CLINICAL TRIAL PROTOCOL

	Document Number:	**c08980589-06**
EudraCT No.: **EU Trial No:**	2016-000499-83	
BI Trial No.:	1386-0004	
BI Investigational Product(s):	BI 1467335	
Title:	A multi-centre, double-blind, parallel-group, randomised, placebo controlled phase II a study to investigate safety, tolerability, pharmacodynamics, and pharmacokinetics of different doses of orally administered BI 1467335 during a 12-week treatment period compared to placebo in patients with clinical evidence of NASH.	
Lay Title:	Different doses of BI 1467335 compared to placebo in patients with clinical evidence of NASH	
Clinical Phase:	IIa	
Trial Clinical Monitor:	Phone: Fax:	
Coordinating Investigator:	Phone: Fax:	
Status:	**Final Protocol (Revised Protocol based on global amendment 5)**	
Version and Date:	**Version 6.0**	**Date: 12 Sep 2018**

CLINICAL TRIAL PROTOCOL SYNOPSIS

Name of company:		**Boehringer Ingelheim**	
Name of finished product:		NA	
Name of active ingredient:		BI 1467335	
Protocol date: 02 Dec 2016	**Trial number:** 1386-0004		**Revision date:** 12 Sep 2018

Title of trial:	A multi-centre, double-blind, parallel-group, randomised, placebo controlled phase II a study to investigate safety, tolerability, pharmacodynamics, and pharmacokinetics of different doses of orally administered BI 1467335 during a 12-week treatment period compared to placebo in patients with clinical evidence of NASH.
Coordinating Investigator:	Phone: Fax:
Trial site(s):	Multicentre Trial conducted in approximately 8 countries / 50 sites
Clinical phase:	IIa
Objective(s):	The primary objective of this study is the proof of mechanism and support of dose finding, together with the safety evaluation in patients with clinical evidence of NASH over different doses of BI 1467335 compared to placebo.
Methodology:	Placebo-controlled, double blind, randomised, parallel design comparison of 5 groups over 12 weeks of treatment.
No. of patients:	
total entered:	Approximately 108
each treatment:	Placebo n_1=28; 1mg BI 1467335 n_2=14; 3mg BI 1467335 n_3=14; 6mg BI 1467335 n_4=14; 10mg BI 1467335 n_5=28
Diagnosis :	Patients with clinical evidence for non-alcoholic-steato-hepatitis based on a) histological evidence or b) hepatic steatosis in combination with hepatic fibrosis.

Name of company:		**Boehringer Ingelheim**	
Name of finished product:		NA	
Name of active ingredient:		BI 1467335	
Protocol date: 02 Dec 2016	**Trial number:** 1386-0004		**Revision date:** 12 Sep 2018

Main criteria for inclusion:	The population will include male and female patients over 18 years of age with clinical evidence for NASH and ALT > 1.5 upper limit normal. Patients with a history of significant alcohol consumption or other forms of chronic liver disease (including liver cirrhosis) will be excluded.
Test product(s):	BI 1467335
dose:	1mg QD, 3mg QD, 6mg QD, 10mg QD
mode of administration:	Tablet, p.o.
Comparator products:	Placebo
dose:	Not applicable
mode of administration:	Tablet, p.o.
Duration of treatment:	12 weeks

Name of company:		**Boehringer Ingelheim**	
Name of finished product:		NA	
Name of active ingredient:		BI 1467335	
Protocol date: 02 Dec 2016	**Trial number:** 1386-0004		**Revision date:** 12 Sep 2018

Endpoints	Primary Endpoint: Plasma AOC3 activity relative to baseline in %, at 24 h post dose, after 12 weeks of treatment Secondary endpoints: • number (%) of subjects with adverse reactions • Relative ALT change from baseline after 12 weeks of treatment • Relative AST change from baseline after 12 weeks of treatment • Relative AP change from baseline after 12 weeks of treatment • Relative γ-GT change from baseline after 12 weeks of treatment • Relative caspase cleaved cytokeratin 18 (M30) change from baseline after 12 weeks of treatment • Relative total cytokeratin 18 (M65) change from baseline after 12 weeks of treatment
Safety criteria:	Adverse event reporting, vital signs and standard laboratory tests
Statistical methods:	For dose-response relationship analysis a non-linear 3-parametric model is used to fit the primary endpoint AOC3 activity relative to baseline, at 24 h post dose, after 12 weeks of treatment with either BI 1467335 or placebo. The dose will be estimated where the fitted mean curve drops below 10%. The secondary biomarker endpoints including ALT, AST, AP, γ-GT, caspase cleaved and total CK18 are evaluated using a mixed effect model for repeated measurements (MMRM). The estimated dose effects at week 12 will then be analysed using the MCPMod approach to test if there is a non-flat dose response relationship between the different doses of BI 1467335 and placebo.

FLOW CHART 1

Trial Period	**Screening[2]**	**Treatment Period**								**Follow-up**
Visit or phone call (☎)	**1**	**2 Baseline**	**3**	**☎ 3A**	**4**	**5**	**6**	**☎ 6A**	**EOT/ ED[6]**	**FU**
Study-Day	-28 to -7	**1**	**15**	**27**	**29**	**43**	**57**	**83**	**85**	**EOT +28**
Week	**-4 to -1**		**2**		**4**	**6**	**8**		**12**	16
Time window (in days)			±3	-2	±3	±3	±3	-2	+3	±3
Patient information & informed consent signed (including informed consent for biobanking[1])	X									
Register Patient in IRT	X									
Randomisation (via IRT)		X								
Demographics	X									
Medical history / baseline conditions	X									
In-/exclusion criteria, incl. quantification of alcohol consumption	X	X[13]								
Clinical imaging (optional)[21]	X[21]									
Concomitant medications	X	X	X		X	X	X		X	X
Height (screening only) / weight / waist and hip circumference[17]	X	X	X		X	X	X		X	X
Vital signs	X	X	X		X	X	X		X	X
Physical examination	X	X	X[14]		X[14]	X[14]	X[14]		X	X
Resting ECG [12]	X	X	X		X	X	X		X	X
Adverse events	X	X	X	X	X	X	X	X	X	X
Pregnancy testing[10, 15]	Xs	Xu	Xu		Xu	Xu	Xu		Xu	Xu
IRT call[3]		X			X		X		X	
Dispense trial medication		X			X		X			
Collect study drug					X		X		X	
Check of medication compliance and dispense/ collect study drug diary [11]		X	X		X	X	X		X	
Telephone contact[16]				X				X		
Infection testing[19]	X									
Safety laboratory tests[15], biomarkers (except screening)	X[20]	X[9]	X		X[9]	X	X		X[9]	X[9]
Biomarker urine collection [22]		X	X		X	X	X		X	X
Sodium citrate coagulation collection[23]		X	X		X		X		X	X
PK sampling[4]		X	X		X	X	X		X	
PG sampling[5]		X							X	
PD sampling[8]		X	X		X	X	X		X	X[18]
Blood samples Biobanking		X							X	

[1] Prior to any study related procedure, may also be done at an extra visit up to 2 weeks before V1.

[2] Screening visit to be performed -28 to -7 days before randomisation visit (V2).

[3] At visits 2, 4 and 6, the respective IMP kit number has to be allocated to the patient via IRT. At Visit EOT/ED the IRT call is performed to close out the patient (Termination of medication).

or

DNA banking, blood samples will be collected at visit 2 and EOT/ED (for details please refer to section 5.5.4).

[6] Also to be completed for patients who are withdrawn or who have discontinued the trial early: in case of early termination, the EOT/ED Visit will be completed instead of the planned treatment period visit. This early discontinuation (ED) visit will include the same procedures as the normal EOT visit except PK, PG and PD sampling. Visit FU should be performed 28 days after the last study drug intake.

For patients who discontinue IMP but are willing to attend scheduled trial visits, only one PK sample and one PD blood sample for measurement of AOC3 activity should be taken and the actual time of the blood draw recorded. Please refer to section 6.2.2.

[8] PD blood samples for measurement of AOC3 activity and concentration. Please see Flow Charts 2, 3, 4 and table 10.1:1 for timing.

For patients who discontinue IMP but are willing to attend scheduled trial visits, only one PD blood sample for measurement of AOC3 activity should be taken and the actual time of the blood draw recorded. Please refer to section 6.2.2.

[10] Pregnancy Testing: Xs =serum testing; Xu= onsite urine testing; Serum pregnancy is done at screening and as a reflex when urine testing is positive.

[11] All patients will complete a study drug diary during the treatment phase to document drug intake and treatment compliance. The diary will be dispensed to the patients at Visit 2 and regularly checked by site-staff at the following visits. Please refer to section 6.2.2

[12] 12-lead ECGs will be recorded at the visits outlined in the flowchart for all patients approximately 90 min after study drug intake. At visits without drug administration ECGs should preferentially be performed prior blood sampling. For detailed timing please refer to Flow Charts 2, 3, 4. ECGs will be recorded after the patients have rested for at least 5 minutes in a supine position. The ECG at the screening visit is regarded as baseline. Please refer to section 5.3.4

[13] Confirm eligibility from Screening visit.

[14] Only symptom-derived physical examination required.

[15] At dosing visits: The respective procedure is to be performed and completed prior to study drug administration.

[17] Waist and hip circumference only at screening and EOT/ED. For detailed instruction how to measure waist and hip circumference see Section 5.3.1.1.

[18] For the timing of this blood sample please refer to table 10.1:1. Not applicable in case of early discontinuation.

[20] If the historic ALT > 1.25 ULN value is older than 3 months prior to screening, the ALT > 1.5xULN must be confirmed via two measurements at least 1 week apart within the screening period. Please refer to sections 3.3.2 and 6.2.1

[21] Clinical imaging (optional): only applicable for patients who don't have the required clinical imaging results suggestive of NASH yet (no more than 3 years prior to screening, according to Inclusion criterion #1). **Not required if histological**

[23] One additional blood sample (sodium citrate coagulation tube) will be taken for platelet-rich plasma (PRP). Please refer to section 5.5.2.

FLOW CHART 2: TIMING OF PROCEDURES*: VISIT 2 AND 4 (DAYS 1 AND 29):

Time relative to drug administration (h)[4]	-24	-0.5	0	0.25	0.5	1	1.5	2	3	6	8
Administer trial medication[1]			X								
PK blood sampling		X		X	X	X	X[3]	X	X	X	X
PK urine collection[2]	<-		->								
AOC 3 PD blood sampling[4]		X[4]			X	X		X	X	X	X

[1] To be done at the clinical site
[2] PK urine collection only to be done on days 28-29. Container should be given to the patient on day 1 (or day 15). Site to call patient on day 27 to remind of 24 h urine collection and study drug intake. Collected urine to be returned to the site on the next morning.
[3] ECG measurement to be performed directly prior to the PK blood sampling at 1.5h.
[4] AOC3 activity measurements at all indicated time points; in addition AOC3 concentration measurement at -0.5h only

FLOW CHART 3: TIMING OF PROCEDURES*: VISIT 3, 5 AND 6 (DAYS 15, 43 AND 57):

Time relative to drug administration (h)	-0.5	0	1	1.5
Administer trial medication[1]		X		
PK blood sampling	X		X	X[2]
AOC 3 PD blood sampling	X[3]			

[1] To be done at the clinical site.
[2] ECG measurement to be performed directly prior to the PK blood sampling at 1.5 h.
[3] AOC3 activity and concentration measurement.

FLOW CHART 4: TIMING OF PROCEDURES*: EOT (DAY 85):

Time relative to drug administration (h) [5]	-0.5	0	0.25	0.5	1	1.5	2	3	6	8	24
Administer trial medication[1]		X									
PK blood sampling	X		X	X	X	X[3]	X	X	X	X	X
PK urine collection[2]		<-	-	-	-	-	-	-	-	-> <-	->
AOC 3 PD blood sampling[4]	X[4]			X	X		X	X	X	X	X

[1] To be done at the clinical site
[2] 24 h urine collection Collection of 0-8 h urine to be performed at the site; collection of 8-24 h urine to be performed by patients and to be returned to the site in the next morning; no overnight stay is required.
[3] ECG measurement to be performed directly prior to the PK blood sampling at 1.5h.
[4] AOC3 activity measurements at all indicated time points; in addition AOC3 concentration measurement at -0.5h only.
[5] Site to call patient on day 83 to remind of study drug intake on day 84.

