CLINICAL STUDY PROTOCOL

Title Page

Protocol Title:

A Phase 1, multiple-dose, single-center, randomized, open-label, 2-period, 2-treatment crossover systemic bioavailability study of diclofenac comparing CCI of AMZ001 (Diclofenac sodium gel CCI) applied once daily CCI versus CCI of Voltaren Emulgel 1.16% Gel (Diclofenac diethylamine gel 1.16%) applied 4 times daily CCI in healthy subjects

Sponsor Protocol Number:	AMZ001-009
Nuvisan Protocol Number:	N-A-PH1-23-070
Investigational Medicinal Products:	AMZ001 (Diclofenac sodium gel CCI Voltaren Emulgel 1.16% Gel (Diclofenac diethylamine gel 1.16% [Name of marketed reference product in Germany: Voltaren Schmerzgel 11,6 mg/g Gel])
Study Phase:	Phase 1
Sponsor:	Amzell B.V. Siriusdreef 31, 2132 WT Hoofddorp The Netherlands
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Regulatory Agency Identifier Number:	EU CT Number: 2024-515954-26-00
Protocol Version:	Version Final 2.0, 10-OCT-2024
Good Clinical Practice (GCP) Statement:	This study will be performed in compliance with ICH GCP guidelines.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviations of PK parameters are provided in Section 10.6.

ACR	American College of Rheumatology
ADaM	Analysis Data Model
ADR	Adverse drug reaction
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
HBsAG	Hepatitis B surface antigen
ANOVA	Analysis of variance
anti-HCV	Hepatitis C antibody
AST	Aspartate aminotransferase
AxMP	Auxiliary medicinal product
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (German Federal Institute for Drugs and Medical Devices)
BID	*"Bis in die"*, i.e., 2 times daily
BMI	Body mass index
BSA	Body surface area
BUN	Blood urea nitrogen
CA	Competent authority
CDISC	Clinical data interchange standards consortium
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval

CIPT	Cumulative irritant patch test
CNS	Central nervous system
CoC	Certificate of compliance
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Coronavirus disease 2019
C(P)K	Creatine (phospho-)kinase
(e)CRF	(Electronic) case report form
CRO	Contract research organization
CSR	Clinical study report
CTFG	Clinical Trial Facilitation Group
CTS	Clinical trial supplies department
CV	Coefficient of variation
DILI	Drug-induced liver injury
DRM	Data Review Meeting
ECG	Electrocardiogram
ED	Early discontinuation
EM(E)A	European Medicines Agency
EOT	End of trial
eSource	Electronic source
EU	European Union
FDA	Food and drug administration
FSH	Follicle stimulating hormone
GCP	Good clinical practice

GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
GLDH	Glutamate dehydrogenase
GLP	Good Laboratory Practice
HBsAG	Hepatitis B virus surface antigen
hCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HRT	Hormonal replacement therapy
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International council on harmonization
(I)EC	(Independent) ethics committee
IMP	Investigational medicinal product
INR	International normalized ratio
IRB	Institutional review board
ISR	Incurred Samples
IUD	Intrauterine device
IUS	Intrauterine system
LAM	Lactational amenorrhea method
LC-MS	Liquid chromatography - mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDH	Lactate dehydrogenase

(L)LOQ	(Lower) limit of quantification
LS	Least square
MedDRA	Medical Dictionary for Regulatory Activities
MS	Mass spectrometry
NIMP	Non-investigational medicinal product
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
OECD	Organization for Economic Cooperation and Development
PD	Pharmacodynamic(s)
PhV	Pharmacovigilance
PK	Pharmacokinetic(s)
QD	*"Quaque die"*, i.e., once daily
QID	*"Quarter in die"*, i.e., 4 times daily
QRS	Part of electrocardiographic wave representing ventricular depolarization
RIPT	Repeat insult patch test
RNA	Ribonucleic acid
RSI	Reference safety information
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SAS	Statistical analysis system
SCR	Screening

SDTM	Study data tabulation model
SmPC	Summary of Product Characteristics
SoA	Schedule of activities
SOC	System organ class
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment emergent adverse event
TID	*"Ter in die"*, i.e., 3 times daily
TMF	Trial master file
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
WHO	World Health Organization
WOCBP	Woman of child-bearing potential
WONCBP	Woman of non-childbearing potential
WOP	Wash-out period

1. Protocol Summary

1.1. Synopsis

Protocol Title:

A Phase 1, multiple-dose, single-center, randomized, open-label, 2-period, 2-treatment crossover systemic bioavailability study of diclofenac comparing CCI of AMZ001 (Diclofenac sodium gel CCI) applied once daily CCI versus CCI of Voltaren Emulgel 1.16% Gel (Diclofenac diethylamine gel 1.16%) applied 4 times daily CCI in healthy subjects

Regulatory Agency Identifier Number:

EU CT Number: 2024-515954-26-00

Rationale:

The aim of the present study is to investigate the systemic bioavailability of CCI of AMZ001, applied once daily CCI compared to CCI of Voltaren Emulgel 1.16% Gel (diclofenac diethylamine gel 1.16%), applied 4 times daily CCI after repeated dosing in healthy subjects for 7 days.

The safety and local tolerability of CCI of AMZ001, applied once daily CCI CCI will be evaluated after repeated dosing in healthy subjects for 7 days.

The dose of AMZ001 has been selected from the outcomes of the previous Phase 1 PK and safety studies, in an attempt to provide comparable systemic diclofenac exposure as Voltaren Emulgel 1.16% Gel.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To investigate the systemic bioavailability of CCI of AMZ001 (diclofenac sodium gel CCI) applied once daily CCI versus CCI of Voltaren Emulgel 1.16% Gel (diclofenac diethylamine gel 1.16%) applied 4 times daily CCI after repeated topical administrations in healthy subjects for 7 days.	Main PK parameter: • AUC_{0-24} of diclofenac in plasma on Day 7
Secondary	
• To determine the PK profile of CCI CCI of AMZ001 (diclofenac sodium gel CCI) and CCI of Voltaren Emulgel 1.16% Gel (diclofenac diethylamine gel 1.16%).	Additional PK parameters: • C_{max}, C_{min}, C_{ave}, t_{max}, t_{min}, PTF, of diclofenac in plasma on Day 7 • $R_{acc(AUC\ 0-24)}$ Day 7 versus Day 1 • Swing = (C_{max} - C_{min}) / C_{min} on Day 7 • AUC_{0-24}, C_{max}, C_{ave}, t_{max}, of diclofenac in plasma on Day 1
• To assess the safety and local tolerability of CCI of AMZ001 (diclofenac sodium gel CCI and CCI of Voltaren Emulgel 1.16% Gel (diclofenac diethylamine gel 1.16%).	• Number of subjects with TEAEs including SAEs • Number of subjects with study intervention-related TEAEs • Assess the skin irritation of each product

Overall Design:

This study will be conducted in a single-center, randomized, open-label, 2-period crossover design. Healthy male and female subjects (18 to 65 years, inclusive) are eligible.

