study treatment must be discontinued under the following circumstances:

- Subject/guardian decision - subjects may choose to discontinue study treatment for any reason at any time.

- The investigator believes that continuation would negatively impact the safety of the subject or the risk/benefit ratio of trial participation.
- CTCAE Grade 3 or higher adverse event unless it can be conclusively shown that AE is not related to study treatment.
- Any protocol deviation that results in a significant risk to the subject's safety.

7.2.1 Withdrawal of informed consent

Subjects may voluntarily withdraw consent to participate in the study for any reason at any time.

Withdrawal of consent from the study is defined as when a subject:

- Does not want to participate in the study anymore and
- Does not want any further visits or assessments and
- Does not want any further study related contacts and
- Does not allow analysis of already obtained biologic material.

In this situation, the investigator must make every effort (e.g. telephone, e-mail, letter) to determine the primary reason for the subject's decision to withdraw his/her consent and record this information.

Study treatment must be discontinued and no further assessments conducted, and the data that would have been collected at subsequent visits will be considered missing.

Further attempts to contact the subject are not allowed unless safety findings require communicating or follow-up.

7.2.2 Lost to follow-up

For subjects whose status is unclear because they fail to appear for study visits without stating an intention to discontinue or withdraw, the investigator should show 'due diligence' by documenting in the source documents steps taken to contact the subject, e.g. dates of telephone calls, registered letters, etc. A subject cannot be formally considered lost to follow-up until his/her scheduled end of study visit would have occurred.

7.3 Cohort stopping rules and Dose escalation decision points

Dose escalation, as well as continued dosing within any given cohort will be placed on hold and may be stopped based on full review of all available clinical safety data and discussion with the Investigator if any of the following occur:

- Two or more subjects experience a similar moderate severity (≥CTCAE Grade 2) or clinically significant non-serious adverse event (based on investigator judgement) which has a reasonable possibility of relation to study drug.

- One or more subjects experience a severe (≥CTCAE Grade 3) non-serious adverse event (based on investigator judgement) which has a reasonable possibility of relation to study drug.
- Two or more subjects with an increase in aminotransferase enzymes (ALT or AST) of greater than 3× ULN or elevation of serum TBL to >2 × ULN.
- The Principal Investigator and the Sponsor consider that the number and/or severity and/or system organ class of adverse events justify discontinuation of drug administration within the cohort.
- The Sponsor unilaterally requests it.

Safety reviews will be conducted in a blinded manner jointly between medically qualified representatives of the Sponsor and Investigator and a joint decision will be made. If a dose level is identified to be intolerable, the preceding dose level will be defined as the maximum tolerated dose.

The severity of adverse events will be graded by the study site Investigator (or the designee) based on clinical judgment and captured in the CRF AE page as per CTCAE 5.0. This information will be used to quantify events that may lead to subject's discontinuation or stopping dose escalation.

If any AE occurs in the sentinel subjects, dosing in remaining subjects in this cohort will be paused until the Investigator and Sponsor discuss the safety and tolerability data from this subject. Dosing can only resume when safety and tolerability of GFH312 has been confirmed.

Unblinding a single subject at site for safety reasons (necessary for subject management) will occur via an emergency system in place at the site. More details see Section 6.5.

7.4 Study stopping rules

The study will be placed on hold and may be stopped based on full review of all available clinical safety data and discussion with the Investigator if any of the following occur:

- Two or more subjects experience a similar AE which was assessed as severe in intensity (≥CTCAE Grade 3), and are considered as potentially related to the study drug.
- One subject experiences any SAE suspected to be related to the study drug.
- The Principal Investigator and the Sponsor consider that the number and/or severity of adverse events justify discontinuation of the study.
- The Sponsor considers that the number and/or severity of AEs, abnormal safety monitoring tests or abnormal laboratory findings justify putting the study on hold.

7.5 Early study termination by the sponsor

The study can be terminated by GenFleet at any time for any reason. This may include reasons related to the benefit/ risk assessment of participating in the study, practical reasons (including slow enrollment), or for regulatory or medical reasons. Should this be necessary, subjects must be seen as soon as possible and treated as a prematurely discontinued subject. The investigator may be informed of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the subject's interests. The investigator will be responsible for informing IRBs/IECs of the early termination of the trial.

8. Procedures and assessments

8.1 Assessments schedule

The full Assessment Schedule is presented at the end of the synopsis section, above.

- Table 1 Assessment Schedule: Part Ia
- Table 2 Assessment Schedule: Part Ib
- Table 3 Assessment Schedule: Part II

Subjects should be seen for all visits/assessments as outlined in the assessment schedule or as close to the designated day/time as possible.

Subjects who prematurely discontinue the study for any reason should be scheduled for a visit as soon as possible, at which time all of the assessments listed for the final visit will be performed.

Every effort will be made to take the pharmacokinetic and pharmacodynamics samples at the protocol specified time. Other assessments, e.g., ECG, vital signs etc. will be taken prior to / after the pharmacokinetic and pharmacodynamics samples. For the timepoint of pre-dose, the window with -2h for ECG and vital signs is acceptable; For the other timepoints, such as post-dose 2h, the window with ±20min for ECG and vital signs is acceptable.