TABLE OF CONTENTS

ABBREVIATIONS

$\%AUC_{tz-\infty}$	the percentage of $AUC_{0-\infty}$ obtained by extrapolation
λ_z	terminal rate constant in plasma
Ae_{t1-t2}	amount of analyte that is eliminated in urine from the time interval t_1 to t_2
A1At	Alpha-1 Antitrypsin
AE	Adverse Event
AESI	Adverse Event of Special Interest
AOC3	amine oxidase copper-containing 3
AUC	Area under the Curve
AUC_{0-24}	area under the concentration-time curve of the analyte in plasma over the time interval from 0 to 24 hours after administration
$AUC_{0-\infty}$	area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
AUC_{0-tz}	area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
AUC_{t1-t2}	area under the concentration-time curve of the analyte in plasma over the time interval t_1 to t_2
b.i.d.	bis in die (twice daily dosing)
BIRDS	Boehringer Ingelheim Regulatory Documents for Submission
BMI	Body Mass Index
C_{pre}	predose concentration of the analyte in plasma immediately before administration
$C_{0.5}$	concentration of the analyte in plasma 0.5 hours after administration
C_1	concentration of the analyte in plasma 1 hour after administration
C_{max}	maximum measured concentration of the analyte in plasma after administration
CCDS	Company Core Data Sheet
CI	Confidence Interval
$CL_{R,\ t1-t2}$	renal clearance of the analyte in plasma from the time point $_{t1}$ to $_{t2}$
CL/F	apparent clearance of the analyte in the plasma after extravascular administration
CML	Local Clinical Monitor
CRA	Clinical Research Associate
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTP	Clinical Trial Protocol
CTR	Clinical Trial Report
D10	Smallest dose where 10 % AOC3 activity is reached
DILI	Drug Induced Liver Injury
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
ED	Early discontinuation
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
EOT	End of treatment
ePRO	Electronic Patient Reported Outcome

EudraCT	European Clinical Trials Database
FAS	Full Analysis Set
FC	Flow Chart
fe_{t1-t2}	fraction of administered drug excreted unchanged in urine from time point t_1 to t_2
FFA	Free Fatty Acids
GCP	Good Clinical Practice
HOMA	Homeostasis Model Assessment
HPC	Human Pharmacology Center
hsCRP	High sensitive C-reactive protein
IB	Investigator's Brochure
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISF	Investigator Site File
i.v.	intravenous
LoEE	List of Essential Element
MedDRA	Medical Dictionary for Drug Regulatory Activities
MCPMod	Multiple Comparison Procedures – Modelling
MMRM	mixed effect model for repeated measurements
MRE	Magnetic Resonance Elastography
MRI-PDFF	Magnetic Resonance Imaging of Proton Density Fat Fraction
MRT_{ex}	mean residence time of the analyte in the body after extravascular administration
MST	Medical Sub team
NASH	Non-alcoholic steatohepatitis
OPU	Operative Unit
PD	Pharmacodynamics
PK	Pharmacokinetics
p.o.	per os (oral)
PCC	Protocol Challenge Committee
PRP	platelet-rich plasma
q.d.	quaque die (once a day)
$R_{A,Cmax}$	accumulation ratio based on C_{max} after multiple doses versus C_{max} after the first dose
$R_{A,AUCt1-t2}$	accumulation ratio based on AUC_{t1-t2} after multiple doses versus AUC_{t1-t2} after the first dose
REP	Residual effect period, after the last dose of medication with measureable drug levels or pharmacodynamic effects still likely to be present
SAE	Serious Adverse Event
s.c.	Subcutaneous
SOC	System Organ Class
SPC	Summary of Product Characteristics
SSAO	semi-carbazide-sensitive amine oxidase
t_{max}	time from dosing to maximum measured concentration of the analyte in plasma

$t_{1/2}$	terminal half-life of the analyte in plasma
TCM	Trial Clinical Monitor
TDMAP	Trial Data Management and Analysis Plan
t.i.d.	ter in die (3 times a day)
TMF	Trial Master File
TMW	Trial Medical Writer
TSAP	Trial Statistical Analysis Plan
US	Ultrasound
V_z/F	apparent volume of distribution during the terminal phase after extravascular administration

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

Boehringer Ingelheim (BI) is developing BI 1467335 (formerly Pharmaxis PXS-4728A), an oral, small-molecule inhibitor of semi-carbazide-sensitive amine oxidase (SSAO) also known as vascular adhesion protein-1 (VAP-1) and amine oxidase copper-containing 3 (AOC3) for the indication non-alcoholic steatohepatitis (NASH).
NASH is characterized histologically by a high level of steatosis, ballooning of hepatocytes, and necroinflammation. AOC3 in liver sinusoidal endothelial cells is responsible for the firm adhesion and transmigration of leukocytes and for the propagation of the inflammatory environment in steatohepatitis. Fibrotic regions of NASH liver sections are strongly positive for AOC3 immune reactivity [R15-5697]. The associated generation of peroxide during the course of amine oxidation is known to activate quiescent stellate cells supporting the differentiation into myofibroblasts and fibrotic tissue generation. Recent findings with AOC3 knockout mice or animals treated with anti-AOC3 antibody displayed reduced hepatic inflammation and fibrosis development in several disease-related models of liver injury [R15 5365] and are supportive of this therapeutic concept. Therefore, targeting inhibiting AOC3 enzymatic activity might be beneficial for patients with steatohepatitis and fibrosis in order to reduce the recruitment of leukocytes into the liver and reduce cytokine and oxygen stress dependent hepatocyte damage and activation of hepatic stellate cells.
With a prevalence of about 20–30% in the general population of Western countries, Non-Alcoholic-Fatty-Liver Disease (NAFLD) is rapidly becoming the most common liver disease worldwide [R15-5365]. While simple hepatic steatosis can have a benign non-progressive course, about 40% of patients with NASH progress [R16-5301].As the disease progresses, significant fibrosis develops in 37-41% of subjects within 15 years. In the United States, NASH is believed to be the most common cause of liver cirrhosis [R15-6070], which is estimated to be the 12th leading cause of death in the US, according to the National Institute of Health [R15-6057]. Patients with NASH are also at increased risk of hepatocellular carcinoma (HCC), even in the absence of cirrhosis [R15-5365]. By 2023, about 13 million patients are projected to have NASH with advanced stages (i.e. ≥ stage 3) of fibrosis (of those, 2.9 million in the US, 3.5 million in EU5, 5 million in China). The risk of liver-related death in Western patients with NASH ranges from 10% over 13.7 years to 18% over 18.5 years [P13-02280].
To date, no approved therapy for liver fibrosis or effective disease modifying regimen for NASH is available. The current standard of care for NASH is weight loss through diet and exercise to improve insulin resistance and lower fat mass, which is a clinically challenging goal to achieve and therefore only affects a minor proportion of the patients [R15-6044].
Persistent inflammation in response to liver injury is the critical factor that drives progression to fibrosis, cirrhosis, and hepatocellular carcinoma [R15-5365]. Inflammation at any site, including the liver, is the result of an accumulation of leukocytes organized into an inflammatory infiltrate. For this to occur, leukocytes must be recruited from the circulation by interactions with the endothelium and positioned within the tissue [R15-6046]. A key protein in promoting the recruitment of leukocytes in liver tissue is AOC3, which is constitutively expressed on human hepatic endothelium. BI 1467335 is an irreversible AOC3 inhibitor with anti-inflammatory and anti-oxidative stress activities that is currently under

development for the treatment of chronic liver disease secondary to Non-alcoholic Steatohepatitis (NASH).

1.2 DRUG PROFILE

BI 1467335 is an AOC3 inhibitor that is being developed for symptomatic treatment of NASH. In humans, NASH is characterized by steatohepatitis, lipotoxicity, necroinflammation, stellate cell differentiation, myofibroblast activation and fibrosis. The potential role of AOC3 in NASH warrants the development of the irreversible AOC3 inhibitor BI1467335 for the treatment of patients with NASH.

2. RATIONALE, OBJECTIVES, AND BENEFIT - RISK ASSESSMENT

2.1 RATIONALE FOR PERFORMING THE TRIAL

Increased plasma levels of ALT in NASH patients are a consequence of the lipotoxicity-induced necroinflammation of the parenchymal liver tissue. Soluble plasma AOC3 (sAOC3) activity is positively correlated with a variety of diseases including atherosclerosis, liver and kidney fibrosis, and septic shock, thereby indicating a potential pivotal role in various forms of inflammation (R15-5801, R15-5774, R15-5773, R15-5766, R15-5767, P15-12198, R15-5771, R15-5772, R15-5802). Inhibition of AOC3, therefore, has the potential to reduce oxidative stress and hepatic inflammation in steatohepatitis, and to improve fibrosis resolution in patients with NASH and ultimately resulting in reduced liver cell damage, thereby reducing elevated plasma ALT levels. This first study in NASH patients is performed to support the proof of mechanism and to allow further insights into the dose-response relationship of BI 1467335.

2.2 TRIAL OBJECTIVES

The primary objective of this study is the proof of mechanism and support of dose finding, together with the safety evaluation in patients with clinical evidence of NASH.
This proof of mechanism study will explore the inhibition of AOC3 activity (% of baseline) after 12 weeks treatment as the primary endpoint.

. This will include the effect of BI 1467335 on reduction of secondary biomarker endpoints (ALT, AST, AP, γ-GT and CK18 fragments).
Safety will be assessed throughout the study to provide key information regarding the use of BI 1467335 in patients with NASH.

The proof of clinical mechanism will be achieved through primary endpoint comparison (inhibition of AOC3 activity (% of baseline)) of the four BI 1467335 doses (1mg QD, 3mg QD, 6mg QD, 10mg QD) and placebo. To this end, the dose-response relationship of the primary endpoint will be analysed by means of non-linear regression.

For the secondary endpoints ALT, AST, γ-GT, AP, caspase cleaved cytokeratin 18 and total cytokeratin 18 a non-flat dose response relationship between the BI doses and placebo will be tested using the multiple comparison procedures and modelling (MCPmod) approach [R10-1424].

2.3 BENEFIT - RISK ASSESSMENT

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

This is a 12-week, multi-centre, randomised, double blind, placebo-controlled, parallel-group comparison in patients with NASH. In total, 108 patients who meet the entry criteria are planned to be randomised in this trial. All eligible patients will be instructed at every visit (including screening) to adhere to their normal dietary and exercise regimen from screening until safety follow-up visit (see inclusion criterion #5 for details). Eligible patients will be randomised to the 12 week double-blind treatment period at Visit 2 and will be assigned to one of the 5 treatment groups (placebo QD, BI 1467335 1 mg QD, BI 1467335 3 mg QD, BI 1467335 6 mg QD or BI 1467335 10 mg QD; each patient will receive one active treatment and/or placebos to match the alternative active treatment). The randomised treatment will be double blind. After the end of the double-blind treatment period, patients will be followed up for additional 4 weeks without study medication. Safety will be evaluated at each visit until end of the observational period which is 28 days after end of treatment or for an appropriately longer time in case of unresolved adverse events.

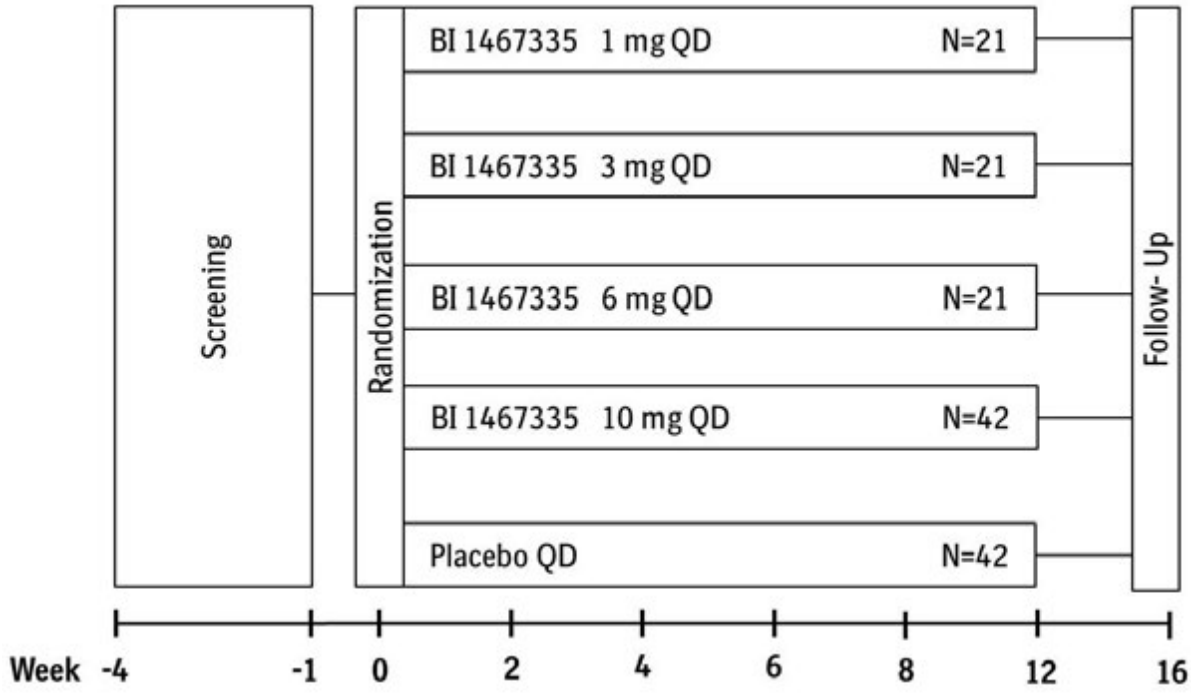


Figure 3.1: 1 Trial Design

3.1.1 Administrative structure of the trial

The trial is sponsored by Boehringer Ingelheim (BI).

A Coordinating Investigator is responsible to coordinate Investigators at different centres participating in this multicentre trial. Tasks and responsibilities are defined in a contract. Relevant documentation on the participating (Principal) Investigators and other important participants, including their curricula vitae, will be filed in ISF.

Boehringer Ingelheim has appointed a Trial Clinical Monitor, responsible for coordinating all required activities, in order to

- manage the trial in accordance with applicable regulations and internal SOPs,
- direct the clinical trial team in the preparation, conduct, and reporting of the trial,
- ensure appropriate training and information of local clinical monitors (CML), Clinical Research Associates (CRAs), and Investigators of participating countries.

The organisation of the trial in the participating countries will be performed by the respective local or regional BI-organisation (Operating Unit, OPU) in accordance with applicable regulations and internal SOPs, or by a Contract Research Organisation (CRO) with which the responsibilities and tasks will have been agreed and a written contract filed before initiation of the clinical trial.
Data Management and Statistical Evaluation will be done by BI according to BI SOPs.

Tasks and functions assigned in order to organise, manage, and evaluate the trial are defined according to BI SOPs. A list of responsible persons and relevant local information can be found in the ISF.
Details of the trial supplies including responsible institutions are given in Section 4 of this protocol.
A central laboratory service and vendors for ECG and IRT (Interactive Response Technology) will be used in this trial. Details will be provided in the applicable manuals, available in the ISF.

3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUP(S)

A randomised, double blind, placebo- controlled design is chosen for this trial in order to evaluate safety, pharmacodynamics, and PK of BI 1467335 in patients with clinical evidence of NASH.
A placebo control arm was chosen to be included in order to evaluate the absolute effects of BI 1467335 on safety and tolerability. This is acceptable as no standard treatment is available.