Brief Summary:

The main purpose of this study in healthy male and female subjects is to investigate the systemic bioavailability of CCI of AMZ001 applied once daily as CCI per knee on both knees compared to CCI, of Voltaren Emulgel 1.16% Gel applied 4 times daily as CCI per knee on both knees for 7 days.

Additionally, the PK profile, and the safety and local tolerability of AMZ001 gel and Voltaren Emulgel 1.16% Gel will be evaluated.

Study details include:

- The study duration will be approximately 9 weeks per subject, depending on the length of the screening- and wash-out periods.
- Two (2) study periods are planned in crossover design, with a wash-out period (WOP) of at least 21 days in-between.
- In each study period, subjects will self-administer AMZ001 gel OR Voltaren Emulgel 1.16% Gel under the supervision of a designated clinical site staff member:
    - One (1) dose CCI of AMZ001 will be applied once daily as CCI per knee on both knees.

      OR
    - One (1) dose CCI of Voltaren Emulgel 1.16% Gel will be applied 4 times daily as CCI per knee on both knees.
- The intervention duration will be 7 days per period, i.e., from Day 1 to Day 7 of Periods 1 and 2.
- The screening examination will be performed within 21 to 2 days prior to the first administration of study intervention.
- Subjects will stay in-house for 9 days per study period. During the WOP, subjects will not be confined to the study site.
- The end of trial (EOT) visit will be performed in the morning of Day 8 of Period 2 and subjects will be discharged afterwards.

Number of Subjects:

A total of 34 subjects are intended to be randomized to study intervention, so that at least 26 subjects will be valid for pharmacokinetic (PK) evaluation of diclofenac.

Subjects who prematurely discontinue the study after having been assigned to study intervention may be replaced if the number of valid subjects for PK becomes or is expected to become less than 26 subjects. Subjects who drop out prior to first administration of study intervention (early dropouts) may be replaced immediately.

Study Intervention Groups and Duration:

For each subject, the study will consist of an ambulatory screening visit (within 21 days to 2 days prior to the first administration of study intervention [until Day -2]), 2 in-house periods of 9 days each (Day -1 to Day 8 of Periods 1 and 2) and a wash-out period of at least 21 days.

In Periods 1 and 2, eligible subjects will be admitted to the study ward 1 day prior to administration of the study interventions (Day -1 of the respective study period).

From Day 1 to Day 7 of Periods 1 and 2, subjects will self-administer AMZ001 gel or Voltaren Emulgel 1.16% Gel under the supervision of a designated clinical site staff member in 1 of the following 2 dosing sequences:

Period 1

(Sequence 1) AMZ001 gel - CCI daily: 1 time daily CCI on each knee for 7 days

(Sequence 2) Voltaren Emulgel 1.16% Gel - CCI daily: 4 times daily CCI on each knee for 7 days

Period 2

(Sequence 1) Voltaren Emulgel 1.16% Gel - CCI daily: 4 times daily CCI on each knee for 7 days

(Sequence 2) AMZ001 gel - CCI daily: 1 time daily CCI on each knee for 7 days

Subjects will remain in-house for 9 days per study period (from Day -1 until discharge in the morning of Day 8) provided there are no medical objections. Periods 1 and 2 will be separated by a wash-out period of at least 21 days, during which the subjects will not be confined to the study site.

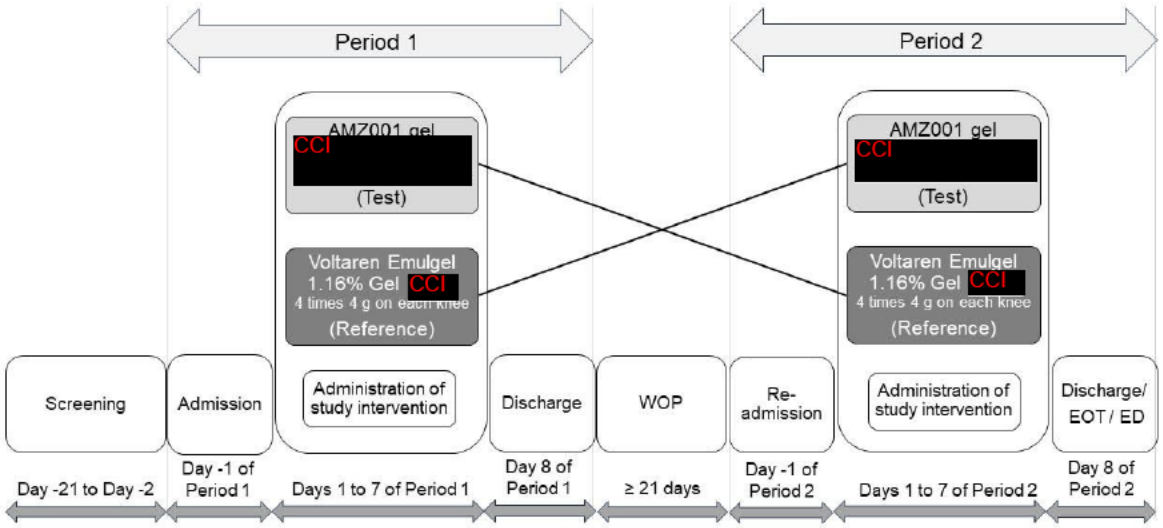
An end of trial (EOT) visit will be performed in the morning of Day 8 of Period 2 and subjects will be discharged afterwards.

The duration of study participation will be up to approximately 9 weeks per subject, depending on the length of the screening period (up to 21 days) and the length of the wash-out period (at least 21 days). For each subject, the study will end with the above-mentioned EOT visit.

Data Monitoring/Other Committee: No

1.2. Schema

Figure 1: Overall Study Design



ED = early discontinuation; EOT = end of trial; WOP = wash-out period

1.3. Schedule of Activities

A schedule of activities (SoA) presenting the timepoints for the study-related measures / actions is provided in Table 1.

- Whenever different assessments and procedures are to be performed at the same nominal time, the following sequence will be followed (except for screening and Day -1 evaluations):
    1. 12-lead ECG (at screening only) / Vital signs;
    2. Blood sampling ([PK and safety]) as close as possible to the nominal time;
    3. Other assessments and procedures;
    4. Assessment of local skin irritation at 30 minutes after application of the gel (first morning dose) in each study period (see Section 8.3.8).

Any assessments / actions at the timepoint of dosing will be performed pre-dose, administration of the study intervention is the last action for the respective timepoint, unless indicated otherwise.

Any meals or water intake will be served after the assessments / actions for a given timepoint are completed.

Assessment time windows will be defined in a deviation manual. Any time deviations falling into the allowed time windows will not be considered a protocol deviation.