When the following assessments are scheduled to be performed at the same time-point, the order of assessments will be as follows: vital signs, then ECG, then blood collection. If subject is unable to provide a safety urine sample at requested time, it is acceptable to collect urine sample before or after the PK/PD sample.

8.2 Informed consent procedures

Eligible subjects may only be included in the study after providing written (witnessed, where required by law or regulation), IRB (Institutional Review Board)/ Independent Ethics Committee (IEC)-approved informed consent.

Informed consent must be obtained before conducting any study-specific procedures (e.g. all of the procedures described in the protocol). The process of obtaining informed consent must be documented in the subject source documents.

Information about common side effects already known about the investigational drug can be found in the Investigator's Brochure (IB). This information will be included in the subject informed consent and should be discussed with the subject during the study as needed.

8.3 Subject screening and Baseline assessments

After written informed consent is provided, subject demographic and baseline characteristic data will be collected on all subjects, including date of birth, age, sex, race, predominant ethnicity. Relevant medical history/current medical conditions data will also be collected until signature of informed consent.

Investigators have the discretion to record abnormal test findings on the medical history CRF, if in their judgment, the test abnormality occurred prior to the informed consent signature.

It is permissible to re-screen a subject if s/he fails the initial screening; however, each case must be discussed and agreed with the Sponsor on a case-by-case basis. Information of screening failures will be recorded in the subject source documents.

8.3.1 Hepatitis screen, HIV screen

All subjects will be screened for HIV, Hepatitis B and C.

8.3.2 Alcohol test, Drug screen, Urine cotinine

All subjects will be screened for substances of abuse and cotinine. Drug screen will include at minimum: amphetamines, barbiturates, cocaine, opiates, tetrahydrocannabinol and benzodiazepines.

8.3.3 Tuberculosis testing

All subjects will be screened for tuberculosis conducted at standard practice of the site.

8.3.4 Pregnancy test

For WOCBP only. Serum pregnancy test at screening, and urine pregnancy test at other required visits. A positive urine test needs to be confirmed with a serum test.

8.4 Safety assessments

8.4.1 Physical examination

A complete physical examination will be conducted at screening and baseline including the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, vascular and neurological. If indicated based on medical history and/or symptoms, rectal, external genitalia, breast, and/or pelvic exams may be performed. During the study physical examination details will be based on investigator’s dissection.

Information for all physical examinations must be included in the source documentation at the study site and will not be recorded the CRF about the details. Significant findings that are

present prior to informed consent are included in the Relevant Medical History CRF. Significant findings observed after informed consent signature which meet the definition of an Adverse Event must be appropriately recorded on the Adverse Event CRF.

8.4.2 Vital signs

Vital signs include body temperature, blood pressure, pulse measurements and respiratory rate. After the subject has been sitting for 3 minutes, with back supported and both feet placed on the floor, systolic and diastolic blood pressure will be measured using an automated validated device, with an appropriately sized cuff. In case the cuff sizes available are not large enough for the subject's arm circumference, a sphygmomanometer with an appropriately sized cuff may be used.

Standing systolic and diastolic blood pressure and pulse should also be obtained at Screening and Baseline, after the subject has been standing for 3 minutes.

If vital signs are out-of-range at screening and initial baseline, the investigator may obtain two additional readings, so that a total of up to three (3) consecutive assessments are made, with the subject seated quietly for approximately five (5) minutes preceding each repeat assessment.

At least the last reading must be within the ranges provided above in order for the subject to qualify.

8.4.3 Height and weight

Height in centimeters (cm) in indoor clothing, but without shoes will be measured. Body weight (to the nearest 0.1 kilogram [kg] in indoor clothing, but without shoes) will be measured. Body mass index (BMI) will be calculated using the following formula:

- Body mass index (BMI) = Body weight (kg) / [Height (m)]^2

8.4.4 Clinical safety laboratory assessments

Protocol required safety laboratory assessments are specified below. The subjects need to be fasted for 8-12 hours prior to collection of safety blood. Assessment schedule details when each assessment is to be performed.

Laboratory Assessments	**Parameters**			
Hematology	Platelet Count		*RBC Indices*:	*WBC count with Differential:*
	RBC Count		MCV	Neutrophils
	Hemoglobin		MCH	Lymphocytes
	Hematocrit			Monocytes
				Eosinophils
				Basophils
Clinical Chemistry	Urea	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose	Calcium	Alkaline phosphatase	Albumin

	Cholesterol	Triglyceride	GGT	C-reactive protein (CRP)
				Anti-nuclear antibody (ANA)
Coagulation Panel	PT, INR, APTT			
Urinalysis	• Specific gravity • pH, leukocytes, nitrite, protein, glucose, ketones, bilirubin, urobilinogen and blood • Microscopic examination (if blood or protein detected ⩾1+)			
Cardiac biomarker	• Serum Troponin			

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the Investigator (e.g., SAE or AE) the results must be recorded in the CRF.

8.4.5 Electrocardiogram (ECG) and Telemetry

A standard 12 lead ECG will be performed, and continuous telemetry will be implemented up to six hours post-dose. Interpretation of the tracing must be made by a qualified physician and documented on the ECG section of the CRF. Each ECG tracing should be kept in the source documents at the study site. Clinically significant abnormalities should be recorded on the relevant medical history/Current medical conditions CRF page prior to signing informed consent and on adverse events page after signing the informed consent.