3.3 SELECTION OF TRIAL POPULATION

In total, 108 patients with clinical evidence of NASH will be randomised to one of the five treatment arms. Approximately 50 sites in multiple countries are planned. Recruitment will be competitive. Patients who discontinue following randomisation will not be replaced and

may not be re-enrolled at a later date. A record is kept of all patients failing to complete all trial visits and their reasons for discontinuation. Permission to randomise more than 20 patients per site must be obtained from the TCM at Boehringer Ingelheim. This will only be allowed after a careful review of the enrolment status.

A log of all patients enrolled into the trial (i.e. who have signed informed consent) will be maintained in the Investigator Site File (ISF) at the investigational site irrespective of whether they have been treated with investigational drug or not.

3.3.1 Main diagnosis for trial entry

The study will include patients with clinical evidence of NASH. This is defined as increase in ALT and diagnosis of NASH (histological evidence of NASH or presence of hepatic steatosis in combination with liver fibrosis (see inclusion criterion #1 for details) within 3 years prior to screening).

Please refer to section 8.3.1 (Source Documents) for the documentation requirements pertaining to the in- and exclusion criteria.

3.3.2 Inclusion criteria

1. Clinical evidence of NASH defined as
   a. histological evidence of NASH (no more than 3 years prior to screening)

   OR

   b. clinical imaging results suggestive of NASH (no more than 3 years prior to screening OR within the screening phase, imaging procedures performed as per local standard)
      i. evidence of hepatic steatosis >5% measured by the MRI-PDFF or assessed as steatosis (raised echogenicity of the liver parenchyma) by ultrasound

      AND

      ii. evidence of liver fibrosis defined as mean stiffness > 3.64 kPa as measured by the MRE protocol or mean stiffness > 7.2 kPa as measured by ultrasound based transient elastography (Fibroscan®)

2. Increased ALT defined as
   a. ALT >1.5 ULN at screening and ALT >1.25 ULN in a local lab within 1 week to 3 months prior screening

   OR

   b. Historic ALT > 1.25 ULN more than 3 months prior to screening and two consecutive ALT > 1.5xULN must be confirmed at least 1 week apart within the screening period

3. Age ≥ 18 and ≤75 years at screening

4. BMI ≥25kg/m^2 and <45kg/m^2 at screening

5. Stable body weight defined as less than 5% change in body weight in the 3 months prior to screening while being treated with the standard of care and not treated with anti-obesity medication at screening.

6. Treatment with Antidiabetic concomitant medication including any insulin regimen needs to be stable for 3 months, and treatment with vitamin E needs to be stable for 6 months prior to informed consent and expected to be stable throughout the trial. All other concomitant medication has to be stable for at least 4 weeks prior screening. Concomitant medications taken to treat acute conditions (e.g. headache, sinusitis) for a short period (< 7 days) are permissible, if not otherwise prohibited. For restricted medications please refer to section 4.2.2.1.

7. For female patients: Women of childbearing potential* can be randomized after a negative pregnancy test and under adequate contraception with two methods, of which at least one is highly effective, during the trial.

   * A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. Tubal ligation is NOT a method of permanent sterilisation. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

8. Signed and dated written informed consent in accordance with GCP and local legislation prior to admission to the trial.

3.3.3 Exclusion criteria

1. Current or history of significant alcohol consumption (defined as intake of >210g/week in males and >140g/week in females on average over a consecutive period of more than 3 months) or inability to reliably quantify alcohol consumption based on investigator judgement.

2. Prior participation in an interventional NASH trial 6 months before baseline or 5 times halflife of the investigational drug, whichever is longer.

3. Prior or planned bariatric surgery during study conduct, except gastric-band surgery more than 2 years prior to screening (including adjustments) with a stable body weight within the last 12 months.

4. Use of drugs historically associated with liver injury, hepatic steatosis or steatohepatitis in the 4 weeks prior to screening; please refer to section 4.2.2.

5. History of liver cirrhosis (fibrosis stage 4), or hepatic decompensation (e.g. ascites, hepatic encephalopathy, variceal bleeding, etc.) or history of other forms of chronic liver disease (for example Hepatitis B, Hepatitis C, autoimmune liver disease, primary biliary sclerosis, primary sclerosing cholangitis, Wilsons disease, hemochromatosis, A1At deficiency, history of liver transplantation).

6. Active known chronic or relevant acute infections, such as HIV (Human Immunodeficiency Virus), \viral hepatitis, or tuberculosis. QuantiFERON® TB test and HBs Ag test will be performed during screening. Patients with a positive test result may participate in the study if further work up (according to local practice/guidelines) establishes conclusively that the patient has no evidence of active infection.

7. Solid liver lesions other than haemangiomas.
   a. Suspicion or diagnosis or history of hepatocellular carcinoma (HCC)

8. eGFR <60ml/min/1.73m^2 at screening (CKD-EPI formula).

9. ALT >5.0 ULN at screening.

10. Platelet count < 150.000/µL

11. Bilirubin level > ULN (except for known Gilbert´s disease with a conjugated bilirubin of < 0.3 mg/dL))

12. Uncontrolled diabetes defined as an HbA1c ≥9.5% in the 3 months prior to or at screening.

13. Diagnosis of a serious or unstable disease including hepatic (other than NASH), renal, gastroenterologic, respiratory, cardiovascular (including ischemic heart disease), endocrinologic, neurologic , psychiatric, immunologic, or hematologic disease and other conditions that, in the clinical judgment of the investigator, are likely to interfere with the analyses of safety and efficacy in this study. Patients with an expected life expectancy of less than 2 years are also excluded.

14. Major surgery (major according to the investigator's assessment) performed within 12 weeks prior to randomisation or planned during study conduct, e.g. hip replacement.

15. Any documented active or suspected malignancy or history of malignancy within 5 years prior to screening, except appropriately treated basal cell carcinoma of the skin or in situ carcinoma of uterine cervix.

16. Patients who must or wish to continue the intake of restricted medications (see section 4.2.2.1) or any drug considered likely to interfere with the safe conduct of the trial.

17. Previous randomisation in this trial.

18. Currently enrolled in another investigational device or drug study, or less than 30 days since ending another investigational device or drug study(s), or receiving other investigational treatment(s).

19. Chronic drug abuse or any condition that, in the investigator's opinion, makes them an unreliable study subject or unlikely to complete the trial.

20. Women who are pregnant, nursing, or who plan to become pregnant while in the trial.

21. Patients with Wolff-Parkinson-White Syndrome, baseline QTc > 450 ms, family history of long QT, or on medication prolonging QT time at screening or planned initiation during the trial.

22. Any other clinical condition that, in the opinion of the investigator, would jeopardize patient safety while participating in this clinical trial.

3.3.4 Removal of patients from therapy or assessments

3.3.4.1 Removal of individual patients

An excessive withdrawal rate can have a severe negative impact on the scientific value of the trial. Every effort should be made to keep patients in the trial as scheduled. This includes careful patient selection and appropriate explanation of the trial requirements and procedures prior to enrolment as well as an explanation of the consequences of premature withdrawal.

An individual patient is to be withdrawn from trial treatment if:

- The patient withdraws consent for trial treatment or trial participation, without the need to justify the decision.
- The patient needs to take concomitant drugs that interfere with the investigational product or other trial medication 4.2.2,
- The patient can no longer be treated with trial medication for other medical reasons (such as surgery, adverse events, other diseases, or pregnancy)
- The patient has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both, the investigator and sponsor representative, is not willing or able to stick to the trial requirements in the future.

3.3.4.1.1 Removal of individual patients in case of increased liver enzymes

In addition, study-specific procedures have been defined in case of increased liver enzymes (AST, ALT and total bilirubin) after randomisation as outlined below [R16-5309].
Please also refer to section 10.3.

Treatment should be temporarily interrupted and the increased laboratory values need to be confirmed by a second measurement (i.e. the patient is informed to stop IMP and to come for an additional unscheduled visit within 48 hours of initial laboratory hepatic injury alert / notification or as soon as possible if timelines cannot be met). Other causes for liver injury should be excluded.

1) **If the values are confirmed or even further increased, IMP will be permanently stopped and the patient is withdrawn from treatment** and will be monitored closely on a weekly basis until resolution or stabilization in the following cases:

AST is normal at baseline and:
- If AST is more than 8x ULN
- If AST is 5-8x ULN for more than 2 weeks

ALT and/or AST are abnormal at baseline and:
- If ALT or AST is more than 8x baseline OR > 500 U/L absolute value, whichever is lower.

After resolution or stabilization the patient is encouraged to continue in the study and complete all visits. If the patient is not willing to continue in the study, the patient should complete the withdrawal- and follow-up protocol study procedures.

2) **If the values are confirmed, IMP will stay interrupted and the patient will be monitored closely** until resolution or stabilization in the following cases:

AST is normal at baseline and:
- If AST is 3x – 8x ULN AND total bilirubin is > 2 mg/dL
- If AST is 3x – 8x ULN OR total bilirubin is > 2 mg/dL
  AND the patient is presenting clinical symptoms of hepatic injury as e.g. encephalopathy, nausea, vomiting, pruritus, severe fatigue, etc.

ALT and AST are abnormal at baseline and:
- If ALT or AST is 5x – 8x baseline OR > 300 U/L absolute value (whichever is lower)
  AND total bilirubin is > 2 mg/dL
- If ALT or AST is 5x – 8x baseline OR > 300 U/L absolute value (whichever is lower) OR total bilirubin is > 2 mg/dL
  AND the patient is presenting clinical symptoms of hepatic injury as e.g. encephalopathy, nausea, vomiting, pruritus, severe fatigue, etc.

If the values return to the baseline value (Visit 2), a re-start of the IMP can be considered after discussion with the sponsor. After re-start of IMP the patient should be monitored on a weekly basis until EOT. In the case the initially elevated liver enzyme, which lead to interruption,

increases again more than 2x baseline, the IMP has to be discontinued permanently.

Given the patient's agreement, the patient will continue the study, or otherwise should be encouraged to undergo the procedures for early treatment discontinuation and follow up as outlined in the Flow Chart (FC) and section 6.2.3.

3.3.4.1.2 Further criteria for removal of individual patients

If a patient becomes pregnant during the trial, the study medication needs to be discontinued, and the patient will complete EOT- and Follow-up Visit procedures. The patient will be followed up until birth or otherwise termination of the pregnancy.

If a patient develops QTc > 500 ms during the trial, the study medication needs to be discontinued, and the patient will complete EOT- and Follow-up Visit procedures.

For all patients the reason for withdrawal (e.g. adverse events) must be recorded in the CRF. These data will be included in the trial database and reported.

Patients who discontinue the trial after receiving the first dose of study medication at visit 2 will not be replaced.

3.3.4.2 Discontinuation of the trial by the sponsor

Boehringer Ingelheim reserves the right to discontinue the trial overall or at a particular trial site at any time for the following reasons:

1. Failure to meet expected enrolment goals overall or at a particular trial site
2. Emergence of any efficacy/safety information invalidating the earlier positive benefit-risk-assessment that could significantly affect the continuation of the trial
3. Violation of GCP, the CTP, or the contract disturbing the appropriate conduct of the trial

The Investigator / the trial site will be reimbursed for reasonable expenses incurred in case of trial termination (except in case of the third reason).

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

Multiple doses of BI 1467335 and/or Placebo to match BI 1467335 will be administered. All products will be supplied by Boehringer Ingelheim.

4.1.1 Identity of the Investigational Medicinal Products

Table 4.1.1: 1 BI 1467335, 1 mg:

Substance:	BI 1467335
Pharmaceutical formulation:	Tablet
Source:	Boehringer Ingelheim Pharma GmbH & Co KG Birkendorfer Strasse 65 D-88397 Biberach a.d. Riss
Unit strength:	1 mg
Posology	QD
Route of administration:	Per os

Table 4.1.1: 2 BI 1467335, 5 mg:

Substance:	BI 1467335
Pharmaceutical formulation:	Tablet
Source:	Boehringer Ingelheim Pharma GmbH & Co KG Birkendorfer Strasse 65 D-88397 Biberach a.d. Riss
Unit strength:	5 mg
Posology	QD
Route of administration:	Per os

Table 4.1.1: 3 Placebo matching BI 1467335, 1 mg:

Substance:	BI 1467335
Pharmaceutical formulation:	Tablet
Source:	Boehringer Ingelheim Pharma GmbH & Co KG Birkendorfer Strasse 65 D-88397 Biberach a.d. Riss
Unit strength:	n.a.
Posology	n.a.
Route of administration:	Per os

Table 4.1.1: 4 Placebo matching BI 1467335, 5 mg:

Substance:	BI 1467335
Pharmaceutical formulation:	Tablet
Source:	Boehringer Ingelheim Pharma GmbH & Co KG Birkendorfer Strasse 65 D-88397 Biberach a.d. Riss
Unit strength:	n.a.
Posology	n.a.
Route of administration:	Per os

4.1.3 Method of assigning patients to treatment groups

All treatments will be double-blind as to be indistinguishable for the patient as well as for the investigator. Patients are randomised to treatment groups at Visit 2. Note that the medication number is different from the patient number (the latter is assigned directly after informed consent was obtained). Site personnel will enter the medication number on the case report form/in the CRF.
During visit 2 eligible patients will be randomised to receive a 2:1:1:1:2 ratio according to a randomisation plan (see figure 3.1: 1 for details). The assignment will occur in a blinded fashion via Interactive Response Technology (IRT).