Table 1: Schedule of Activities for Periods 1 and 2

Procedure	SCR[A]	Study Day								EOT/ ED[C,D]	Notes
	-21 to -2	-1[B]	1	2	3	4	5	6	7	8	
Informed consent	X										To be obtained prior to any screening activity. Refer to Section 10.1.
Ambulatory visit	X										
Hospitalization / In-house stay		X→	→	→	→	→	→	→	→	→X	Day -1: Admission. Day 8: Discharge after completion of Day 8 assessments.
Inclusion/exclusion criteria	X										Refer to Sections 5.1.1 and 5.2.1.
Confirmation of eligibility		X[E]									[E] Recheck of clinical status on Day -1or Day 1 of Period 1 before randomization, and on Day -1 of Period 2. Refer to Sections 5.1.2 and 5.2.2.
Demographic data	X										Refer to Section 8.1.1
Medical history	X	X									Events existing prior to informed consent signature, which are resolved before first study intervention. Including history of illegal drugs, alcohol, tobacco, and caffeine use. Refer to Section 8.1.3
Prior medication	X	X									Any medication (including prescription, non-prescription drugs, dietary and herbal supplements) taken which had been stopped prior to the first administration of study intervention.
Comprehensive physical examination	X										Refer to Section 8.3.1.

Procedure	SCR[A]	Study Day								EOT/ ED[C,D]	Notes
	-21 to -2	-1[B]	1	2	3	4	5	6	7	8	
Abbreviated physical examination		X								X[D]	Refer to Section 8.3.1.
Height, weight	X										BMI will be calculated. Refer to Section 8.3.1.
Pregnancy test (WOCBP only)	X[F]	X[G]								X[G]	[F] SCR: Serum pregnancy test; [G] Day -1 of both periods, and Day 8 of Period 2 (EOT/ ED visit): Urine pregnancy test. Refer to Section 8.3.5.
TSH	X										Refer to Section 10.2.
Serology	X										HIV1+2 Ab+HIV1 p24 Ag, Hepatitis B virus surface Ag and Hepatitis C virus Ab. Refer to Section 10.2.
Safety laboratory assessments	X									X[D]	Hematology, clinical chemistry, urinalysis under fasted conditions. Refer to Section 10.2.
Urine drug and cotinine screen and alcohol breath test	X	X									Refer to Section 8.3.7 and Section 10.2.
12-lead ECG	X										For details refer to Section 8.3.3.
Vital signs including body temperature	X	X								X[D]	For details refer to Section 8.3.2.
Randomization		X[H]									[H] Within the afternoon of Day -1 of Period 1 until pre dose Day 1 of Period 1, before any Day 1 procedures. Refer to Section 6.3.
Weight of study intervention			X	X	X	X	X	X	X		Before and after dosing. Refer to Section 6.1

Procedure	SCR[A]	Study Day								EOT/ ED[C,D]	Notes
	-21 to -2	-1[B]	1	2	3	4	5	6	7	8	
Study intervention administration			X	X	X	X	X	X	X		AMZ001: [redacted] once daily per knee as specified. Voltaren Emulgel: [redacted] 4 times daily per knee, as specified. Refer to Section 6.1.
PK blood sampling			X[I,J]	X[K,L]	X[L]	X[L]	X[L]	X[L]	X[I,J]		[I]Day 1 and Day 7: Pre-dose (prior to morning dose) and 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, 16 h, 18 h, 20 h, and 24 h (on Days 2 and 8) post (first daily) application. [J]Day 1 and Day 7: Prior to Voltaren Emulgel administration, if applications overlap with PK collections. [K]Day 2 pre-dose = same PK collection as 24 h post application on Day 1. [L]: Prior to morning dose. Refer to Section 8.5.
Baseline treatment area assessment	X	X									Refer to Section 8.1.4 and Section 8.3.8 for dermal response score.
Assessment of local skin irritation			X	X	X	X	X	X	X	X[D]	At 30 minutes after application of the gel (first morning dose) in each study period. Refer to Section 8.3.8 for dermal response score.
(S)AE review	X	X	X→	→	→	→	→	→	→	→X	Any event with start after ICF signature. Refer to Sections 8.4 and 10.5.

Procedure	SCR[A]	Study Day								EOT/ ED[C,D]	Notes
	-21 to -2	-1[B]	1	2	3	4	5	6	7	8	
Concomitant medication review			X→	→	→	→	→	→	→	→X	Any medication taken at/after time of first study intervention, regardless of whether it had started prior to the study or not. Refer to Section 6.9.

[A]: A SCR: Only performed prior to Period 1

[B]: Day -1: Repeated after WOP, prior to Period 2.

[C]: EOT: Only performed in Period 2.

[D]: ED: All attempts shall be made to complete the following: Reason for withdrawal; assessment of skin reaction; IMP container weight; physical examination; vital signs; clinical laboratory safety tests; AEs and concomitant medication.

Abbreviations used in Table 1:

AE=adverse event; BMI=body mass index; ECG=electrocardiogram; ED=early discontinuation; h=hour; HIV=human immunodeficiency virus; ICF=informed consent form; min=minute; PK=pharmacokinetic(s); SAE=serious adverse event; SCR=Screening; TSH=thyroid-stimulating hormone; WOCBP=women of childbearing potential; WOP=wash-out period.

Legend to study SoA provided in Table 1 above:

X measure / action to be done at the time point indicated
→ measure / action to be done continuously, starting from the time point indicated

2. Introduction

Diclofenac is widely employed in the treatment of pain and inflammation. It is a member of the aniline phenylacetate class of acidic non-steroidal anti-inflammatory drugs (NSAIDs). The combination of the lipid solubility of the phenylacetate moiety along with its solubility in alkaline and other salts equips diclofenac with highly favorable physicochemical properties for penetrating through membranes (Rainsford et al., 2008).

Osteoarthritis (OA) is a chronic disease characterized by the breakdown and eventual loss of the cartilage of one or more joints. Cartilage is a protein substance that functions as a protective cushion between the adjoining bones. This degenerative joint disease increases with age, is more prevalent when there is physical stress on the joint (for example, athletes such as hockey players suffer from knee and hip joint pain and inflammation, others may suffer from OA due to obesity), and is the leading cause of chronic musculoskeletal pain and disability in elderly populations (Zhang et al., 2010). This progressive, complex, multifactorial, disease affects up to 50% of the adult population over the age of 65 and pain is the most significant symptom. In addition to pain, stiffness, and limitations of functional activities of daily living are experienced, including rising from a chair, walking, balancing, and using stairs. OA commonly affects the hands, feet, spine, and large weight-bearing joints, such as the hips and knees (Song et al., 2006; Johnson et al., 2014; Allen et al., 2015).

There are two distinct types of OA. Primary (idiopathic) OA is defined as a degenerative disorder of aging. Secondary OA is associated with an apparent cause for the breakdown of cartilage, such as injury or overuse, heredity, obesity, other diseases (e.g., hemochromatosis, acromegaly) and types of arthritis (e.g., rheumatoid arthritis).