Clinically significant findings must be discussed with the sponsor.

The CRF will contain:

- date and time of ECG
- heart rate
- PR interval
- QT interval (QTcF)
- QRS duration

8.5 Pharmacokinetics

8.5.1 Sample collection

Part I:

- Blood

For all cohorts, whole blood sample will be collected for pharmacokinetics (PK) analyses of GFH312. Refer to Table 7 for PK sampling timepoints.

For the food effect cohort, following a high-fat diet, whole blood sample will be collected at the same timepoints in Table 7.

For food effect period (Part Ib), only whole blood for PK is applicable.

- Cerebrospinal fluid (CSF)

One cohort will be selected to collect CSF samples (approximately 5 mL/timepoint) for concentrations detection of GFH312. Refer to Table 7 for CSF sampling timepoints. The timepoints of CSF sampling will be adjusted according to the plasma PK data of the first cohort in Part I, if necessary.

The time window of CSF sampling is ±30 min.

- Urine

Starting from the third cohort, urine samples will be collected before dosing and during the periods of 0-12, 12-24, 24-48, and 48-72 h post-dose, for concentrations detection of GFH312.

Table 7 Blood and CSF sampling plan for Part I

Day	Whole blood[1]		CSF[2]		Urine	
1	Pre-dose	X			Pre-dose	X
	0.5 ± 10 min	X			0-12 h	X
	1 ± 10 min	X				
	2 ± 10 min	X				
	4 ± 15 min	X	4 ± 30min	X (first 4 subjects)		
	6 ± 15 min	X				
	8 ± 30 min	X	8± 30min	X (second 4 subjects)		
	12 ± 30 min	X				
2	24 ± 1 h	X			12-24 h	X
3	48 ± 1 h	X			24-48 h	X
4	72 ± 1 h	X			48-72 h	X

[1] The remaining samples can be used to evaluate the metabolites of GFH312 and to explore potential biomarkers in plasma.

[2] The remaining samples can be used to explore potential biomarkers in CSF.

Part II:

- Blood

For all cohorts, whole blood sample will be collected for pharmacokinetics (PK) analyses of GFH312. Refer to Table 8 for PK sampling timepoints.

- CSF

One cohort will be selected to collect CSF samples (approximately 5 mL/timepoint) for concentrations detection of GFH312. Refer to Table 8 for CSF sampling timepoints.

Table 8 Blood and CSF sampling plan for Part II

Day	Whole blood[1]		CSF[2]	
1	Pre-dose	X		
	0.5 ± 10 min	X		
	1 ± 10 min	X		
	2 ± 10 min	X		
	4 ± 15 min	X		
	8 ± 30 min	X		
	12 ± 30 min	X		
2	24 ± 1 h (pre-dose)	X		

7	Pre-dose	X		
13	Pre-dose	X		
14	Pre-dose	X		
	0.5 ± 10 min	X		
	1 ± 10 min	X		
	2 ± 10 min	X		
	4 ± 15 min	X	4 ± 30 min	X[3]
	8 ± 30 min	X		
	12 ± 30 min	X		
15	24 ± 1 h	X		

[1] The remaining samples can be used to evaluate the metabolites of GFH312 and to explore potential biomarkers in plasma.

[2] The remaining samples can be used to explore potential biomarkers in CSF.

[3] If necessary, the timepoint of CSF sampling will be adjusted according to the data from Part I.

Instructions for collection and handling of biological samples will be provided by the Sponsor. The actual date and time of each sample will be recorded.

If operationally possible, an unscheduled extra PK blood sample may be collected whenever the investigator considers necessary.

8.5.2 **Bioanalysis method**

Plasma, CSF, and urine samples will be analyzed by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, with the lowest limit of quantification (LLOQ) of 10 ng/mL or lower. In the statistical summary, the concentration value lower than LLOQ will be treated as 0 and expressed as 'BQL' in the data list.

This study is the first-in-human trial of GFH312, plasma and urine samples will be tested for metabolite identification analysis to investigate whether there are unique metabolites in human.

Refer to the laboratory manual for the processing method of bio-samples.

8.5.3 **Calculation of pharmacokinetic parameters**

The non-compartmental model will be used to calculate the PK parameters of GFH312 after single and multiple doses. The main PK parameters include AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, CL/F, V_d/F, CL_r and $t_{1/2}$ for single dose (fed and fasted), while AUC_{0-tau}, $C_{max,ss}$, $C_{min,ss}$, T_{max}, CL/F, V_d/F, $t_{1/2}$ and R_{acc} for multiple doses. CSF/plasma concentration ratio will also be calculated.

The drug concentration data below LLOQ before T_{max} will be marked as 0 when calculating PK parameters, and BQL after T_{max} will not be included in the calculation of PK parameters. The linear trapezoidal method will be used to calculate AUC. During the calculation of $t_{1/2}$, the data of at least 3 time points after T_{max} should be used. $T_{1/2}$ will not be reported if the adjusted R^2 value of the regression analysis is <0.75.