4.1.4 Drug assignment and administration of doses for each patient

IRT will allocate medication kit numbers at Visit 2, 4, and 6. The amount of trial medication dispensed and returned will be recorded on drug accountability forms.
For blinding reasons all treatments will consist of five tablets verum or placebo to be taken within 10-15 minutes in the morning depending on the treatment arm. This will not be changed during the entire study treatment period (V2-EOT).
For days without site visits, patients should be instructed to take the tablets orally with water in the morning before breakfast at approximately the same time every day without food (defined as no food intake until 60 mins after dosing). If a dose is missed by more than 8hrs, that dose should be skipped and the next dose should be taken as scheduled. No double doses should be taken and dose reductions are not permitted. Patients should be instructed to bring all unused drug and empty study blister to the study site.
Patients should be instructed NOT to take their study medication on the morning of scheduled trial visits. The first dose of study medication will be taken at the visit 2 under supervision of the investigator or site staff. At all site visits the morning dose of the investigational drug will be taken during the visit under supervision of the investigator or relevant site staff. The actual visit date and time of study drug administration at the trial visit will be recorded in the eCRF at each visit. Patients should be fasted at the beginning of the study visit. Patients who erroneously take the morning dose of study medication before coming to the clinic at a visit with scheduled PK samples should have the visit rescheduled as soon as possible, ideally on the following day. Patients will record the administration dates and times of all doses with the help of a diary as outlined in the Flow Chart and in Section 6.2.2.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

Patients, investigators and everyone involved in trial conduct or analysis or with any other interest in this double-blinded trial will remain blinded with regard to the randomized treatment assignments until after database lock. Please refer to Section 4.1.5.2 for rules of breaking the code for patients in emergency situations.

The randomization code will be kept secret by Clinical Trial Support up to database lock. The randomization codes will be provided to bioanalytics prior to last patient out to allow for the exclusion from the analyses of PK samples taken from placebo patients and to a member of the unblinded team in preparation of a possible interim PK/PD analysis. Bioanalytics will not disclose the randomization code or the results of their measurements until the trial is officially unblinded.

A possible interim PK/PD analysis will be performed by an unblinded team independent from the trial and project teams to maintain the blinded conduct of the study. Results of the interim analysis will only be presented as aggregated data and not on subject level to maintain blinding. The logistical aspects of conducting the interim PK / PD analysis, and the plan to access of interim results will be described in the Interim Analysis Logistics Plan and Results Access Plan.

4.1.5.2 Unblinding and breaking the code

Emergency unblinding will be available to the Investigator / Pharmacist / investigational drug storage manager via IRT. It must only be used in an emergency situation when the identity of the trial drug must be known to the Investigator in order to provide appropriate medical treatment or otherwise assure safety of trial participants. The reason for unblinding must be documented in the source documents and/or appropriate CRF page along with the date and the initials of the person who broke the code.

Due to the requirements to report Suspected Unexpected Serious Adverse Reactions (SUSARs), it may be necessary for a representative from Boehringer Ingelheim' s Pharmacovigilance group to access the randomisation code for individual patients during trial conduct. The access to the code will only be given to authorised Pharmacovigilance representatives and not be shared further.

4.1.6 Packaging, labelling, and re-supply

The investigational products will be provided by BI or a designated CRO. They will be packaged and labelled in accordance with the principles of Good Manufacturing Practice (GMP). Re-supply to the sites will be managed via an IRT system, which will also monitor expiry dates of supplies available at the sites.
For details of packaging and the description of the label, refer to the ISF.

4.1.7 Storage conditions

Drug supplies will be kept in their original packaging and in a secure limited access storage area according to the recommended storage conditions on the medication label. IMP needs to be shipped and stored under refrigerated conditions (2-8°C / 36-46°F). A temperature log must be maintained for documentation.

If the storage conditions are found to be outside the specified range, the local clinical monitor (as provided in the list of contacts) must be contacted immediately.

4.1.8 Drug accountability

The Investigator, Pharmacist or investigational drug storage manager will receive the investigational drugs delivered by the sponsor when the following requirements are fulfilled:

- Approval of the trial protocol by the IRB / ethics committee
- Availability of a signed and dated clinical trial contract between the sponsor and the head of the investigational site,
- Approval/notification of the regulatory authority, e.g. competent authority,
- Availability of the curriculum vitae of the principal Investigator,
- Availability of a signed and dated clinical trial protocol
- Availability of the proof of a medical license for the principal Investigator
- Availability of Form 1572

The Investigator, Pharmacist or investigational drug storage manager must maintain records of the product's delivery to the trial site, the inventory at the site, the use by each patient, and the return to the sponsor or warehouse / drug distribution centre or alternative disposal of unused products. If applicable, the sponsor or warehouse / drug distribution centre will maintain records of the disposal.

These records will include dates, quantities, batch / serial numbers, expiry ('use- by') dates, and the unique code numbers assigned to the investigational product and trial patients. The Investigator / Pharmacist / investigational drug storage manager will maintain records that document adequately that the patients were provided the doses specified by the CTP and reconcile all investigational products received from the sponsor. At the time of return to the sponsor< and/or >appointed CRO, the Investigator / Pharmacist / investigational drug storage manager must verify that all unused or partially used drug supplies have been returned by the clinical trial patient and that no remaining supplies are in the Investigator's possession.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

There are no special emergency procedures to be followed.

4.2.2 Restrictions, warnings and precautions

4.3 TREATMENT COMPLIANCE

Patients are requested to bring all remaining trial medication including empty package material with them when attending visits.
Based on tablet counts, treatment compliance will be calculated as the number of tablets taken, divided by the number of tablets which should have been taken according to the scheduled period, multiplied by 100. Compliance will be verified by the on-site monitor authorised by the sponsor.

$$\text{Treatment compliance (\%)} = \frac{\text{Number of tablets actually taken} \times 100}{\text{Number of tablets which should have been taken}}$$

If the number of doses taken is not between 80-120%, site staff will explain the patient the importance of treatment compliance.

5. VARIABLES AND THEIR ASSESSMENT

5.1 TRIAL ENDPOINTS

5.1.1 Primary Endpoint(s)

The primary endpoint is the plasma amine oxidase copper-containing 3 (AOC3) activity relative to baseline in %, 24 h post dose, after 12 weeks of treatment. The baseline is defined as the last AOC3 activity measurement prior to administration of any randomised study medication.

5.1.2 Secondary Endpoint(s)

Safety and tolerability will be assessed based on the number (%) of subjects with adverse reactions.

The secondary biomarker endpoints will be assessed based on the
relative ALT change from baseline after 12 weeks of treatment
relative AST change from baseline after 12 weeks of treatment
relative AP change from baseline after 12 weeks of treatment
relative γ-GT change from baseline after 12 weeks of treatment
relative caspase cleaved cytokeratin 18 (M30) change from baseline after 12 weeks of treatment
relative total cytokeratin 18 (M65) change from baseline after 12 weeks of treatment

5.2 ASSESSMENT OF EFFICACY

Not Applicable

5.3 ASSESSMENT OF SAFETY

5.3.1 Physical examination

A full physical examination will be performed at the visits indicated in the Flow Chart. This should be performed according to medical standards and usually includes (but is not necessarily limited to) a review of the following organ systems: General appearance (including Skin), Head-Eyes-Ears-Nose-Throat (HEENT), Chest (including Pulmonary and Heart), Abdomen, Extremities, Urogenital and neurological assessment (basic mental status, cranial nerves, motor system, sensation, cerebellum/coordination). Clinically relevant abnormal findings noticed after randomisation will be reported as (S)AEs. At all other visits according to the Flow Chart a symptom-derived physical examination should be performed. This includes vital sign assessment as well as an evaluation of the organ systems associated with AE(s) symptoms or laboratory abnormalities.
Clinically significant abnormal findings will be reported as baseline conditions or AE's.

5.3.1.1 Waist and hip circumference

Waist and hip circumference will be measured at screening and EOT/ED visit.
Waist circumference measurements should be made around a patient's bare midriff, after the patient exhales while standing without shoes and with both feet touching and arms hanging freely. The measuring tape should be placed perpendicular to the long axis of the body and horizontal to the floor and applied with sufficient tension to conform to the measurement surface. Waist circumference should be determined by measuring the midpoint between the lowest rib and the iliac crest.
Hip circumference measurement should be taken around the widest portion of the buttocks.

5.3.1.2 Body weight

The scale used to capture body weight for each patient should remain consistent during the trial. In order to get comparable body weight values, it should be performed in the following way:
- Fasting (except for the screening visit);
- After the urine sampling (body weight after bladder voiding);
- Shoes and coat/jackets should be taken off; and
- Pockets should be emptied of heavy objects (i.e. keys, coins etc.)

5.3.2 Vital Signs

Systolic and diastolic blood pressure (BP), respiratory frequency (RF) and pulse rate (PR) will be measured after the patient has rested for at least 5 min in the sitting position.

Measurement of vital signs should precede blood sampling to avoid the impact of blood sampling on the vital measurements. The measured vital signs will be documented in the source documents and recorded in the eCRF.

5.3.3 Safety laboratory parameters.

Parameters that will be determined during the trial conduct are listed in Table 5.3.3: 1 and will be collected by the trial site at the time points indicated in the Flow Chart. All analyses will be performed by a central laboratory. Patients have to be fasted (except for the screening visit) for at least 8 h before the blood sampling for the safety laboratory. The respective reference ranges and details about sample handling shipment and results reporting will be provided in the ISF (Lab Manual).
Clinically significant abnormal findings will be reported as baseline conditions or AE's.

Table 5.3.3: 1 Safety laboratory parameters – whole blood, serum or plasma

Hematology	
Haematocrit	
Haemoglobin	WBC / Leukocytes
MCV, MCH, RDW, MCHC	Platelet Count / Thrombocytes
Red Blood Cells (RBC) / Erythrocytes	Differential Automatic (relative and absolute count): Neutrophils, Eosinophils, Basophils, Monocytes, Lymphocytes
Coagulation	
Activated Partial Thromboplastin Time (aPTT)	
Prothrombin Time (INR)	
Fibrinogen	
Clinical chemistry	
Albumin	
Alkaline phosphatase	Creatine kinase (CK)
γ-GT (gamma-glutamyl transferase)	CK-MB, troponin I (reflex tests if CK is elevated)
ALT (alanine aminotransaminase, SGPT)	eGFR*
AST (aspartate aminotransaminase, SGOT)	Fasting Plasma Glucose
Bicarbonate	Ferritin
Bilirubin Total	Plasma Insulin
Bilirubin Direct	Serum Free fatty acids
Bilirubin Indirect	Lactate dehydrogenase (LDH)
Calcium	Lipase
Chloride	Magnesium
Creatinine	Phosphate
hsC-reactive protein	Potassium
	Protein total
	Sodium
	Urea (BUN)
	LDL/HDL and total cholesterol
	Triglycerides
	TSH

* Estimated Glomerular filtration rate as assessed by the CKD-EPI formula (2009).

Urine chemistry

Urine creatinine (for urine biomarker assessment, please see section 5.5.2)

Pregnancy Test

- Urine Pregnancy test[1]
- Serum Pregnancy test[2]

[1] Urine pregnancy test performed on-site at all dosing visits (pre-dose) as well as EOT and FU (only for female patients of childbearing potential).

[2] Serum pregnancy test at screening as well as confirmation of positive urine pregnancy test (only for female patients of child bearing potential at screening as well as reflex for positive urine pregnancy test).

5.3.4 Electrocardiogram

Printed paper traces from 12-lead ECGs in triplicate will be recorded at the visits outlined in the flowchart for all patients approximately 90 min after study drug intake. At visits without drug administration ECGs should preferentially be performed prior blood sampling. Please refer to the Flow Charts 1, 2, 3 and 4 for exact timing. ECGs will be recorded after the patients have rested for at least 5 minutes in a supine position. Six limb leads, as specified by Einthoven (I, II and III) and Goldberger (aVR, aVL, aVF), and six pre-cordial leads (V1–V6), according to Wilson, will be used. ECGs may be repeated for quality reasons and the repeated recording used for analysis.
In the event of any clinical cardiac symptoms (e.g. suspicion of heart rhythm disorders or cardiac ischaemia), an additional ECG will be recorded at the investigator´s discretion.
At screening visit all triplet ECGs will be printed and evaluated (signed, dated and commented upon) by the treating physician/ investigator and will be filed in the patient's source documents. This will be used as baseline before the first drug administration.
At all other visits, only the first of the three replicate ECGs at a single assessment time will be evaluated by the treating physician/ investigator and stored locally. The remaining second and third replicate ECGs will be printed and kept together with other medical records of the patient for additional analyses if required at a later time point.

All ECGs recorded during trial conduct including the baseline ECG will also be transmitted to a vendor for central evaluation and stored at an external central ECG database. Only the first of the three replicate ECGs at a single assessment time will be centrally evaluated and transferred to the sponsor. The results of the centralized evaluation will be sent from the ECG core lab to the sponsor according to a pre-specified data transmission agreement. The remaining second and third replicate ECGs will only be stored at the ECG vendor for additional analyses if required at a later time point. The central reader's evaluation of the tracing is considered the official reading for the trial.

In case of discrepancies between investigator and central reading, the central evaluation will be valid.
Clinically significant abnormal findings will be reported either as baseline condition (if identified at the screening visit) or otherwise as adverse events and will be followed up and/or treated locally until normal or stable condition.

Information about the details of ECG collection will be provided in the ISF.

5.3.5 Assessment of adverse events

5.3.5.1 Definitions of AEs

Adverse event
An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Serious adverse event

A serious adverse event (SAE) is defined as any AE which:
- results in death,
- is life-threatening, this refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if more severe.
- requires inpatient hospitalisation or
- prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity, or
- is a congenital anomaly / birth defect,
  or
- is to be deemed serious for any other reason if it is an important medical event when based upon appropriate medical judgment which may jeopardise the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions.

Medical and scientific judgement should be exercised in deciding whether other situations should be considered serious reactions, such as important medical events that might not be immediately life threatening or result in death or hospitalisation but might jeopardise the patient or might require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or

development of dependency or abuse. Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

AEs considered "Always Serious"
Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the duration between discontinuation of the drug and must be reported as described in 5.3.5.2, subsections "AE Collection" and **AE reporting to sponsor and timelines"**
In accordance with the European Medicines Agency initiative on Important Medical Events, Boehringer Ingelheim has set up a list of further AEs, which by their nature, can always be considered to be "serious" even though they may not have met the criteria of an SAE as defined above.

The latest list of "Always Serious AEs" can be found in the EDC system. A copy of the latest list of "Always Serious AEs" will be provided upon request. These events should always be reported as SAEs as described above.