Amzell is introducing a novel transdermal hydroalcoholic gel formulation with diclofenac sodium as the active ingredient. This topical formulation is designed for rapid and efficient diclofenac absorption with low systemic side effects. Its higher drug strength (CCI diclofenac sodium) combined with an efficient topical delivery system allows AMZ001 to effectively relieve localized pain with a once daily application improving patient convenience.

2.1. Study Rationale

The aim of the present study is to investigate the systemic bioavailability of CCI of AMZ001, applied once daily CCI compared to CCI of Voltaren Emulgel 1.16% Gel (diclofenac diethylamine gel 1.16%), applied 4 times daily CCI after repeated dosing in healthy subjects for 7 days.

The safety and local tolerability of CCI of AMZ001, applied once daily (CCI CCI), will be evaluated after repeated dosing in healthy subjects for 7 days.

The dose of AMZ001 has been selected from the outcomes of the previous Phase 1 PK and safety studies, in an attempt to provide comparable systemic diclofenac exposure as Voltaren Emulgel 1.16% Gel.

In line with the current EMA guidance on topically applied products (EMA, 2010, EMA, 2018), the present study is conducted to provide evidence that the systemic diclofenac exposure for the test product, AMZ001, compared to the reference product, Voltaren Emulgel 1.16% Gel, is similar.

2.2. Background

Non-steroidal anti-inflammatory drugs (NSAIDs) are often employed for symptomatic relief of mild-to moderate pain associated with OA. Traditional orally ingested NSAIDs are responsible for an increase in incidence of gastrointestinal (GI) and cardiovascular adverse effects (FitzGerald et al. 2001; García Rodríguez et al., 2008). The acidic molecules in NSAIDs directly irritate the gastric mucosa and the inhibition of the COX-1 and COX-2 isoenzymes decreases the levels of protective prostaglandins. Common GI adverse drug reactions (ADRs) include dyspepsia, diarrhea, nausea/vomiting, and gastric ulceration/bleeding (Sostres et al., 2013; Laine, 2003). A previous study demonstrated that NSAIDs with a long half-life and/or slow-release formulation are associated with coincident inhibition of COX-1 and COX-2 isozymes which result in an increased risk of upper GI bleeding and perforation (González et al., 2010).

The dose-dependent risk of orally administered NSAIDs has triggered the development of different formulations, including topically applied formulations. The transcutaneous administration of NSAIDs offers advantages over systemically administered medications, including a lower total systemic daily dose for patients to ameliorate pain symptoms, site-specific delivery, and the circumvention of first-pass hepatic metabolism. In patients with chronic OA, the American College of Rheumatology (ACR) and the Osteoarthritis Research Society International (OARSI) generally recommend oral treatments (acetaminophen, NSAIDs) and topical NSAIDs equally, favoring topical therapy for patients who have pre-existing GI risk, exhibit multiple co-morbidities, or are 75 years of age and older (Hochberg et al., 2012; Nelson et al., 2014; Whelton, 1999). The concern for GI, hepatic, and renal toxicity is appropriate in such situations.

Topical NSAIDs are useful for the local treatment of acute and chronic musculoskeletal conditions (Gøtzsche, 2007; Derry et al., 2015; Persson et al., 2016). These formulations penetrate the skin and permeate to tissues or joints, providing site-specific delivery of the drug to target areas and high local concentrations to exert a therapeutic effect. Topical formulations effectively lead to lower circulating levels of these drugs, thus minimizing the risk of harmful effects, and simultaneously provides symptom control comparable with oral counterparts (Klinge et al., 2013).

In a pharmacokinetic (PK) study, subjects treated with diclofenac sodium 0.1% gel had only 1 to 3% of systemic exposure to diclofenac compared with the recommended 75 mg daily dose of oral diclofenac sodium, despite the fact that the subjects had a first-degree sunburn (Magnette et al., 2004). A similar observation was made by Kienzler and colleagues comparing the systemic

bioavailability and pharmacodynamics of topical diclofenac sodium 1% gel with oral diclofenac sodium in healthy subjects. Systemic exposure with the topical preparation was 5- to 17-fold lower than with oral diclofenac. Topical diclofenac did not inhibit platelet aggregation and was devoid of GI AE compared with oral diclofenac (Kienzler et al., 2010).

In a randomized, double-blind, vehicle-controlled trial of 492 adult patients, topical treatment with diclofenac sodium 1% gel achieved statistically and clinically significant improvements of pain over a three-month treatment period (Barthel et al., 2009). Equivalence studies between topical diclofenac gel or solution was shown to be as effective as oral diclofenac or ibuprofen in patients with OA of the hand or knee, but with fewer systemic AE (Zacher et al., 2001; Roth et al., 2011).

Overall, the data demonstrate that topical diclofenac is superior to placebo and comparable to oral diclofenac in the treatment of patients with knee OA, with a lower incidence of GI complaints compared to oral therapy. Topical agents may offer a safe, well-tolerated and effective alternative to systemic therapies in the treatment of patients with chronic, localized musculoskeletal injuries (Galer, 2011).

AMZ001 is a novel high strength, discrete, odorless, and non-invasive topical gel formulation for once daily application, containing CCI diclofenac sodium.

The pharmacology and toxicology of diclofenac is well-known. Therefore, no nonclinical pharmacodynamic, pharmacokinetic, single dose toxicity, genotoxicity, carcinogenicity as well as reproductive and developmental studies have been conducted with AMZ001. A detailed summary of the available nonclinical information on AMZ001 (diclofenac gel CCI formulation) is provided in Table 2 and in Section 5.1 of the IB.

Table 2: Summary on nonclinical information

Study Type		Study Design
Local tolerance and Dermal Sensitization Studies	Non-GLP Dermal Tolerability Studies in Minipigs	1. AMZ001 CCI 0.5mL/site BID, TID and QID for 14 days 2. AMZ001 CCI) 4mg/cm^2 BID, TID and QID for 21 days 3. AMZ001 (CCI 4mg/cm^2 TID for 28 days
	GLP Dermal Irritation Study in Rabbits	1. Diclofenac Gel CCI 16 mg/cm2 QD for 7 days
	GLP Dermal Sensitization Studies in Guinea Pigs	1. AMZ001 CCI) as per OECD Guidance test No 406 2. AMZ001 CCI Placebo as per OECD Guidance test No. 406 with Various Myristyl Alcohol Strength
Repeat Dose Dermal Toxicity	GLP Diclofenac Gel CCI 9-months Chronic Dermal Toxicity Study in Gottingen Minipigs with a 3 Month Interim Necropsy and a 4-Week Recovery Period	1. AMZ001 CCI : BID and TID 2. AMZ001 Placebo: BID for 9 months

BID=2 times daily; GLP=Good Laboratory Practice; OECD=Organization for Economic Cooperation and Development; TID=3 times daily; QD=once daily; QID=4 times daily.