8.6 Pharmacodynamics

8.6.1 PBMC biomarkers

In this study, human whole blood for individual healthy subjects will be collected to isolate PBMCs (peripheral blood mononuclear cells) for pharmacodynamic (PD) biomarker assessment. The PD marker evaluated is phospho-RIPK1(pRIPK1) S166 expression levels. In Part I, PMBCs will be harvested at pre-dose, and 2, 4, 8, 12, 24, 48 hours after dosing. In Part II, at both Day 1 and Day 14, PMBCs will be harvested at pre-dose, 2, 4, 8, 12, 24 hours after dosing, respectively. At Day 7, PBMCs will be harvested only at pre-dose.

Table 9 Blood sampling plan for PD

Day	**Time points** (hours post dosing)	**Whole blood**
Part I		
1	Pre-dose	X
	2 ± 10 min	X
	4 ± 15 min	X
	8 ± 30 min	X
	12 ± 30 min	X
2	24 ± 1 h	X
3	48 ± 1 h	X
Part II		
1	Pre-dose	X
	2 ± 10 min	X
	4 ± 15 min	X
	8 ± 30 min	X
	12 ± 30 min	X
2	24 ± 1 h (pre-dose)	X
7	Pre-dose	X
14	Pre-dose	X
	2 ± 10 min	X
	4 ± 15 min	X
	8 ± 30 min	X
	12 ± 30 min	X
15	24 ± 1 h	X

In order to better define the PD profile, the timing of the sample collection may be altered based on emergent data. The number of samples/blood draws and total blood volume collected will not exceed those stated in the protocol.

8.6.2 CSF biomarkers

In this study, expression changes of a cytokine panel including but not limited to MIP-1α/β, IL-35, IL-23, IL-6, IL-2, IL-33 and IL-1b from CSF samples will be evaluated for individual subjects between pre-dosing and post-treatment. In Part II, the pre-dosing samples will be collected at Day 1 before dosing in the morning. The post-treatment samples are the PK leftover samples (minimum requirement 200μl, 4h post dosing at Day 14). The time points may be adjusted to align with PK sampling if necessary.

Table 10 CSF sampling plan in Part II for biomarker study

Day	Time points (hours post-dose)	CSF[1]
1	Pre-dose	X
14	4 (± 30 min)	X[2]

[1] The remaining samples can be used to explore the other potential biomarkers in CSF. It is acceptable to collect pre-dose CSF samples on Day -1.

[2] This CSF sampling timepoint for biomarker study will be aligned with CSF sampling for PK. If the sampling timepoint for PK changes, the one for biomarker study will change accordingly.

Subjects will lie on their back for at least two hours and be monitored for signs of neurological problems after lumbar puncture for CSF sampling.

8.7 Other assessments

8.7.1 Suicidality assessment

GFH312 has not been shown observations associated with an increased risk of suicidal thinking or behavior in the preclinical studies. As it is considered to be a CNS-active drug., GenFleet considers it important to monitor for such events in the multiple-dose part of this study per Food and Drug Administration (FDA) guidance.

The Columbia-Suicide Severity Rating Scale (C-SSRS) is a questionnaire that prospectively assesses Suicidal Ideation and Suicidal Behavior. The C-SSRS must be administered at specified visits, and any unscheduled visits at the investigator's discretion. More instructions see section 9.7.

8.7.2 Meal record

Meal records are required for domiciled meals on the days indicated in the Assessment schedule.

9. Adverse event reporting

9.1 Adverse events (AEs)

9.1.1 Definition of AEs

An AE is any untoward medical occurrence in a study subject administered a product; the event need not necessarily have a causal relationship with the treatment or usage. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. The time period for collecting AEs for each subject begins from the time the subject provides written informed consent, until the end of safety follow-up (30 calendar days after the last administration of GFH312). Examples of AEs include, but are not limited to:

- Aggravation of existing (prior to enrollment of study) medical conditions/ diseases (including exacerbation of symptoms, signs, laboratory abnormalities);
- Any new occurrence of AEs: any adverse medical conditions that newly occur (including symptoms, signs, newly diagnosed diseases);
- Abnormal laboratory test findings of clinical significance.

9.1.2 Severity Assessment

For severity of each AE, refer to the 5-grade scale developed from NCI-CTCAE v5.0. For AEs not included in NCI-CTCAE v5.0, the severity of each AE will be graded based on the general guidelines in Table **11.**

Table 11 The Judgment Standard on Severity of AE

Grade	Clinical Description of Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL. Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL. Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE.

Abbreviations: AE=adverse event, ADL=Activities of Daily Living.

9.1.3 Causality Assessment

An investigator's causality assessment is the determination of whether there exists a reasonable possibility that GFH312 caused or contributed to an AE, such as whether the

occurrence of AE follows a reasonable temporal sequence from administration of GFH312, the properties of GFH312, toxicological and pharmacological effects of GFH312, the use of concomitant medications, subjects' underlying diseases, medical history, family history and dechallenge and rechallenge reactions, etc. Generally the facts (evidence) or arguments to suggest a causal relationship should be provided.

The causality of AE with GFH312 administration will be assessed as 'related' and 'unrelated'.

9.2 Serious adverse events (SAEs)

9.2.1 Definition of SAE

An SAE is any untoward medical occurrence at any dose that:

- Results in death;
- Is life-threatening (immediate risk of death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity (substantial disruption of the ability to conduct normal life functions);
- Results in congenital anomaly/birth defect;
- Is considered to be medically significant or requires intervention to prevent one of the outcomes listed above.