Adverse events of special interest (AESIs)
The term AESI relates to any specific AE that has been identified at the project level as being of particular concern for prospective safety monitoring and safety assessment within this trial, e.g. the potential for AEs based on knowledge from other compounds in the same class.
AESI need to be reported to the sponsor's Pharmacovigilance Department within the same timeframe that applies to SAE, please see above.
The following are considered as AESIs:

Hepatic injury
A hepatic injury is defined by the following alterations of hepatic laboratory and clinical parameters as defined by the removal and stopping criteria in section 3.3.4.1.1 and section 10.3.

These lab findings constitute a hepatic injury alert and the patients showing these lab abnormalities need to be followed up according to the "DILI checklist" provided in the ISF. In case of clinical symptoms of hepatic injury (e.g. encephalopathy, nausea, vomiting, pruritus, severe fatigue, icterus, etc.) without lab results (ALT, AST, total bilirubin) available, the Investigator should make sure these parameters are analysed, if necessary in an unscheduled blood test. Should the results meet the criteria of hepatic injury alert, the procedures described in the DILI checklist should be followed.

Intensity of AEs

The intensity (severity) of adverse events should be classified and recorded in the (e)CRF according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 dated 14 June 2010 [R10-4848].

Causal relationship of AEs

The definition of an adverse reaction implies at least a reasonable possibility of a causal relationship between a suspected medicinal product and an adverse event. An adverse reaction, in contrast to an adverse event, is characterised by the fact that a causal relationship between a medicinal product and an occurrence is suspected.
Medical judgment should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history.

Arguments that may suggest that there is a reasonable possibility of a causal relationship could be:

1. The event is consistent with the known pharmacology of the drug
2. The event is known to be caused by or attributed to the drug class.
3. A plausible time to onset of the event relative to the time of drug exposure.
4. Evidence that the event is reproducible when the drug is re-introduced
5. No medically sound alternative aetiologies that could explain the event (e.g. pre-existing or concomitant diseases, or co-medications).
6. The event is typically drug-related and infrequent in the general population not exposed to drugs (e.g. Stevens-Johnson syndrome).
7. An indication of dose-response (i.e. greater effect size if the dose is increased, smaller effect size if dose is diminished).

Arguments that may suggest that there is no reasonable possibility of a causal relationship could be:

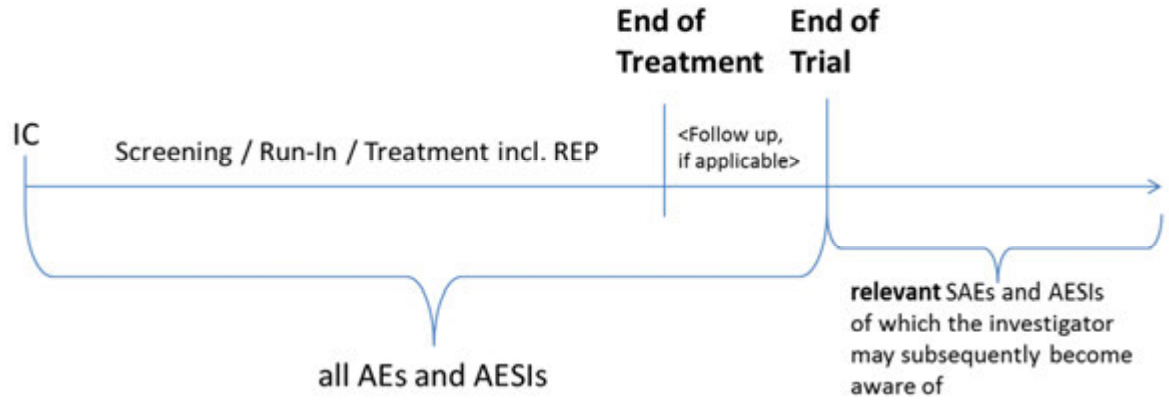
1. No plausible time to onset of the event relative to the time of drug exposure is evident (e.g. pre-treatment cases, diagnosis of cancer or chronic disease within days / weeks of drug administration; an allergic reaction weeks after discontinuation of the drug concerned)
2. Continuation of the event despite the withdrawal of the medication, taking into account the pharmacological properties of the compound (e.g. after 5 half-lives).
   Of note, this criterion may not be applicable to events whose time course is prolonged despite removing the original trigger.
3. Additional arguments amongst those stated before, like alternative explanation (e.g. situations where other drugs or underlying diseases appear to provide a more likely explanation for the observed event than the drug concerned).
4. Disappearance of the event even though the study drug treatment continues or remains unchanged.

5.3.5.2 Adverse event collection and reporting

AE Collection
The Investigator shall maintain and keep detailed records of all AEs in their patient files. The following must be collected and documented on the appropriate CRF(s) by the Investigator:

- From signing the informed consent onwards through the Residual Effect Period (REP), until individual patient's end of trial:
  - all AEs (serious and non-serious) and all AESIs.

  However, if an individual patient discontinues trial medication prematurely but stays in the trial (i.e. if further visits incl. telephone visits, or vital status assessments are planned) from then on and until the individual patient's end of the trial the Investigator must report related SAEs and related AESIs.
- After the individual patient's end of trial:
  the Investigator does not need to actively monitor the patient for AEs but should only report relevant SAEs and relevant AESIs of which the Investigator may become aware of.



As the REP is to be explored in this trial, it is considered as the entire FU period from last dose administration of trial medication until individual patient's end of trial.
All AEs which occurred through the treatment phase until entire Follow-up period will be considered as on treatment, please see section 7.3.4.

AE reporting to sponsor and timelines
The Investigator must report SAEs, AESIs, and non-serious AEs which are relevant for the reported SAE or AESI, on the BI SAE form via fax immediately (within 24 hours) to the sponsor's unique entry point (country specific contact details will be provided in the ISF). The same timeline applies if follow-up information becomes available. In specific occasions the Investigator could inform the sponsor upfront via telephone. This does not replace the requirement to complete and fax the BI SAE form.

With receipt of any further information to these events, a follow-up SAE form has to be provided. For follow-up information the same rules and timeline apply as for initial information.

Information required
For each AE, the Investigator should provide the information requested on the appropriate CRF pages and the BI SAE form. The Investigator should determine the causal relationship to the trial medication.
The following should also be recorded as an (S)AE in the CRF and SAE form (if applicable):

- Worsening of the underlying disease or of other pre-existing conditions
- Changes in vital signs, ECG, physical examination and laboratory test results, if they are judged clinically relevant by the Investigator.

  If such abnormalities already pre-exist prior trial inclusion they will be considered as baseline conditions.

All (S)AEs, including those persisting after individual patient's end of trial must be followed up until they have resolved, have been sufficiently characterised, or no further information can be obtained.

Pregnancy
In rare cases pregnancy may occur in a clinical trial. Once a patient has been enrolled into this clinical trial and has taken trial medication, the Investigator must report immediately (within 24 hours) a potential drug exposure during pregnancy (DEDP) to the sponsor's unique entry point (country-specific contact details will be provided in the ISF). The Pregnancy Monitoring Form for Clinical Trials (Part A) should be used.

The outcome of the pregnancy associated with the drug exposure during pregnancy must be followed up and reported to the sponsor's unique entry point on the Pregnancy Monitoring Form for Clinical Trials (Part B).
The ISF will contain the Pregnancy Monitoring Form for Clinical Trials (Part A and B).

As pregnancy itself is not to be reported as an AE, in the absence of an accompanying SAE and/or AESI, only the Pregnancy Monitoring Form for Clinical Trials and not the SAE form is to be completed. If there is an SAE and/or AESI associated with the pregnancy an SAE form must be completed in addition.

5.5 ASSESSMENT OF BIOMARKER(S)

5.5.2 Biochemical and cellular biomarkers

Concentration and activity of AOC3 in plasma will be measured at the time points indicated in the Flow Charts 1, 2, 3, 4 and in Section 10.1. The values determined prior to the first dosing of BI 1467335 will be defined as baseline.
AOC3 activity per time point will be presented relative to baseline. AOC3 concentration will be presented as absolute values and relative to baseline.

The following parameters will be determined for plasma AOC3 activity relative to baseline, if feasible; after multiple dosing the parameters will be denoted with the index 'N', where N is the number of doses administered.

E_{max} (maximum plasma AOC3 activity relative to baseline within the dosing interval)

E_{min} (minimum plasma AOC3 activity relative to baseline within the dosing interval)

t_{max} (time from dosing to reach E_{max})

t_{min} (time from dosing to reach E_{min})

$E_{pre,N}$ (plasma AOC3 activity relative to baseline prior to the N_{th} dose of BI 1467335)

.

For the secondary endpoints ALT, AST, ALP, γ-GT, caspase cleaved cytokeratin 18 (M30) and total cytokeratin 18 (M65) samples will be collected as described in the Flow Chart 1.

Relative changes from baseline in ALT, AST, AP,γ-GT and Cytokeratine18 fragments after 2, 4, 8, 12 weeks of treatment and 4 weeks after last dose will be determined.

For the endpoints and exploratory biomarkers clear endpoint definitions and further details on evaluation will be provided in the TSAP.

5.5.3 Methods of sample collection

For the measurement of the activity and the concentrations of AOC3 4.0 ml of blood will be taken from an antecubital or forearm vein into a K2-EDTA anticoagulant blood drawing tube at the time points indicated in the Flow Chart. 2 x 4.0 ml will be taken at Visit 2 to have a back-up sample for the pre-dose value.
Blood will be withdrawn by means of either an indwelling venous catheter or by venepuncture with a metal needle.

For the secondary endpoints and the exploratory biomarkers evaluation approximately 23 ml of blood (10 ml for K2-EDTA blood and 10 ml for serum and 3ml for sodium citrate coagulation tube) will be taken at the time points indicated in the Flow Chart 1. Blood will be withdrawn by means of either an indwelling venous catheter or by venepuncture with a metal needle.
Further details on collection, handling, storage and processing of the blood sample will be provided in a separate laboratory instruction manual.

All left over samples will be stored and used for not yet specified explorative investigations. These samples will be stored for up to three years after the end of the clinical trial and may be used for not yet specified biomarker analyses to enable further characterization of metabolic diseases and their progress, as well as method development and evaluation. Results of these assessments will not be part of the Clinical Trial Report.

5.5.5 Appropriateness of Measurements

All measurements performed during this trial are standard measurements and will be performed in order to monitor subjects'safety and to determine pharmacokinetic and pharmacodynamic parameters in an appropriate way. The scheduled measurements will allow monitoring of changes in vital signs, standard laboratory values, and ECG parameters that might occur as a result of administration of trial medication. The safety assessments are standard, are accepted for evaluation of safety and tolerability of an orally administered drug, and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in Section 5.4 are generally used assessments of drug exposure.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

All patient visits should be scheduled according to the Flow Chart. Each visit date (with its window) is to be counted from Day 1. If any visit has to be rescheduled, subsequent visits should follow the original visit schedule. The trial medication packs contain sufficient medication to allow for these time windows.
All trial visits should be initiated preferentially in the morning starting before 9:00 AM.
Patients should be instructed to avoid intake of the morning dose of the study medication at home at scheduled visit days as they will be dosed whilst at the study site.
Study measurements and assessments according to the Flow Chart scheduled to occur before study drug administration on Day 1, 15, 29, 43, 57 and 85 are to be performed and completed within a 2 h-period prior to the study drug administration.
Unscheduled visits will be possible at the discretion of the investigator at any time in order to check the safety of the patient.
If the reason for removal of a patient from the treatment is an adverse event or an abnormal laboratory test result, the patient must be followed until complete resolution or stabilization of the event or until follow-up is agreed adequate by the Investigator and BI Clinical Monitor.

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening

Screening Period
All patients must sign an Informed Consent consistent with ICH-GCP guidelines and the local legislation prior to any study specific procedures. Once they have consented, the patient is considered to be enrolled in the trial and have started screening. The patient should be recorded on the enrolment log and be registered in IRT as a screened patient. Patients will be assigned a patient number and enrolment must be recorded in eCRF pages. The Screening period is defined as the period from the Screening visit to Randomisation (first study drug administration). The screening period should be no longer than 28 days and no less than 7 days and will be used to assess eligibility of the patients. Thus patients will not be randomized until all screening procedures are completed and results are reviewed to verify study eligibility. Please refer to Section 8.1.
For the comprehensive list of the trial procedures required at the Screening Visit (Visit 1) please refer to the Flow Chart.
Patients are allowed to repeat lab testing once during the screening period.
For those patients with historic ALT > 1.25 ULN value more than 3 months prior to screening, the ALT > 1.5x ULN must be confirmed via two measurements at least 1 week apart within the screening period.

Patients without required clinical imaging results suggestive of NASH (no more than 3 years prior to screening, according to inclusion criterion #1), clinical imaging can be performed as optional procedure during the screening period. The imaging procedures will be performed according to local standards. Please refer to Flow Chart 1.
No imaging is required if histological evidence of NASH (no more than 3 years prior to screening) is available. Please refer to section 3.3.2

Demographics
Information on race will be collected because this is required for the calculation of eGFR (CKD-EPI formula).

Re-screening
Patients who screen-fail the study after Visit 1 should be registered in the IRT as a screen failure within the protocol defined screening period.
Re-screening of not yet randomised patients can be allowed in exceptional cases but should be discussed on a case-by-case basis between the study site, Monitor staff and with the TCM.

IRT
All patients that are screened must be registered with IRT. If the patient results in a screen failure, IRT should be notified as soon as possible and within the 28- day screening period.
Details of IRT procedures can be found in the IRT manual located in the ISF

6.2.2 Treatment period

Visits 2, 3, 4, 5, 6 and EOT
The treatment period consists of a maximum of 6 visits (visits 2 - EOT). Visit 2 is the randomisation visit and visit EOT is the end of treatment visit, where the last dose of medication will be administered. Procedures are to be performed according to the flowcharts 1, 2, 3, 4, section 10.1 and the respective protocol sections.