To date, 5 Phase 1 studies have been completed in healthy subjects:

- A 21-day, randomized, controlled study to evaluate the skin irritation potential of AMZ001 (diclofenac sodium CCI), using a Cumulative Irritant Patch Test (CIPT) design, in 43 healthy subjects, 34 of whom completed the trial (AMZ001-001)
- A randomized, controlled study to evaluate the sensitizing potential of AMZ001 using a Repeat Insult Patch Test (RIPT) design in 200 healthy subjects (AMZ001-002)
- An open-label, fixed-sequence, active control, multiple-dose study to investigate the relative bioavailability, safety, and tolerability of three different doses (4.6, 9.2, and 13.8 g/day) of AMZ001 topical gel in comparison to Voltaren (diclofenac gel 1%) after a 5-day treatment regimen in healthy subjects (study 000084)
- A Phase 1, open-label PK and safety study of AMZ001 (Diclofenac Gel CCI) in comparison with Voltaren (diclofenac gel 1%) in healthy subjects (AMZ001-005; Kousba, 2018).
- A Phase 1, single-center, randomized, open-label, bioavailability and safety three period crossover multiple-dose study comparing two dose levels (6 ml [5.5 g] and CCI of AMZ001 (diclofenac sodium gel CCI) and one dose level (CCI of Voltaren arthritis

pain gel (diclofenac sodium gel 1%) in healthy subjects after multiple-dose topical administrations for 7 consecutive days in healthy subjects (AMZ001-008).

In addition, one (1) 4-week Phase 2/3 efficacy study in 444 patients of 3 different countries (United States, Denmark, and Czech Republic) has been successfully completed:

- A multicenter, placebo-controlled, double-blind, randomized trial of AMZ001 for the treatment of knee OA symptoms (NCT03691844). The trial also included a single-blind component, consisting of Voltaren. The aim of the trial was to evaluate the efficacy and safety and tolerability of once or twice daily application of AMZ001 during a 28-day period in subjects with radiographic and symptomatic knee OA in either one or both knees (AMZ001-006; Bihlet, 2020).

Overall, the safety profile of the active ingredient diclofenac and the excipients used in AMZ001 are well known. The clinical trials conducted with AMZ001, as listed above, confirmed the safety in the intended indication.

More detailed information on the chemistry, pharmacology, efficacy, and safety of AMZ001 can be found in the current IB.

2.3. Benefit/Risk Assessment

2.3.1. Risk Assessment

This study will be performed in healthy subjects who will not have medical or health benefits from administration of AMZ001 or Voltaren Emulgel 1.16% Gel. Given the well-known safety profile of diclofenac, the risks related to treatment with topical diclofenac gel at the proposed dose are regarded as low.

However, due to the alcohol content of study interventions, frequent applications to the skin may cause application site reactions including pruritus, erythema, dryness, and urticaria. There is also a risk of infection at site of puncture for blood draws. During this clinical study, safety will be monitored on an ongoing basis.

The excipients used for the formulation of AMZ001 are well known and have been used in previously conducted studies as well as in already EU-approved topical products, such as Testavan (SmPC Testavan, 2023).

No Serious Adverse Reaction (SAR) was observed in the performed clinical trials with AMZ001. One (1) SAE “cerebrovascular accident” occurred in the CIPT study and was considered to be unlikely related to treatment, and the subject was discontinued from the study. No treatment related SAEs have been reported to date (see Section 7.10 of the IB).

Relevant emerging safety data, e.g., serious adverse events (SAEs), suspected unexpected serious adverse reactions (SUSARs), and serious safety-related protocol deviations, which are affecting the benefit/risk assessment of the study will be communicated as soon as possible between the

Sponsor, the study site and investigators and study subjects (more details of the communication process are given in Section 8.4.4). Regular telephone conferences with the site will be held to discuss any upcoming relevant safety information during the conduct of the study.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of diclofenac can be found in the current IB of AMZ001, and the SmPC of Voltaren Emulgel 1.16% Gel (Name of marketed reference product in Germany: Voltaren Schmerzgel 11,6 mg/g Gel). If IB/SmPC are updated during the study, the new version(s) will be made available to the investigators and filed in the study file.

Key risks that subjects in the study might be exposed to may arise from study-related procedures as well as from the administration of study interventions, as outlined in the tabular summary below (see Table 3):

Table 3: Summary on key risks

Identified and Potential Risks of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention AMZ001		
Increased risk for thrombotic events, myocardial infarction, and stroke in patients with known history of cardiovascular disease.	Risks are described in the current version of the reference safety information (IB) for similar types of diclofenac-containing gels or for NSAIDs, including diclofenac.	Subjects will be on ward during administration period of study intervention. Specific in- and exclusion criteria to be applied (see Section 5.1 and 5.2). Regular checking for AEs and local tolerability (skin reactions) by staff (see Sections 1.3, 8.3.8, and 8.4). Equipment and trained staff present to treat potential adverse effects. Regular safety laboratory assessment (see Sections 1.3 and 10.2). Regular monitoring of vital signs (see Sections 1.3 and 8.3.2). Specific restrictions are in place (see Section 5.3.4). Specific withdrawal criteria are in place (see Section 7.2.1).
Risk for GI perforation, bleeding, and ulceration.		
Risk for hypertension or exacerbation of hypertension.		
Risk for edema.		
Risk for serious skin reactions such as toxic epidermal necrolysis, Steven-Johnson Syndrome and exfoliative dermatitis.		
Risk for anemia, and prolonged bleeding.		
Precipitation of bronchospasm in patients suffering from or with a previous history of bronchial asthma.		
Risk for photosensitivity reactions.		
Study Intervention Voltaren Emulgel Gel		
Skin and subcutaneous tissue disorders		Subjects will be on ward during administration period of study intervention. Specific in- and exclusion criteria to be applied (see Section 5.1 and 5.2). Regular checking for AEs and local tolerability (skin reactions) by staff (see Sections 1.3, 8.3.8, and 8.4). Specific restrictions are in place (see Section 5.3.4). Specific withdrawal criteria are in place (see Section 7.2.1).
Rash, eczema, erythema, dermatitis (including dermatitis contact), pruritus.	Risk is described as common (≥1/100, <1/10) in the current example SmPC.	
Desquamation, edema, skin dehydration	Risk is described as uncommon (≥1/1000, <1/100) in the current example SmPC.	
Dermatitis bullous.	Risk is described as rare (≥1/10.000, <1/1000) in the current example SmPC.	
Photosensitivity reaction.	Risk is described as very rare (<1/10.000) in the current example SmPC.	

continued

Identified and Potential Risks of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention Voltaren Emulgel Gel (continued)		
Infections and infestations		
Rash pustular.	Risk is described as very rare (<1/10.000) in the current example SmPC.	
Immune system disorders		
Hypersensitivity (including urticaria), angioneurotic oedema.	Risk is described as very rare (<1/10.000) in the current example SmPC.	
Respiratory, thoracic, and mediastinal disorders		
Asthma.	Risk is described as very rare (<1/10.000) in the current example SmPC.	
Gastrointestinal disorders		
Gastrointestinal disturbances	Risk is described as very rare (<1/10.000) in the current example SmPC.	
Study Procedures		
Complications from indwelling catheters	Local reactions, infections, nerve, or tissue damage may occur (rare).	Standard medical care to be applied when catheters are used.
Allergic reactions to ECG electrodes or dressing adhesive	Local intolerance may occur (rare).	Standard medical care to be applied when local intolerance occurs.