9.2.2 Hospitalization

Hospitalization is defined as any initial admission (even less than 24h) in a hospital or equivalent healthcare facility, or any prolongation of an existing admission.

Hospitalization does not include the following:

- Rehabilitation facilities;
- Nursing homes;
- General emergency admission;
- Same-day surgeries (as outpatient/same-day/ambulatory procedures).

Hospitalization or prolongation of hospitalization in the absence of a precipitating clinical AE is not in itself an SAE. Examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new AE or with a worsening of the preexisting condition (e.g., for workup of a persistent pretreatment laboratory abnormality);
- Administrative admission (e.g., for yearly physical examination);
- Protocol-specified admission during a study (e.g., for a procedure required by the study protocol);

- Optional admission not associated with a precipitating clinical AE (e.g., for elective cosmetic surgery);
- Preplanned treatments or surgical procedures. These should be noted in the baseline documentation for the entire protocol and/or for the individual subject;
- Admission exclusively for the administration of blood products.

Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the reporting period should be reported if the AE requirements are met, and the resulting appendectomy should be recorded as treatment of the AE.

9.2.3 SAE Reporting Requirements

All SAEs should be reported to Genfleet Safety by the investigator on the 'Clinical Trial Serious Adverse Event (SAE) Report Form' (signed and dated) within 24h of awareness, regardless of whether this is an initial report or a follow-up report. The investigator should also report the SAEs to relevant organizations in a timely manner as required by local regulations. In countries or regions outside China, SAEs should be reported in accordance with the most stringent standards in accordance with local regulations.

SAEs occurring in a subject after the active collection period has ended are reported to GenFleet Safety if the investigator becomes aware of them; at a minimum, all SAEs that the investigator believes have at least a reasonable possibility of being related to GFH312 must be reported to GenFleet Safety. The detailed record content of SAE should include symptoms, severity, causality with GFH312, time of onset, time of treatment, action taken with GFH312, follow-up time and method as well as outcome.

The sponsor's email to receive safety reports (for SAEs or exposure during pregnancy) in this study: drugsafety@genfleet.com.

9.3 Liver safety monitoring

To ensure subject safety and enhance reliability in determining the hepatotoxic potential of an investigational drug, a standardized process for identification, monitoring and evaluation of liver events has to be followed.

9.3.1 Definition of liver events

Please refer to Appendix 2 for complete definitions of liver events.

9.3.2 Follow-up for liver events

Every liver event as defined in Appendix 2 should be followed up by the investigator or designated personal at the trial site, as summarized below and detailed in Appendix 2.

- Repeating liver chemistry tests (ALT, AST, TBL, PT/INR, ALP and G-GT) to confirm elevation within 48-72 hours. These liver chemistry repeats should be performed using the local laboratory used by the site. Repeat laboratory test results must be entered on the appropriate unscheduled local laboratory CRF.
- If the initial elevation is confirmed, close observation of the subject will be initiated, including consideration of treatment interruption if deemed appropriate.
- Discontinuation of the investigational drug (refer to Section 7.2), if appropriate.
- Hospitalization of the subject if appropriate.
- Causality assessment of the liver event.
- Thorough follow-up of the liver event should include:
    i. If total bilirubin is elevated > 2 × ULN, fractionation into direct and indirect bilirubin is required. To rule out muscular origin of transaminase elevations, CPK should be measured along with liver chemistry tests. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic. Retesting should be continued up to resolution.
    ii. Obtaining a more detailed history of symptoms and prior or concurrent diseases.
    iii. Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
    iv. Exclusion of underlying liver disease.
    v. Imaging such as abdominal US, CT or MRI, as appropriate.
    vi. Obtaining a history of exposure to environmental chemical agents.
    vii. Considering gastroenterology or hepatology consultations.

All follow-up information, and the procedures performed must be recorded as appropriate in the CRF. If live event is associated with any SAE, please refer to 9.2.3 SAE reporting section.

9.4 Renal safety monitoring

Every renal laboratory trigger or renal event must be followed up by the investigator or designated personnel at the trial site. Recommended follow-up assessments are listed in Appendix 3.

Whenever a renal event is identified, a detailed subject history and examination are indicated to identify, document and potentially eliminate risk factors that may have initiated or contributed to the event:

- Blood pressure assessment (after 5 min rest, with an appropriate cuff size)
- Signs and symptoms such as fever, headache, shortness of breath, back or abdominal pain, dysuria, hematuria, dependent or periorbital edema

- Changes in blood pressure, body weight, fluid intake, voiding pattern, or urine output
- Concomitant events or procedures such as trauma, surgical procedures, cardiac or hepatic failure, contrast media or other known nephrotoxin administration, or other potential causes of renal dysfunction, e.g., dehydration, hemorrhage, tumor lysis

All follow-up information, and the procedures performed must be recorded as appropriate in the CRF. If renal event is associated with any SAE, please refer to 9.2.3 SAE reporting section.

9.5 Reporting of Overdose

Overdose is defined as any dose accidentally or intentionally exceeding the planned dose. If an overdose results in an AE, the AE must be recorded on the AE CRF. If overdose is associated with any SAE, please refer to 9.2.3 SAE reporting section.