Safety laboratory testing
Patients should be fasted (no food and only water) for at least 8 hours prior to the start of procedures until 60 mins after drug intake. If a patient comes in non-fasted where a fasting condition is required, the visit should be performed, the non-fasted condition documented on the laboratory requisition, and the patient reminded about the expected condition.

Pregnancy testing
Urine pregnancy testing for all women of childbearing potential will be conducted on-site prior to every dosing and must be negative to further treat the patient. A positive urine test must be confirmed with a serum pregnancy test.

Randomisation (Visit 2)
Randomisation via IRT and administration of the first dose of study medication will occur at Visit 2.

Study drug diary
The study patients will be instructed to complete a study drug diary during the treatment phase to document drug intake (date and time) and number of the tablet row indicating the day of intake between the on-site visits.
The diary will be dispensed to the patients at Visit 2 and the patient´s entries will be checked by site-staff during the consecutive treatment visits. Selected entries (drug administration date and time 3 days prior to each visit) will be entered by site-staff into the CRF.

Pharmacokinetic and biomarker blood sampling
The blood sampling times given in Section 10.1 are the recommended sampling times relative to the drug administration and should be adhered to as closely as possible. The actual times of drug administration and blood samplings will be recorded in the CRF.
Please refer to the Flow Charts 1, 2, 3 and 4 for exact timing.

Telephone contacts
Day 27/ Phone Call 3A:
The site staff should call the patients on day 27 to remind them of the urine collection starting directly after the drug administration at day 28 (24h prior to planned visit 4/ day 29 drug administration) and study drug intake.
Day 83/ Phone Call 6A:
The site staff should call the patients on day 83 to remind them of study drug intake on day 84. Please refer to section PK urine collection above and Flow Charts 1, 4 and section 10.1.

End of Treatment (EOT) Visit/ Early Discontinuation (ED):

If the regular end of the treatment period at week 12 is reached, the EOT Visit will be completed. The time interval for the regularly scheduled EOT Visit is 4 days, i.e. Day 85+3. The overall duration of the treatment period (randomisation to EOT) should not be less than 85 days.
If the patient refuses to continue the treatment in the trial or must stop treatment the early discontinuation (ED) Visit will be completed instead of the planned treatment period visit. This early discontinuation visit will include the same procedures as the normal EOT visit except drug administration, PK, PG and PD sampling. Visit FU should be performed no later than four weeks after the last study drug intake.

It is important to distinguish between premature study drug discontinuation and complete withdrawal of consent to participate in further study procedures. Patients not willing to continue study drug intake should be asked to further attend scheduled trial visits, follow-up visits and assessments until the end of the trial unless they withdraw consent to participate in the study. For patients who discontinue IMP but are willing to attend scheduled trial visits, the early discontinuation (ED) Visit will be completed according to FlowChart instead of the next planned treatment period visit. All following visits should be performed according to the initial study schedule (without study drug administration). In addition to the safety sampling, only one PK sample and one PD blood sample for measurement of AOC3 activity should be taken at each visit on which PK and/or PD sampling was originally planned and the actual time of the blood draw recorded. One of these remaining visits should be performed no later than four weeks after the last study drug intake.
Should it not be possible to attend all visits, at least phone contacts should occur at the scheduled visits time points. It is vital to explain to these patients the importance to continue trial participation.

All unused study medication will be collected and the study drug diary will be checked by the site. Procedures are to be performed according to the Flow Chart.

6.2.3 Follow Up Period and Trial Completion

Follow-up (FU):
This visit will be performed 28±3 days after EOT (see Flow Chart 1 and Section 10.1 for procedures to be performed).
In case of early discontinuation this visit will include the same procedures as the normal FU visit except PG and PD sampling.
All abnormal values (including laboratory parameters) that are judged clinically relevant by the investigator will be monitored using the appropriate tests until a return to a medically acceptable level is achieved. Adverse events persisting after trial completion must be monitored until they have normalised or have been sufficiently characterised.

Trial completion:
Trial completion is defined as patients completing the FU visit within the specified window per the flow chart and who have not discontinued drug prematurely.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN - MODEL

See Section 3.1 for details on the design of the study.
See Section 2.2 for details on the objective of the study.

Primary endpoint is the plasma AOC3 activity relative to baseline in %, 24 hours post dose, after 12 weeks of treatment. Baseline is defined as the last measured plasma AOC3 activity prior to administration of any randomised study medication.

The dose-response relationship of the primary endpoint will be analysed using a nonlinear regression model applied to the AOC3 activity at week 12. The fitted regression model will be used to derive the smallest dose where the mean plasma AOC3 activity curve drops below 10% (D10). This corresponds to:

$$\mathrm{D10} = \min\{d \in [0,10] | \, \widehat{f(d)} \leq 10\%\}$$

where d is the dose and $\widehat{f()}$ is the fitted dose-response curve described in Section 7.3.1.

The analyses of the secondary biomarker endpoints ALT AST, AP, γ-GT and CK18 fragments will follow the MCPmod approach allowing for the simultaneous evaluation of different potential dose response patterns, whilst protecting the overall probability of type I error (one-sided alpha of 0.05).

7.2 NULL AND ALTERNATIVE HYPOTHESES

In this trial it is not planned to test any statistical hypotheses in a confirmatory sense. Where statistical testing is used it will have to be interpreted in the perspective of the exploratory character of the study.

No hypothesis testing will be used for the primary analysis of the primary endpoint.
The analyses of the secondary biomarker endpoints ALT, AST, AP, γ-GT and CK18 fragments contain hypotheses testing. The null hypothesis is that there is a flat dose response pattern across placebo and any dose of BI 1467335 within the tested dose range (0-10mg). The alternative hypothesis is that there is a non-flat dose response pattern indicating a benefit of BI 1467335 compared to placebo.

7.3 PLANNED ANALYSES

The following patient analysis sets are defined for this trial:

- Treated Set (TS): includes all patients who signed informed consent and were treated with at least one dose of the trial medication. Patients in TS are analyzed under the actual trial medication received at randomisation. The TS is used for safety analyses as well as demographics and baseline characteristics.

- Full Analysis Set (FAS): includes all patients in treated set who had non-missing baseline and at least one non-missing post-baseline and on-treatment measurement on any biomarker endpoint. Patients in FAS are analyzed according to the intent-to-treat principle, i.e., patients in FAS are analyzed under the randomized trial medication.

- Per protocol set (PPS): includes all patients from the FAS without important protocol violations (IPV) leading to exclusion. This includes:

Compliance to randomised treatment within 80% -120% (inclusive)
At least 80% treatment compliance within 4 weeks before last visit

Data from subjects who are screened but not randomised will be listed but not included in any summary statistics or inferential statistics. Specifications of important protocol violations leading to exclusion will be provided in the TSAP.

Further Analysis sets will be defined in the TSAP.

7.3.1 Primary endpoint analyses

The primary analysis of the primary endpoint will be based on the PPS.

The subject specific plasma AOC3 activity at time t relative to baseline in % is calculated via

$$\%AOC3a_t := \left[\left(AOC3a_t - AOC3a_{t,back}\right)/\left(AOC3a_{base} - AOC3a_{base,back}\right)\right] * 100$$

Where $AOC3a_t$ is the AOC3 activity measured at time t, $AOC3a_{t,back}$ is the background noise at time t, $AOC3a_{base}$ is the AOC3 activity measured at baseline and $AOC3a_{base,back}$ is the background noise at baseline.

As the primary endpoint should show a reduction in AOC3 activity with higher doses, a decreasing 3-Parameter regression curve will be used of the form

$$\%AOC3a_{12,i} = \frac{TOP * D_i^N}{ED50^N + D_i^N} + \varepsilon_i$$

where

$\%AOC3a_{12,i}$	is the response value of patient i, 24h after drug administration at week 12 (EOT visit),
TOP	is the highest AOC3 activity,
D_i	is the administered drug amount to patient i
$ED50$	is the dose, which produces half of *TOP*
N	is the slope factor determining the steepness of the dose-response curve,
ε_i	is the random error term of patient i.

Note that the dose-response relationship is assumed to asymptotically approach 0% at high doses.

As it is expected to see a higher variability in AOC3 activity measurements in placebo treated patients then in patients treated with BI 1467335 the model fit will use power of mean variance estimates (POM) to account for heterogeneity.

In case of the 24h value after drug administration at week 12 is missed, the -0:30 values at week 12 can be used instead. In case of other missing AOC3 activity values, except of missing baseline where no imputation is done, multiple imputation will be used. After exploring the missing data mechanism and observed measurements on the blinded data the variable AOC3 activity relative to baseline in % will be included in the imputation model.
If the data is monotone missing, a regression model will be used for imputation including baseline AOC3 activity, and AOC3 activity relative to baseline measurements predose (-0:30h relative to drug administration) at Visit 4 and 6 and 24h relative to drug administration at visit 3,5 and EOT by treatment.
In the case of non-monotone missing, a Markov chain Monte Carlo (MCMC) step will be used to create monotone missing data in multiple datasets. The regression method will then be used to complete the imputation in each dataset. For each imputed complete dataset, the decreasing 3-parameter curve model will be used for the analysis. The results will be pooled following the standard multiple imputation procedure.

The dose where the model fit drops below 10% and the 95% confidence interval will be determined (D10). The fitted curve will be graphically displayed.

7.3.2 Secondary endpoint analyses

The number (%) subjects with adverse reactions will be evaluated descriptively on the TS. For more details see Section 7.3.4.

The primary analysis of the secondary biomarker endpoints will be first analysed using a repeated measurement analysis (MMRM) on the PPS. The relative ALT (AST, AP, γ-GT and CK18 fragments respectively) changes from baseline at visit 3,4,5,6 and EOT will be evaluated on log scale using an MMRM accounting for the following sources of variation: 'baseline', 'visit', 'treatment', 'visit*treatment' and 'visit*baseline' interaction as fixed effect as well as the random 'subject' effect. The unstructured covariance structure will be used to model the within patient measurements. To estimate denominator degrees of freedom the Kenward-Roger approximation will be used.
The dose-response relationship of the week 12 estimates from MMRM will then be analysed using MCPMod. A set of plausible dose response patterns is considered with pre-specified parameters define:

The following model assumptions have been selected to cover a plausible and diverse range of dose response patterns when having matching Placebo and active BI 1467335 doses of 1 mg, 3 mg, 6 mg and 10 mg:

- Linear: no assumptions needed
- Linear logistic no assumptions needed
- Quadratic: 90 % of the maximum effect is achieved at 7 mg
- Exponential: 90 % of the maximum effect is achieved at 7 mg
- Emax: 90 % of the maximum effect is achieved at 7 mg
- Sigmoidal Emax: 30 % of the maximum effect is achieved at 3 mg and 90 % of the maximum effect is achieved at 7 mg
- Logistic: 30 % of the maximum effect is achieved at 3 mg and 90 % of the maximum effect is achieved at 7 mg
- Betamod: 90 % of the maximum effect is achieved at 5 mg, maximum effect is achieved at 4 mg and maximal dose is 10 mg

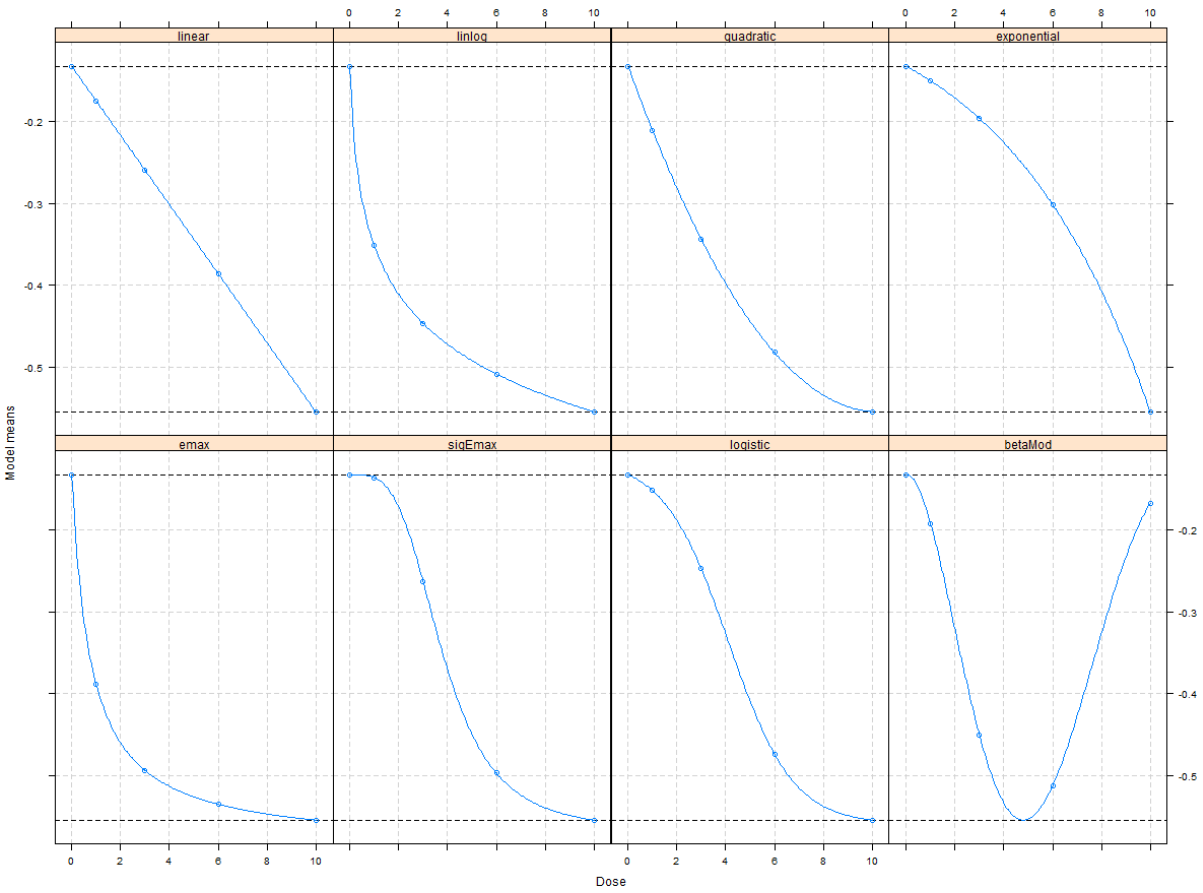


Figure 7.3.2: 1 Defined MCPMod shapes to be used.