AE=adverse event; ECG=electrocardiogram; GI=gastrointestinal h=hour; IB=investigator's brochure; SmPC=summary of product characteristics.

2.3.2. Warnings and Precautions

According to the current version of the reference safety information (IB) for AMZ001, subjects should be advised to avoid direct solar radiation onto the application area during treatment and avoid gel contact with the eyes. Subjects should also be advised to wash hands thoroughly after the gel is rubbed into the skin. Specific restrictions are in place to address this issue in this study (see Section 5.3.4).

According to the current SmPC of Voltaren Emulgel 1.16% Gel (Name of marketed reference product in Germany: Voltaren Schmerzgel 11,6 mg/g Gel), the possibility of systemic side effects from application of diclofenac gel (e.g., renal, hepatic, or gastrointestinal side effects, systemic hypersensitivity reactions) cannot be excluded, if the preparation is used on large areas of skin and over a prolonged period of time. The study intervention may cause mild, localized skin irritation

in some people due to its content of propylene glycol. In addition, the medicine contains fragrance with benzyl benzoate, benzyl alcohol, citral, citronellol, coumarin, d-limonene, eugenol, farnesol, geraniol, and linalool, which may cause allergic reactions.

Voltaren Emulgel should be applied only to intact, non-diseased skin and not to skin wounds or open injuries. It should not be allowed to come into contact with the eyes or mucous membranes and should not be ingested. Treatment should be discontinued if a skin rash develops after applying the product. Subjects should be warned against excessive exposure to sunlight in order to reduce the incidence of photosensitivity. Some possibility of gastro-intestinal bleeding in those with a significant history of this condition has been reported in isolated cases. Specific exclusion criteria and restrictions are in place to address these issues in this study (see Section 5.2 and Section 5.3.4).

Diclofenac is contraindicated during the third trimester of pregnancy with reference to experience from treatment with NSAIDs with systemic uptake. Data from epidemiological studies suggest an increased risk of miscarriage and of cardiac malformation and gastroschisis after use of a prostaglandin synthesis inhibitors in early pregnancy. The systemic concentration of diclofenac is lower after topical administration, compared to oral formulations. To address this issue, adequate contraceptive measures have been defined for female subjects of childbearing potential (see Sections 5.1 and 10.4). No increased risk of major congenital abnormalities was found in the offspring of 705 fathers using NSAIDs in a large cohort study. Males trying to conceive can continue to take NSAIDs, such as diclofenac (Hui, 2022). Therefore, no specific contraception measures are required for male subjects with a WOCBP female partner (see Section 5.1).

2.3.3. Benefit Assessment

Healthy subjects may expect no direct benefits from participating in this clinical study. However, thorough medical check-ups may be an advantage.

2.3.4. Overall Benefit Risk Conclusion

Given the well-known safety profile of diclofenac, the risks related to treatment with topical diclofenac gel at the proposed dose are regarded as low. Overall, the risks posed to the subjects participating in the study are deemed low and ethically justifiable, and medical surveillance is considered adequate to ensure safety of the subjects.

Risk minimization measures routinely implemented in Phase 1 clinical studies are considered adequate, including exclusion criteria for laboratory parameters and observation of vital signs. Administration will be discontinued in case of events that unacceptably endanger the safety of the subjects (Section 7).

This clinical study will start after the favorable opinion of the IEC and once permission of the CA has been obtained. All investigations will be conducted in compliance with the clinical study protocol, ICH GCP, and any additional applicable regulatory requirements.

3. Objectives and Endpoints

Table 4: Objectives and Endpoints

Objectives	Endpoints
Primary	
• To investigate the systemic bioavailability of CCI) of AMZ001 (diclofenac sodium gel CCI) applied once daily (CCI CCI) versus CCI of Voltaren Emulgel 1.16% Gel (diclofenac diethylamine gel 1.16%) applied 4 times daily (CCI CCI) after repeated topical administrations in healthy subjects for 7 days.	Main PK parameter: • AUC_{0-24} of diclofenac in plasma on Day 7
Secondary	
• To determine the PK profile of CCI CCI of AMZ001 (diclofenac sodium gel CCI) and CCI of Voltaren Emulgel 1.16% Gel (diclofenac diethylamine gel 1.16%).	Additional PK parameters: • C_{max}, C_{min}, C_{ave}, t_{max}, t_{min}, PTF, of diclofenac in plasma on Day 7 • $R_{acc(AUC\ 0-24)}$ Day 7 versus Day 1 • Swing = $(C_{max} - C_{min}) / C_{min}$ on Day 7 • AUC_{0-24}, C_{max}, C_{ave}, t_{max}, of diclofenac in plasma on Day 1
• To assess the safety and local tolerability of CCI of AMZ001 (diclofenac sodium gel CCI) and CCI of Voltaren Emulgel 1.16% Gel (diclofenac diethylamine gel 1.16%).	• Number of subjects with TEAEs including SAEs • Number of subjects with study intervention-related TEAEs • Assess the skin irritation of each product
Other	
• To assess further included safety parameters.	• Various safety parameters (e.g., vital signs, laboratory tests, ECGs).

For definitions of PK endpoints refer to Section 8.5.2.

4. Study Design

4.1. Overall Design

The study will be conducted in a single-center, randomized, open-label, 2-period crossover, active-controlled design (see Figure 1).

The study will investigate the systemic bioavailability, safety, and tolerability of 1 dose CCI CCI of AMZ001 applied once daily CCI) compared to 1 dose CCI of Voltaren Emulgel 1.16% Gel applied 4 times daily CCI after repeated dosing in healthy subjects for 7 days.

All subjects will undergo a screening examination within 21 days prior to first administration of study intervention. Eligible subjects will be admitted to the study ward 1 day prior to administration of the study intervention (Day -1 of Period 1). Subjects who are in good general health, meet all the inclusion criteria and none of the exclusion criteria, will be allocated a randomization number. Eligibility check and randomization may be performed within the afternoon of Day -1 of Period 1 until pre-dose of Day 1 of Period 1, before any Day 1 procedures.