9.6 Reporting of Pregnancy

If a female subject is pregnant during the study, the subject must discontinue GFH312 administration immediately and withdraw from the study; if a male subject's partner becomes pregnant during the study, the subject can continue the study. The investigator must report the pregnancy to the sponsor within 24 hours of awareness using the Pregnancy Report/Follow-up Form, and to the relevant institutions in a timely manner per local requirement.

The investigator should follow up on the pregnancy outcome (e.g., any early termination of pregnancy, or a live birth) until 1 month after delivery, and notify the sponsor and the ethics committee (or other organizations as required by local regulations) of the pregnancy outcome. If the pregnancy outcome meets the criteria for an SAE (i.e., ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly), the investigator should follow the procedures for reporting SAEs.

If a subject experiences an SAE during pregnancy, both the pregnancy and the SAE should be reported following the procedures for reporting SAEs, please refer to 9.2.3 SAE reporting section.

9.7 Prospective suicidality assessment

In study part II, the C-SSRS, which uses a semi-structured interview to probe subject responses, will be administered by an individual who has received training and certification in its administration. At the first study visit, the 'baseline/screening' version of the C-SSRS, will be administered. This version assesses Suicidal Ideation and Suicidal Behavior during the subject's lifetime and during a predefined period. At Day 7 and Day 14 visits, the 'since last visit' version will be administered.

If, at any time after screening and/or baseline, the score is 'yes' on item 4 or item 5 of the Suicidal Ideation section of the C-SSRS or 'yes' on any item of the Suicidal Behavior section,

the subject must be referred to a mental health care professional for further assessment and/or treatment. The decision on whether the study treatment should be discontinued is to be taken by the investigator in consultation with the mental health professional to whom the subject is referred.

In addition, if a subject answers 'yes' to one of the questions in the Suicidal Behavior section, an SAE must be reported. All events of 'Non-Suicidal Self-Injurious Behavior' (question also included in the Suicidal Behavior section) should be reported as AEs and assigned the appropriate severity grade.

9.8 Collection and Follow-Up of AEs/SAEs

AE collection starts from the time that the subject signs the informed consent to participate in the study and continues until the end of safety follow-up (30 calendar days after the last administration of GFH312) (refer to Table 12).

At each study visit, the investigator should assess whether any subjective AEs have occurred. Each AE must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline and/or to Grade≤1, or there is a satisfactory explanation for it (such as lost to follow-up, death), or the event is finally determined to be unrelated to GFH312 or study procedures by the end of safety follow-up. Every effort should be made to ensure that the subject achieves the best outcome and definite causality assessment is obtained.

All AEs should be recorded in the AE page of the eCRF in details, including: event term, start and stop date, severity, seriousness, the causal relationship between the event and investigational drugs, actions taken with GFH312, as well as final results and outcomes.

Table 12 AE/SAE Pregnancy Collection

Duration	Collection Request
From the subject signs the Informed Consent Form (ICF) until the end of safety follow-up.	All AE/SAEs/Pregnancies Pregnancies: collection starts from the first administration of the investigational product.
Outside the above period	SAEs which are considered related to the investigational product.

Abbreviations: AE=adverse event, SAE=serious adverse event.

10. Data review and database management

10.1 Site monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, a GenFleet representative or the delegation will review the protocol and CRFs with the investigators and their staff. During the study, the field monitor will visit the site regularly to check the completeness of subject's records, the accuracy of entries on the CRFs, the adherence to the

protocol and to Good Clinical Practice, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits.

The investigator must maintain source documents for each subject in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information on CRFs must be traceable to these source documents in the subject's file. The investigator must also keep the original informed consent form signed by the subject.

The investigator must give the monitor access to all relevant source documents to confirm their consistency with the CRF entries. No information in source documents about the identity of the subjects will be disclosed.

10.2 Data collection

Information to be originally captured in the source documentation and reviewed shall include details of the subject visit and the protocol required assessments performed as a part of these visits, Medical History, and Concomitant Medications. The source data will be verified by the study clinical research associate (CRA).

All data should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification.

10.3 Database management

All data should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification.

Contract Research Organization (CRO) staff, working on behalf of GenFleet, review the data entered into the CRFs by investigational staff for completeness and accuracy and instruct the site personnel to make any required corrections or additions. Queries are sent to the investigational site using an electronic data query. Designated investigator site staff is required to respond to the query and confirm or correct the data. Concomitant medications entered into the database will be coded using the World Health Organization (WHO) Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

At the conclusion of the study, the occurrence of any emergency code breaks will be determined after return of all code break reports and unused drug supplies to GenFleet. The occurrence of any protocol deviations will be determined. After these actions have been completed and the database has been declared to be complete and accurate, it will be locked

and the treatment codes will be unblinded and made available for data analysis. Any changes to the database after that time can only be made by approval from GenFleet management.

11. Data analysis

The two parts of this study will be reported separately. Placebo subjects will be pooled within the single and multiple dose cohorts. For food effect bioavailability cohort, data will be divided into two parts, i.e., fasted and fed.

11.1 Analysis Sets

For all analysis sets, subjects will be analyzed according to the study treatment received.