If there is a at least one significant model, the null hypothesis of a flat dose-response relationship for the secondary endpoint ALT (for AST, AP, γ-GT and CK18 fragments respectively) jointly for each of the candidate dose-response models with a contrast test controlled for the family-wise error rate (α = 0.05 1-sided) will be rejected.
When the null hypothesis is rechecked, the best-fitting model from the above set of eight models can be refitted to the data without any parameter assumptions to generate new estimates of the model parameters from the data. The target dose will be obtained via model averaging across the significant models based on Akaike Information Criterion (AIC) (the smaller the AIC value the better the model fit). The target dose(s) can then be determined from that model by incorporating information on the minimum clinically relevant effect as well as safety information.

Further details on the primary analysis, will be provided in the TSAP.

7.3.4 Safety analyses

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Standard BI summary tables and listings will be produced. All adverse events with an onset between start of treatment and individual patient's end of trial will be assigned to the treatment period for evaluation.
The analysis will be done by 'treatment at onset'.
All treated patients will be included in the safety analysis (analysis on TS). In general, safety analyses will be descriptive in nature and will be based on BI standards. No hypothesis testing is planned.

Statistical analysis and reporting of adverse events will concentrate on treatment-emergent adverse events. To this end, all adverse events occurring between start of treatment and individual patient's end of trial will be considered 'treatment-emergent'. Adverse events that start before first drug intake and deteriorate under treatment will also be considered as 'treatment-emergent'.

Frequency, severity, and causal relationship of adverse events will be tabulated by system organ class and preferred term after coding according to the current version of the Medical Dictionary for Drug Regulatory Activities (MedDRA). Frequency tables for all adverse events, protocol-specified AESI, serious adverse event (SAE), adverse event leading to death, adverse event leading to discontinuation, investigator assessed drug-related adverse event and serious adverse event will be generated for treatment-emergent adverse events.

Laboratory data will be analysed both quantitatively as well as qualitatively. The latter will be done via comparison of laboratory data to their reference ranges. Values outside the reference range as well as values defined as clinically relevant will be highlighted in the listings. Treatment groups will be compared descriptively with regard to distribution parameters as well as with regard to frequency and percentage of patients with abnormal values or clinically relevant abnormal values.

Vital signs, physical examinations, or other safety-relevant data observed at screening, baseline, during the course of the trial and at the end-of-trial evaluation will be assessed with regard to possible changes compared to findings before start of treatment.

7.4 INTERIM ANALYSES

An interim PK/PD analysis may be performed for model development purposes of the population PK and PK/PD analysis and to support dose decision for future trials. The interim analysis will focus on PK and AOC3 inhibition; other secondary biomarker endpoints may be evaluated in addition. The exact time point for data cut-off will be aligned with other studies investigating BI 1467335 and general project planning. Details of this analysis will be defined in the interim PK/PD analysis plan.

The result of the interim analysis will not be used to make any adaptions of the trial like sample size adjustments.

For steps to maintain blinding during the interim analysis see Section 4.1.5.1.

7.5 HANDLING OF MISSING DATA

Primary and secondary biomarker endpoints

Every effort will be made to collect complete data at the specified time points.

If missing values still occur they will be handled as described in the corresponding section.

Safety

With respect to safety evaluations, it is not planned to impute missing values.

7.6 RANDOMISATION

Patients will be randomised in blocks to double-blind treatment in a 2:1:1:1:2 ratio (Placebo, 1 mg, 3 mg, 6mg and 10 mg respectively). The randomisation of patients to the treatment groups will be performed via an interactive response technology (IRT).
BI will arrange for the randomisation and the packaging and labelling of trial medication. The randomisation list will be generated using a validated system, which involves a pseudo-random number generator so that the resulting treatment will be both reproducible and non-predictable. The block size will be documented in the CTR. Access to the codes will be controlled and documented.

7.7 DETERMINATION OF SAMPLE SIZE

The sample size calculation is based on both, plasma AOC3 activity relative to baseline and ALT change from baseline, the latter being the biomarker with the least favorable ratio of anticipated effect size to variance.

Under the assumptions given in detail below, a sample size of 98 with an allocation ratio of 2:1:1:1:2 for Placebo, 1 mg, 3 mg, 6mg and 10 mg respectively is appropriate for both the estimation of D10 of plasma AOC3 activity relative to baseline and the MCPMod analysis of biomarkers. This assumes 98 evaluable patients for the primary and secondary endpoints and thus around 108 will need to be randomized. For the dose assessment the lowest (Placebo) and highest (10 mg BI 1467335) dose groups will have the highest impact on the analysis. Due to the nature of the dose- response-relationship, the biggest difference is anticipated between Placebo and the 10 mg group. The power analysis and simulation via MCPMod supported the 2:1:1:1:2 allocation ratio instead of equal allocations (see also Table 7.7:1).

ALT

Sample size is based on four assumptions:

- an assumed maximum effect size of BI 1467335 vs. placebo of 30% for relative change from baseline in ALT after 12 weeks of treatment
- a target dose of 20% difference to placebo can be estimated
- the target dose lies within the tested dose range
- MCPMod shapes and guesstimates as described below

The defined response patterns and model assumptions from section 7.3.2 are used.

Further assumptions are a mean baseline ALT of 80 U/L, a placebo effect at week 12 of -10 U/L and an effect of active treatment at week 12 of -34 U/L, i.e. the true difference between the relative change from baseline between placebo and active treatment at week 12 is assumed to be 30%. Further on, it is assumed to have a constant coefficient of variation over the whole ALT range of 40/80 = 0.5. This assumption is based on external data from CENTAUER, GOLDEN and ARAMCHOL (R16-5489; R18-2779; R18-2781; mean baseline ALT of 63-78 and respective SD of 31-44) and on blinded estimated SD for baseline ALT from 61 patients from this study.

On log scale this corresponds to a change from baseline in the placebo group of -0.14 and in the active treatment group of -0.56, i.e. a difference of 0.42.

An autoregressive correlation structure is assumed with constant variability of ALT on log scale regardless of visit and exponentially declining correlation with distance. The correlation between measurements at visits four weeks apart are assumed to be 0.7. Consequently, correlation between baseline and week 12 is 0.34

MCMod will be applied to change from baseline of ALT at week 12 on log scale. It is investigated if (i) the MCPMod testing step rejects the null hypothesis of no dose-response relationship at one-sided alpha = 0.05, and (ii) a target dose of 20% difference to placebo, corresponding to a Delta on log-scale of 0.26, can be estimated and lies within the tested dose range.

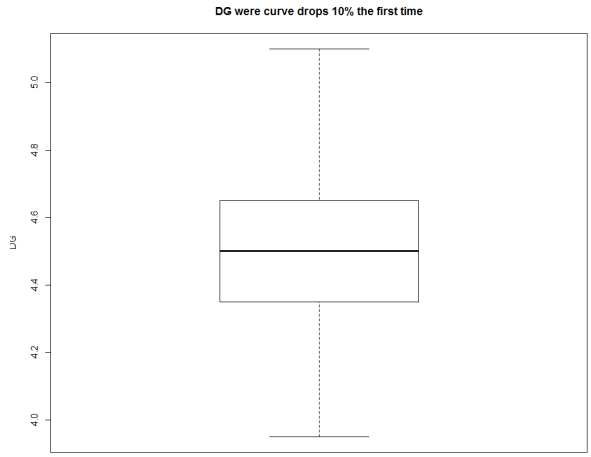
Simulations show that under the assumptions given above and assuming a true effect of 30% (Situation 3), the probability to fulfil the two conditions is approx. 84% with a sample size of 98 using an allocation ratio of 2:1:1:1:2 for doses Placebo, 1 mg, 3 mg, 6mg and 10 mg respectively..

Table 7.7:1 Possible scenarios to fulfil the two conditions using the assumptions define above.

SD	N per arm (total)	True assumed effect	probability to fulfil the two conditions
40	28 : 14 : 14 : 14 : 28 (98)	Situation 1 with true assumed effect of 0.001%	5.3%
		Situation 2 with true assumed effect of 20%	50.4%
		Situation 3 with true assumed effect of 30%	84.8%

Using these parameter estimates, datasets with 98 patients in a 2:1:1:1:2 ratio were simulated (1000 repeats). The D10 of the fitted nonlinear curves using simulated data was between 3.95 mg and 5.1 mg, the 5% and 95% quantiles of the distribution being 4.2 mg and 4.8 mg, respectively. This precision is considered to be sufficient for the purpose of the trial.

Figure 7.7:1: Boxplot of estimated D10 using 1000 simulated datasets.



Simulation shows that a 10% drop out rate does not decrease the precision of D10 estimation to an unacceptable degree.

The calculations have been performed using R version 3.3.2 as well as SAS Version 9.4. The R and SAS codes for the sample size calculations will be documented in a separate file and stored in the TMF.

8. INFORMED CONSENT, TRIAL RECORDS, DATA PROTECTION, PUBLICATION POLICY

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Tripartite Guideline for Good Clinical Practice (GCP), relevant BI Standard Operating Procedures (SOPs), the EU regulation 536/2014 and other relevant regulations.

Standard medical care (prophylactic, diagnostic and therapeutic procedures) remains in the responsibility of the treating physician of the patient.

The Investigator will inform the sponsor immediately of any urgent safety measures taken to protect the trial subjects against any immediate hazard, and also of any serious breaches of the protocol or of ICH GCP*.

The Boehringer Ingelheim transparency and publication policy can be found on the following web page: trials.boehringer-ingelheim.com. The rights of the Investigator and of the sponsor with regard to publication of the results of this trial are described in the Investigator contract. As a rule, no trial results should be published prior to finalization of the Clinical Trial Report.

8.1 TRIAL APPROVAL, PATIENT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institutional Review Board (IRB) / Independent Ethics Committee (IEC) and competent authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.
Prior to patient participation in the trial, written informed consent must be obtained from each patient (or the patient's legally accepted representative) according to ICH / GCP and to the regulatory and legal requirements of the participating country. Each signature must be personally dated by each signatory and the informed consent and any additional patient-information form retained by the Investigator as part of the trial records. A signed copy of the informed consent and any additional patient information must be given to each patient or the patient's legally accepted representative.
The Investigator must give a full explanation to trial patients based on the patient information form. A language understandable to the patient should be chosen, technical terms and expressions avoided, if possible. The patient must be given sufficient time to consider participation in the trial. The Investigator obtains written consent of the patient's own free will with the informed consent form after confirming that the patient understands the contents. The Investigator must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent.
Re-consenting may become necessary when new relevant information becomes available and should be conducted according to the sponsor's instructions.

The consent and re-consenting process should be properly documented in the source documentation.

8.2 DATA QUALITY ASSURANCE

A quality assurance audit/inspection of this trial may be conducted by the sponsor, sponsor's designees, or by IRB / IEC or by regulatory authorities. The quality assurance auditor will have access to all medical records, the Investigator's trial-related files and correspondence, and the informed consent documentation of this clinical trial.

8.3 RECORDS

Case Report Forms (CRF) for individual patients will be provided by the sponsor. See Section 4.1.5.2 for rules about emergency code breaks. For drug accountability, refer to Section 4.1.8.

8.3.1 Source documents

In accordance with regulatory requirements the Investigator should prepare and maintain adequate and accurate source documents and trial records that include all observations and other data pertinent to the investigation on each trial subject. Source data as well as reported data should follow good documentation practices and be attributable, legible, contemporaneous, original and accurate. Changes to the data should be traceable (audit trail). Data reported on the CRF must be consistent with the source data or the discrepancies must be explained.
The current medical history of the subject may not be sufficient to confirm eligibility for the trial and the Investigator may need to request previous medical histories and evidence of any diagnostic tests. In this case the Investigator must make three documented attempts to retrieve previous medical records. If this fails a verbal history from the patient, documented in their medical records, would be acceptable.
Before providing any copy of patients' source documents to the sponsor the investigator must ensure that all patient identifiers (e.g. patient's name, initials, address, phone number, social security number) have properly been removed or redacted to ensure patient confidentiality.

If the patient is not compliant with the protocol, any corrective action e.g. re-training must be documented in the patient file.
For the CRF, data must be derived from source documents, for example:

- Patient identification: gender, year of birth (in accordance with local laws and regulations)
- Patient participation in the trial (substance, trial number, patient number, date patient was informed)
- Dates of Patient's visits, including dispensing of trial medication
- Medical history (including trial indication and concomitant diseases, if applicable)
- Medication history

- Adverse events and outcome events (onset date (mandatory), and end date (if available))
- Serious adverse events (onset date (mandatory), and end date (if available))
- Concomitant therapy (start date, changes)
- Originals or copies of laboratory results and other imaging or testing results, with proper documented medical evaluation (in validated electronic format, if available)
- Completion of Patient's Participation in the trial (end date; in case of premature discontinuation document the reason for it).
- Prior to allocation of a patient to a treatment into a clinical trial, there must be documented evidence in the source data (e.g. medical records) that the trial participant meets all inclusion criteria and does not meet any exclusion criteria. The absence of records (either medical records, verbal documented feedback of the patient or testing conducted specific for a protocol) to support inclusion/exclusion criteria does not make the patient eligible for the clinical trial.