From Day 1 to Day 7 of Periods 1 and 2, subjects will self-administer AMZ001 gel or Voltaren Emulgel 1.16% Gel under the supervision of a designated clinical site staff member in 1 of the following 2 dosing sequences (see Figure 1):

Period 1

(Sequence 1) AMZ001 gel - CCI daily: 1 time daily CCI on each knee for 7 days

(Sequence 2) Voltaren Emulgel 1.16% Gel - CCI daily: 4 times daily CCI on each knee for 7 days

Period 2

(Sequence 1) Voltaren Emulgel 1.16% Gel - CCI daily: 4 times daily CCI on each knee for 7 days

(Sequence 2) AMZ001 gel - CCI) daily: 1 time daily CCI for 7 days

Blood samples for measurement of plasma concentration of diclofenac will be collected during the 1st and 7th day of treatment (Days 1 and 7) of each period at timepoints outlined in the SoA (see Section 1.3). Trough samples will be collected in the mornings of Day 2 to Day 6 in addition.

Subjects will be discharged from the ward on Day 8 of Period 1 and re-admitted on Day -1 of Period 2. Periods 1 and 2 will be separated by a wash-out period of at least 21 days during which the subjects will not be confined to the study site.

An end of trial (EOT) visit will be performed in the morning of Day 8 of Period 2 and subjects will be discharged afterwards.

In both study periods, the total in-house period for each subject will be at least 9 days per period, with at least 8 overnight stays.

Detailed information on study procedures is provided in the SoA for all study days (see Section 1.3).

Further information on study interventions and application instructions is provided in Section 6.1.

The duration of the study will be approximately 9 weeks per subject, depending on the length of the screening period (up to 21 days) and the length of the wash-out period (at least 21 days). For each subject, the study will end with the above-mentioned EOT. See Section 1.3 (SoA).

The total duration of the study will be approximately 3 months.

Planned sample size: A sufficient number of subjects will be screened and approximately 34 subjects will be randomized to ensure that at least 26 subjects are available for evaluation.

Minimum number of valid subjects with complete study interventions: 26 subjects.

Interim analyses are not planned.

4.2. Scientific Rationale for Study Design

4.2.1. Sex and Age Distribution

The envisaged indication for AMZ001 is relief of the pain associated with arthritis in males and females.

Arthritis includes approximately 100 conditions that affect the joints and surrounding tissues. In a recent evaluation, approximately 21.2% of U.S. adults aged ≥18 years had diagnosed arthritis. Nearly one half of adults with arthritis (48.3%) were aged ≥65 years, and 40.0% were aged 45 to 64 years (Fallon, 2023).

This Phase 1 study will primarily focus on the evaluation of PK parameters. In order to reduce variability not related to differences between products, the study will be performed in healthy male and female volunteers from 18 to 65 years of age. In line with the guideline on the investigation of bioequivalence (EMA, 2010), the model, *in vivo* healthy volunteers, is regarded as adequate to detect formulation differences and to allow extrapolation of the results to populations for which the reference medicinal product is approved (e.g., for the elderly).

Adequate contraceptive methods will be applied in female subjects of child-bearing (see Sections 5.1 and 10.4). Possible differences between sexes will not be evaluated, as there are no gender differences in the pharmacokinetics of diclofenac (Voltaren Emulgel Product Monograph, 2017).

4.3. Justification for Dose

Higher concentration and absorption characteristics of the AMZ001 formulation will provide a delivery of diclofenac that allows for once daily application compared to dosing 4 times daily recommended for the reference product, Voltaren Emulgel 1.16% Gel.

With CCI of AMZ001 CCI a total daily dose of CCI of diclofenac sodium will be applied onto the skin. With CCI of Voltaren Emulgel 1.16% Gel CCI CCI), a total daily dose of CCI of diclofenac sodium will be administered.

Comparative efficacy trials support the proposal of once daily dosing for AMZ001 (Bihlet et al. 2020). Using already completed PK studies, a PK modelling and simulation has allowed to estimate the appropriate dose (CCI) that is expected to provide similar exposure as Voltaren Emulgel 1.16% Gel, despite the difference in the number of daily applications (1 time versus 4 times daily) and the lower daily diclofenac dose with AMZ001 CCI diclofenac sodium) compared to Voltaren Emulgel 1.16% Gel CCI diclofenac sodium).

4.4. Start / End of Study Definition

The study start is defined as the date of the first informed consent signature of the first subject.

Subjects are considered to have completed the study, if they have completed all phases of the study including the last scheduled procedure shown in the SoA (refer to Section 1.3).

The end of the study is defined as the date of the last scheduled procedure shown in the SoA for the last subject.

Information on study termination or discontinuation criteria is provided in Section 7.

5. Study Population

No subject may be randomly assigned to study intervention unless adherence to all eligibility criteria as given in Sections 5.1 and 5.2 is established.

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

Individuals who do not meet the eligibility criteria due to medical findings will be advised to consult a doctor as applicable.

5.1. Inclusion Criteria

A subject is eligible to be included in the clinical study only, if all of the following criteria apply:

5.1.1. Inclusion Criteria to be Checked at Screening

Age

1. Age of 18 to 65 years inclusive, at the time of signing the informed consent.

Weight

2. Body mass index (BMI) within the range of 18.0-30.0 kg/m^2 (inclusive) at screening.

Sex and Contraceptive/ Barrier Requirements

3. Male and female.

Contraceptive use by men and women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

a. Male Subjects:

No specific contraception measures are required for male subjects with a WOCBP female partner.

b. Female Subjects:

- A female subject is eligible to participate if she is not pregnant or breastfeeding, and one of the following conditions applies:
    - Is a WONCBP as defined in Section 10.4.

      OR
    - Is a WOCBP and using a contraceptive method that is highly effective (with a failure rate of <1% per year), preferably with low user dependency, as described in Section 10.4 without interruption, during trial participation and until 30 days after the last administration of study intervention and agrees not to donate eggs (ova, oocytes) for the purpose of reproduction during this period. The Investigator should evaluate the potential

for contraceptive method failure (e.g., noncompliance, recently initiated) in relationship to the first dose of study intervention.

- A WOCBP must have a negative highly sensitive serum pregnancy test at screening. Additional requirements for pregnancy testing during and after study intervention are provided in Sections 5.1.2 and 8.3.5.
- The Investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy.

Type of Subject and Disease Characteristics

4. Subject is overtly healthy as determined by medical evaluation including medical history, full physical examination, vital signs, and ECG.
5. Subject is free of any systemic or dermatologic disorder and chronic or acute infections, which, in the opinion of the Investigator, may interfere with the study results or increase the risk of adverse events.

Drugs and Stimulants

6. Subject is a non-smoker, former smoker or stable non-smoker (= 0 cigarettes, pipes, cigars, or others) for at least 3 months prior to screening. Subjects must also have abstained from use of other nicotine containing products (e.g., nicotine patch, chewing gum or e-cigarettes) for at least 3 months before screening.

Informed Consent

7. Capable of giving signed informed consent as described in Section 10.1, which includes compliance with the requirements and restrictions listed in the ICF and in this protocol prior to any clinical study specific procedure.