- Full analysis Set (FAS) consists of all subjects who have received any study drug which includes GFH312 and placebo.
- Safety set (SS) will include all subjects from FAS who have received any study drug (GFH312 or placebo) and had at least one valid post-baseline safety assessment.
- The PK analysis set (PKAS) consists of all subjects with at least one sample providing evaluable PK data after drug administration and no protocol deviations with relevant impact on PK data.

11.2 Safety Evaluation

The primary objective of this study is to evaluate the safety and tolerability of single and multiple doses of GFH312 in healthy subjects. The primary variables include all safety assessments such as AEs, vital signs, ECG, safety laboratory, and physical exam.

Adverse Events (AEs)

All information obtained on adverse events will be displayed by treatment and subject. The number and percentage of subjects with adverse events will be tabulated by system organ class, preferred term and severity with a breakdown by treatment. A subject with multiple adverse events within a system organ class or preferred term is only counted once. For food effect bioavailability cohort, an adverse event starting in one period and continuing into the next period is counted only in the onset period.

Vital Signs

Summary of vital signs and change from baseline by treatment and visit/time point will be provided. All vital signs data will be listed by treatment and subject.

ECG and telemetry

All ECG and telemetry data will be listed by treatment, subject and visit/time point. Summary statistics will be provided by treatment and visit/time point.

Safety Laboratory

Summary of all laboratory parameters and change from baseline by treatment and visit/time point will be provided. All laboratory data will be listed by treatment and subject.

Physical Exam

Summary of subjects experiencing normal/abnormal physical examination findings by treatment and visit/time point. A by-subject listing of physical examinations will be provided.

11.3 Pharmacokinetic Analysis

11.3.1 Pharmacokinetic Concentrations

GFH312 plasma，CSF and urine concentration data will be listed by treatment, subject, and nominal sampling time point, and actual sampling time. Descriptive summary statistics will be provided by treatment and nominal sampling time point. Summary statistics will include mean (arithmetic and geometric), standard deviation (SD), coefficient of variation (CV) (arithmetic and geometric), median, minimum and maximum. The individual and mean plasma concentration-time plots will be provided in linear scale and semi-logarithmic scale.

11.3.2 Pharmacokinetic Parameters

Pharmacokinetic parameters will be calculated as described in Section 8.5 and will be listed by treatment and subject. Descriptive statistics of pharmacokinetic parameters will include mean (arithmetic and geometric), SD, CV (arithmetic and geometric), median, minimum and maximum. When a geometric mean will be presented it will be stated as such. For T_{max}, only median, minimum and maximum values will be presented.

11.3.3 Pharmacokinetic Analysis

An exploratory assessment of dose-proportionality will be performed (if applicable). Dose-proportionality will be assessed for C_{max} and AUC_{last} for single dose administration; AUC_{tau} for multiple dose administration.

A power model (PK parameter = α dose β) used to evaluate dose proportionality of GFH312 exposure. Individual concentration values will be used to perform a least-squares linear regression analysis, using the formula log_pkvar=A log_dose + B, where 'log_pkvar' represents the natural log transformed C_{max}, AUC_{last} or AUCtau and 'log_dose' represents the natural log transformed dose. An estimate of the slope of the regression line and corresponding 90% confidence interval (CI) will be obtained.

11.4 Exploratory Variables Analysis

All pharmacodynamic/biomarker data will be listed by treatment, subject, and visit/time point. Summary statistics will be provided by treatment and visit/time point. Graphical presentation may be applied as appropriate.

11.5 Sample Size Calculation

Eight subjects per each cohort in Part I and Part II will be included. This sample size is typical in first-in-human studies and is deemed sufficient to meet the objective of safety and tolerability assessment. No formal sample size calculation has been performed also no power evaluation is provided due to lack of variability information.

At a sample size of 6 subjects on active drug per each cohort, an adverse event of an underlying occurrence rate of 30% or higher would have ≥88% probability that at least one subject will report an adverse event.

11.6 Interim analyses

No formal interim analyses are planned for this study.

12. Ethical considerations

12.1 Regulatory and ethical compliance

This clinical study was designed and shall be implemented and reported in accordance with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice (GCP), with applicable local regulations, and with the ethical principles laid down in the Declaration of Helsinki.

12.2 Informed consent procedures

Eligible subjects may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC-approved informed consent. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the subject source documents.

GenFleet will provide to investigators in a separate document a proposed informed consent form that complies with the ICH Good Clinical Practice guideline and regulatory requirements and is considered appropriate for this study. Any changes to the proposed consent form suggested by the investigator must be agreed to by GenFleet before submission to the IRB/IEC, and a copy of the approved version must be provided to the GenFleet designated monitor after IRB/IEC approval.

12.3 Responsibilities of the investigator and IRB/IEC

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB) before study start. A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to

GenFleet before study initiation. Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to GenFleet representatives and designated agents, IRBs/IECs/REBs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform GenFleet immediately that this request has been made.

12.4 Publication of study protocol and results

Upon study completion and finalization of the study report the results of this trial may be submitted for publication.