8.3.2 Direct access to source data and documents

The sponsor will monitor the conduct of the trial by regular on-site monitoring visits and in-house data quality review. The frequency of on-site monitoring will be determined by assessing all characteristics of the trial, including its nature, objective, methodology and the degree of any deviations of the intervention from normal clinical practice.
The Investigator /institution will allow on-site trial-related monitoring, audits, IRB / IEC review and regulatory inspections. Direct access must be provided to the CRF and all source documents/data, including progress notes, copies of laboratory and medical test results, which must be available at all times for review by the CRA, auditor and regulatory inspector (e.g. FDA). The CRA and auditor may review all CRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in section 8.3.1. The sponsor will also monitor compliance with the protocol and ICH GCP.
An adaptive approach to clinical trial monitoring will be utilised. The sponsor will perform a risk assessment of the trial to determine the extent and nature of monitoring required in order to ensure the reliability and robustness of the results. Regular review of risk reports will provide sponsor oversight during trial conduct and direct monitoring activities to the areas of greatest risk which have the most potential impact to subject safety and data quality.
The Investigator /institution will allow on-site trial-related monitoring, audits, IRB / IEC review and regulatory inspections. Direct access should be granted to all source documents (paper and e-records) including progress notes, copies of laboratory and medical test results. The CRA and auditor may review all CRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in section 8.3.1. The sponsor will also monitor compliance with the protocol and ICH GCP.

8.3.3 Storage period of records

Trial site(s):
The trial site(s) must retain the source and essential documents (including ISF) according to the national or local requirements (whatever is longer) valid at the time of the end of the trial.
Sponsor:
The sponsor must retain the essential documents according to the sponsor's SOPs.

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

BI is responsible to fulfil their legal regulatory reporting obligation and in accordance to the requirements defined in this CTP.

8.5 STATEMENT OF CONFIDENTIALITY AND PATIENT PRIVACY

Individual patient data obtained as a result of this trial is considered confidential and disclosure to third parties is prohibited with the exceptions noted below and in 5.5.4. Patient privacy will be ensured by using patient identification code numbers.
Data protection and data security measures are implemented for the collection, storage and processing of patient data in accordance with the principles 6 and 12 of the WHO GCP handbook.
Treatment data may be given to the patient's personal physician or to other appropriate medical personnel responsible for the patient's welfare. Data generated as a result of the trial need to be available for inspection on request by the participating physicians, the sponsor's representatives, by the IRB / IEC and the regulatory authorities.

8.6 TRIAL MILESTONES

The **start of the trial** is defined as the date when the first patient in the whole trial signs informed consent. **The end of the trial** is defined as the date of the last visit of the last patient in the whole trial ("Last Patient Out"). The "**Last Patient Drug Discontinuation"** (LPDD) date is defined as the date on which the last patient at an individual trial site ends trial medication (as scheduled per protocol or prematurely). Individual Investigators will be notified of SUSARs occurring with the trial medication until 30 days after LPDD at their site.
Early termination of the trial is defined as the premature termination of the trial due to any reason before the end of the trial as specified in this protocol.
Temporary halt of the trial is defined as any unplanned interruption of the trial by the sponsor with the intention to resume it.
Suspension of the trial is defined as an interruption of the trial based on a Health Authority request.
The IEC / competent authority in each participating EU member state will be notified about the trial milestones according to the respective laws.
A final report of the clinical trial data will be written only after all patients have completed the trial in all countries (EU or non-EU) to incorporate and consider all data in the report.

The sponsor will submit to the EU database a summary of the final trial results within one year from the end of a clinical trial as a whole, regardless of the country of the last patient (EU or non-EU).

9. REFERENCES

9.1 PUBLISHED REFERENCES

R10-1424 Pinheiro J, Bornkamp B, Bretz F. Design and analysis of dose-finding studies combining multiple companrisons and modelling procedures. J Biopharm Stat. 2006. 16(5): 639–656.

P13-02280 Torres DM, Williams CD, Harrison SA. Features, diagnosis, and treatment of nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2012. 10(8): 837-858.

R10-4848 Common terminology criteria for adverse events (CTCAE): version 4.0 (NIH publication no. 09-5410, published: May 28, 2009 (v4.03: June 14, 2010), revised June 2010, reprinted June 2010). http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf. 2010.

R15-1863 Inoue T, Morita M, Tojo T, Nagashima A, Moritomo A, Miyake H. Novel1H-imidazol-2-amine derivatives as potent and orally active vascular adhesion protein-1 (VAP-1) inhibitors for diabetic macular edema treatment. Bioorg Med Chem. 2013. 21(13): 3873-3881.

R15-1868 Foot JS, Deodhar M, Turner CI, et al. The discovery and development of selective 3-fluoro-4-aryloxyallylamine inhibitors of the amine oxidase activity of semicarbazide-sensitive amine oxidase/vascular adhesion protein-1 (SSAO/VAP-1). Bioorg Med Chem Lett. 2012. 22(12): 3935-3940.

R15-2803 Stolen CM, Marttila-Ichihara F, Koskinen K, et al. Absence of the endothelial oxidase AOC3 leads to abnormal leukocyte traffic in vivo. Immunity. 2005. 22(1): 105-115.

R15-5365 Weston CJ, Shepherd EL, Claridge LC et al. Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. J Clin Invest. 2015. 125(2): 501-520.

R15-5652 Bonder CS, Norman MU, Swain MG, et al. Rules of recruitment for Th1 and Th2 lymphocytes in inflamed liver: a role for alpha-4 integrin and vascular adhesion protein-1. Immunity. 2005. 23: 153-163.

R15-5654 Koskinen K, Vainio PJ, Smith DJ, et al. Granulocyte transmigration through the endothelium is regulated by the oxidase activity of vascular adhesion protein-1 (VAP-1). Blood. 2004. 103(9): 3388-3395.

R15-5697 Karim S, Liaskou E, Fear J, Garg A, Reynolds G, Claridge L, et al. Dysregulated hepatic expression of glucose transporters in chronic disease: contribution of semicarbazide-sensitive amine oxidase to hepatic glucose uptake. Am J Physiol. 2014. 307(12): G1180-G1190.

R15-6056 O'Rourke AM, Wang EY, Miller A, Podar EM, et al. Anti-inflammatory effects of LJP 1586 [Z-3-fluoro-2-(4-methoxybenzyl)allylamine hydrochloride], an amine-based inhibitor of semicarbazide-sensitive amine oxidase activity. J Pharmacol Exp Ther. 2008. 324(2): 867-875.

R15-6057 Murphy SL, Xu J, Kochanek KD. Deaths: final data for 2010. Nat Vital Stat Rep. 2013. 61(4): 1-117.

R15-6058 Merinen M, Irjala H, Salmi M, Jaakkola I, Hanninen A, Jalkanen S. Vascular adhesion protein-1 is involved in both acute and chronic inflammation in the mouse. Am J Pathol. 2005. 166(3): 793-800.

R15-6059 Lee WY, Salmi M, Kelly MM, Jalkanen S, Kubes P. Therapeutic advantage of anti-VAP-1over anti-α4 integrin antibody in concanavalin A-induced hepatitis. Hepatology. 2013. 58(4): 1413-1423.

R15-6044 Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012. 55(6): 2005-2023.

R15-6045 Marttila-Ichihara F, Smith DJ, Stolen C, et al. Vascular amine oxidases are needed for leukocyte extravasation into inflamed joints in vivo. Arthritis Rheum. 2006. 54(9): 2852-2862.

R15-6046 Lalor PF, Edwards S, McNab G, et al. Vascular adhesion protein-1 mediates adhesion and transmigration of lymphocytes on human hepatic endothelial cells. J Immunol. 2002. 169(2): 983-992.

R15-6047 Salter-Cid LM, Wang E, O'Rourke AM, et al. Anti-inflammatory effects of inhibiting the amine oxidase activity of semicarbazide-sensitive amine oxidase. J Pharmacol Exp Ther. 2005. 315(2): 553-562.

R15-6048 Xu HL, Salter-Cid L, Linnik MD, Wang EY, Paisansathan C, Pelligrino DA. Vascular adhesion protein-1 plays an important role in postischemic inflammation and neuropathology in diabetic, estrogen-treated ovariectomized female rats subjected to transient forebrain ischemia. J Pharmacol Exp Ther. 2006. 317(1):19-29.

R15-6070 Dharel N, Fuchs M. Nonalcoholic fatty liver disease − a major public health challenge for the 21st century. JSM Gastroenterol Hepatol. 2014. 2(2): 1018.

R15-5801 Aalto K, Maksimow M, Juonala M, et al. Soluble vascular adhesion protein- 1 correlates with cardiovascular risk factors and early atherosclerotic manifestations. Arterioscler Thromb Vasc. Biol. 2012. 32: 523-532.

R15-5774 Li HY, Jiang YD, Chang TJ, et al. Serum vascular adhesion protein-1 predicts 10-year cardiovascular and cancer mortality in individuals with type 2 diabetes. Diabetes. 2011. 60(3): 993-999.

R15-5773 Li HY, Wei JN, Lin MS, et al. Serum vascular adhesion protein-1 is increased in acute and chronic hyperglycemia. Clinica Chimica Acta. 2009. 404: 149-153.

R15-5766 Lin MS, Li HY, Wei JN, et al. Serum vascular adhesion protein-1 is higher in subjects with early stages of chronic kidney disease. Clin Biochem. 2008. 41: 1362-1367.

R15-5767 Madej A, Reich A, Orda A, Szepietowski JC. Expression of Vascular Adhesion Protein-1 in Atopic Eczema. Int Arch Allergy Immunol. 2006. 139(2): 114-121.

P15-12198 Kurkijärvi R, Yegutkin GG, Gunson BK, Jalkanen S, Salmi M, Adams DH. Circulating soluble vascular adhesion protein-1 accounts for the increased serum monoamine oxidase activity in chronic liver disease. Gastroenterology. 2000. 119(4): 1096-1103.

R15-5771 Kurkijärvi R, Adams DH, Leino R, Mottonen T, Jalkanen S, Salmi M. Circulating form of human vascular adhesion protein-1 (VAP-1): increased serum levels in inflammatory liver diseases. J. Immunol. 1998. 161: 1549-1557.

R15-5772 Kurkijärvi R, Jalkanen S, Isoniemi H, Salmi M. Vascular adhesion protein-1 (VAP-1) mediates lymphocyte-endothelial interactions in chronic kidney rejection. Eur J Immunol. 2001. 31: 2876-2884.

R15-5802 Boomsma F, van den Meiracker AH, Winkel S, et al. Circulating semicarbazide-sensitive amine oxidase is raised both in type I (insulindependent), in type II (non-insulin-dependent) diabetes mellitus and even in childhood type I diabetes at first clinical diagnosis. Diabetologia. 1999. 42: 233-237.

R16-5301 McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol. 2015. 62: 1148-1155.

R16-5309 Chalasani N, Regev A. Drug induced liver injury in patients with preexisting chronic liver disease in drug development – how to identify and manage? Gastroenterology. 2016.

R16-5489 Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, et al, GOLDEN-505 Investigator Study Group
Elafibranor, an agonist of the peroxisome proliferator-activated receptor-alpha and -delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening.
Gastroenterology 150 (5), 1147 - 1159 (2016)

R18-2779 Safadi R, Konikoff FM, Mahamid M, Zelber-Sagi S, Halpern M, Gilat T, et al, FLORA Group
The fatty acid-bile acid conjugate Aramchol reduces liver fat content in patients with nonalcoholic fatty liver disease.
Clin Gastroenterol Hepatol 12 (12), 2085 - 2091 (2014)

R18-2781 Friedman SL, Ratziu V, Harrison SA, Abdelmalek MF, Aithal GP, Caballeria J, et al
A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis.
Hepatology 67 (5), 1754 - 1767 (2018)

10. APPENDICES

10.1 PHARMACOKINETIC AND BIOMARKER SAMPLING TIME POINTS

Table 10.1:1 Blood sampling scheme for pharmacokinetic and pharmacodynamic/biomarker blood samples

Visit No.	Day	Time point	Time rel. to drug admin. [h]	CRF Time / planned time [h:min]	PK samples	AOC3 activity / concentration
2	1	Prior to first BI 1467335 dose (baseline)	-0.5	-0:30	X	X
		BI 1467335 administration	0	0:00		
			0.25	0:15	X	
			0.5	0:30	X	X
			1	1:00	X	X
			1.5	1:30	X	
			2	2:00	X	X
			3	3:00	X	X
			6	6:00	X	X
			8	8:00	X	X
3	15		-0.5	335:30	X	X
		BI 1467335 administration	0	336:00		
			1	337:00	X	
			1.5	337:30	X	

Table 10.1:1 continued Blood sampling scheme for pharmacokinetic and pharmacodynamic/biomarker blood samples

Visit No.	Day	Time point	Time rel. to drug admin. [h]	CRF Time / planned time [h:min]	PK samples	AOC3 activity / concentration
4	29		-0.5	671:30	X	X
		BI 1467335 administration	0	672:00		
			0.25	672:15	X	
			0.5	672:30	X	X
			1	673:00	X	X
			1.5	673:30	X	
			2	674:00	X	X
			3	675:00	X	X
			6	678:00	X	X
			8	680:00	X	X
5	43		-0.5	1007:30	X	X
		BI 1467335 administration	0	1008:00		
			1	1009:00	X	
			1.5	1009:30	X	
6	57		-0.5	1343:30	X	X
		BI 1467335 administration	0	1344:00		
			1	1345:00	X	
			1.5	1345:30	X	

Table 10.1:1 continued Blood sampling scheme for pharmacokinetic and pharmacodynamic/biomarker blood samples

Visit No.	Day	Time point	Time rel. to drug admin. [h]	CRF Time / planned time [h:min]	PK samples	AOC3 activity / concentration
EOT	85		-0.5	2015:30	X	X
		BI 1467335 administration	0	2016:00		
			0.25	2016:15	X	
			0.5	2016:30	X	X
			1	2017:00	X	X
			1.5	2017:30	X	
			2	2018:00	X	X
			3	2019:00	X	X
			6	2022:00	X	X
			8	2024:00	X	X
			24	2040:00	X	X
EOT + 28	113		672	2688:00		X

10.3 INCREASED LIVER ENZYMES PROCEDURE

Please also refer to section 3.3.4.1.1.

Figure 10.3: 1 study- specific procedures in case of further increased liver enzymes after randomisation.