13. Reference

1. Christofferson, D.E., Y. Li, and J. Yuan, Control of life-or-death decisions by RIP1 kinase. Annu Rev Physiol, 2014. 76: p. 129-50.
2. Onuora, S., New autoinflammatory disease caused by non-cleavable RIPK1 variants. Nat Rev Rheumatol, 2020. 16(2): p. 61.
3. Yuan, J., P. Amin, and D. Ofengeim, Necroptosis and RIPK1-mediated neuroinflammation in CNS diseases. Nat Rev Neurosci, 2019. 20(1): p. 19-33.
4. Degterev, A., D. Ofengeim, and J. Yuan, Targeting RIPK1 for the treatment of human diseases. Proc Natl Acad Sci USA, 2019. 116(20): p. 9714-9722.
5. Newton, K., et al., RIPK3 deficiency or catalytically inactive RIPK1 provides greater benefit than MLKL deficiency in mouse models of inflammation and tissue injury. Cell Death Differ, 2016. 23(9): p. 1565-76.
6. Weisel K, Scott NE, Tompson DJ, et al. Randomized clinical study of safety, pharmacokinetics, and pharmacodynamics of RIPK1 inhibitor GSK2982772 in healthy volunteers. Pharmacol Res Perspect. 2017;5(6):e00365.

Appendix 1 Columbia Suicide Severity Rating Scale (C-SSRS)

To be provided separately.

Appendix 2: Liver event definitions and follow-up requirements

Table 13 Liver event definitions

Definition	Thresholds
Potential Hy's law cases	• ALT or AST > 3 × ULN and TBL > 2 × ULN without initial increase in ALP to > 2 × ULN
ALT or AST elevation with coagulopathy	• ALT or AST > 3 × ULN and INR > 1.5 (in the absence of anticoagulation)
ALT or AST elevation accompanied by symptoms	• ALT or AST > 3 × ULN accompanied by (general) malaise, fatigue, abdominal pain, nausea, or vomiting, or rash, or eosinophilia
Isolated ALT or AST elevation	• ALT or AST > 8 × ULN • 5 × ULN < ALT/AST ≤ 8 × ULN • 3 × ULN < ALT/AST ≤ 5 × ULN
Isolated ALP elevation	• ALP > 2 × ULN (in the absence of known bone pathology)
Others	• Any clinical event of jaundice (or equivalent term) • Any adverse event potentially indicative of liver toxicity

Table 14 Actions required for liver events

Criteria	Actions required
Potential Hy's Law case	• Discontinue the study treatment immediately • Hospitalize, if clinically appropriate • Establish causality
ALT or AST elevation with coagulopathy	
ALT or AST elevation accompanied by symptoms	
Isolated ALT or AST elevation > 8 × ULN	
Jaundice	
Isolated ALT or AST elevation > 5 to ≤ 8 × ULN	• If confirmed, consider interruption or discontinuation of study drug • If elevation persists for more than 1 week, discontinue the study drug • Establish causality
Isolated ALT or AST elevation > 3 to ≤ 5 × ULN	• Monitor liver chemistry tests two or three times weekly
Isolated ALP elevation	• Repeat liver chemistry tests within 48-72 hours • If elevation is confirmed, measure fractionated ALP; if >50% is of liver origin, establish hepatic causality
Any AE potentially indicative of liver toxicity	• Consider study treatment interruption or discontinuation • Hospitalize if clinically appropriate

Appendix 3 Specific renal alert criteria and actions

Table 15 Specific renal alert criteria and actions

Criteria	Action required
Serum creatinine (sCr) increase 25 – 49% compared to baseline	• Consider causes and possible interventions • Follow up within 2-5 days
Serum creatinine increase ≥ 50%	• Consider causes and possible interventions • Repeat assessment within 24-48h if possible • Consider drug interruption or discontinuation unless other causes are diagnosed and corrected • Consider hospitalization and specialized treatment
Protein-creatinine or albumin-creatinine ratio increase ≥ 2-fold; or new onset dipstick proteinuria ≥ 1+; or Albumin-creatinine ratio (ACR) ≥ 30 mg/g or ≥ 3 mg/mmol; or Protein-creatinine ratio (PCR)≥ 150 mg/g or >15 mg/mmol	• Consider causes and possible interventions • Assess serum albumin & serum protein • Repeat assessment to confirm • Consider drug interruption or discontinuation unless other causes are diagnosed
New onset glucosuria on urine dipstick (unless related to concomitant treatment, diabetes)	• Blood glucose (fasting) • Serum creatinine • Urine albumin-creatinine ratio
New hematuria on dipstick	• Urine sediment microscopy • Assess sCr and urine albumin-creatinine ratio • Exclude infection, trauma, bleeding from the distal urinary tract/bladder, menstruation • Consider bleeding disorder

Table 16 Follow-up of renal events

Action	Follow up
Assess, document and record in the Case Report Form (CRF). Review and record possible contributing factors to the renal event (co-medications, other co-morbid conditions) and additional diagnostic procedures (MRI etc) in the CRF.	• Urine dipstick and sediment microscopy • Blood pressure and body weight • Serum creatinine, electrolytes (sodium, potassium, phosphate, calcium), bicarbonate and uric acid • Urine output
Monitor subject regularly (frequency at investigator's discretion) until:	• Event resolution: (sCr within 10% of baseline or protein-creatinine ratio within 50% of baseline); or • Event stabilization: sCr level with ±10% variability over last 6 months or protein-creatinine ratio stabilization at a new level with ±50% variability over last 6 months.