5.1.2. Inclusion Criteria to be Re-checked upon Admission and Re-Admission (Day -1 of Periods 1 and 2)

Sex and Contraceptive/Barrier Requirements

8. No changes in medical conditions or prior/concomitant therapy further to sex and contraceptive/barrier requirements compared to screening.
9. A WOCBP must have a negative highly sensitive urine pregnancy test on Days -1, within 24 hours before the first dose of study intervention of each study period, (see Section 8.3.5).
    - If a urine test cannot be confirmed as negative (e.g., an ambiguous result), a serum pregnancy test is required. In such cases, the subject must be excluded from participation if the serum pregnancy result is positive.

Type of Subject and Disease Characteristics

10. Subject is overtly healthy as determined by medical evaluation including check of changes in medical history compared to screening, abbreviated physical examination, and vital signs.
11. No changes in type of subject and disease characteristics compared to screening.

Drugs and Stimulants

12. No changes in drugs and stimulants compared to screening.

5.2. Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

5.2.1. Exclusion Criteria to be Checked at Screening

Medical Conditions

13. Any visible skin disease, skin lesions, wounds, or a significant amount of hair at the application sites (both knees).
14. Any history or evidence of any clinically relevant cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrinologic, hematologic, immunologic, metabolic, genitourinary, pulmonary, neurologic, dermatologic, musculoskeletal, psychiatric and/or other major disease as determined by medical evaluation (including [abbreviated] physical examination) capable of significantly altering the absorption, metabolism, or elimination of drugs; constituting a risk when taking the study intervention; or interfering with the interpretation of data.
15. Any known or suspected malignancy, excluding basal cell cancer unless it is associated with the treatment area.
16. Any gastrointestinal bleeding issues, e.g., Gastroesophageal Reflux Disease, Peptic Ulcer Disease.
17. Any hospital admission or major surgery within 30 days prior to randomization.
18. History or current evidence of ongoing hepatic disease or impaired hepatic function at screening as indicated by diagnostic assessments.
19. History or current evidence of renal disease or impaired renal function at screening as indicated by diagnostic assessments.
20. Any clinically relevant history of allergic conditions requiring hospitalization or prolonged systemic treatment (including drug allergies, drug hypersensitivity, asthma, angioedema, urticaria, eczema, acute rhinitis precipitated by acetylsalicylic acid or other NSAIDs, allergies requiring therapy with corticosteroids or anaphylactic reactions), excluding allergic contact

sensitizations (e.g., nickel allergy). Subjects with uncomplicated seasonal allergic rhinitis can be accepted only if the expected allergy season is clearly outside enrolment/ treatment periods.

21. Known or suspected hypersensitivity to diclofenac, or any components of the formulation used (i.e., diethylene glycol monoethyl ether, myristyl alcohol, ethyl alcohol, hydroxypropyl cellulose, diethylamine, carbomers, cetomacrogol, cocoyl caprylocaprate, isopropyl alcohol, propylene glycol, liquid paraffin, fragrance with benzyl benzoate, benzyl alcohol, citral, citronellol, coumarin, d-limonene, eugenol, farnesol, geraniol, linalool), aspirin, Xarelto, coumadin, or other non-steroidal anti-inflammatory drugs (NSAIDs), including Cyclooxygenase-2 (COX-2) inhibitors.
22. Contraindications for the use of study interventions.
23. Any clinically relevant chronic or acute infectious illnesses or febrile infections within 2 weeks prior to the first scheduled administration of study intervention.
24. Evidence of COVID-19 signs or symptoms or confirmed COVID-19 infection within the last 2 weeks prior to screening.

Prior/Concomitant Therapy

25. Treatment with systemic or local diclofenac within 30 days of enrollment or during the study (except for study interventions).
26. Use of any concomitant medication or any drugs / medicines (including over-the-counter medication, dietary supplements, natural and herbal remedies) within 2 weeks before the first scheduled administration of study intervention or within less than 10 times the elimination half-life of the respective drug (whichever is longer) or is anticipated to require concomitant medication during the 2-week period or at any time throughout the study.

    Occasional use of Paracetamol (acetaminophen) 500 mg is allowed (up to 1000 mg daily, medicinal products in their original packaging, approved and marketed in Germany).

    Oral, injectable, implantable, and topical contraceptives as outlines in Section 10.4 and hormone replacement therapy are permitted, as long as the female subject is on stable treatment for at least 3 months and continues treatment throughout the study.

    Single intake of other medication is only permitted, if it is judged by the Investigator to have no clinical relevance and if it is not expected to confound the interpretation of the study results.
27. Use of any topical medication, cosmetics, cream, ointments, lotions on the treatment site 2 weeks prior to enrollment through EOT visit, see Table 5 of the study protocol.

Prior/Concurrent Clinical Study Experience

28. Use of any investigational drug or participation in any clinical study within 30 days or 10 half-life times (if known) of study drug administered in a previous trial, whichever is longer, prior to the expected date of first administration of study intervention.

Diagnostic assessments

29. Positive for hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies (anti-HCV) and anti-human immunodeficiency virus antibodies (anti-HIV 1 and 2 and HIV 1-p24 antigen) at screening;

30. Positive screen for alcohol, drugs of abuse and cotinine test at screening.

31. Supine systolic blood pressure >140 mmHg or <90 mmHg; diastolic blood pressure >90 mmHg or <50 mmHg and pulse rate <50 bpm or >90 bpm, and tympanic body temperature of <35.9 and >37.6°C at screening.

32. 12-lead ECG with clinically relevant abnormality or showing a QTcF >450 ms for male and >470 ms for female subjects, PR >215 ms, or QRS >120 ms at screening.

33. Elevations in alanine aminotransferase (ALT) ≥1.1 x ULN, aspartate aminotransferase (AST) ≥1.2 x ULN, serum bilirubin ≥1.2 x ULN, creatinine ≥1.1 x ULN, blood urea nitrogen (BUN) ≥35 mg/dL, or presence of clinically significant abnormal urinary constituents (e.g., albuminuria) at screening.

34. Elevations in more than one of the following lab values: Gamma-glutamyl transferase (GGT) ≥1.5 ULN; alkaline phosphatase (ALP) ≥1.5 ULN; creatine kinase (CK) ≥3 ULN at screening. A single deviation from the above values is acceptable and will not exclude the candidate, unless specifically advised by the Investigator.

Other Exclusions

35. Any use of drugs-of-abuse or alcohol abuse within 6 months prior to screening. Higher than low-risk alcohol consumption i.e., consumption of an average weekly alcohol intake of >14 units/week for men (daily dose of >24 g, weekly dose of >168 g) and >7 units/week for women (daily dose of >12 g, weekly dose of >84 g). One unit (12 g) corresponds to 0.3 L of beer/day or 0.12 L of wine/day or 1 glass (at 2 cL) of spirits/day.

36. Excessive consumption of caffeine- or xanthine-containing food or beverages (>5 cups of coffee a day or equivalent) or inability to stop consuming from 48 hours prior to first planned administration of study intervention.

37. Intake of specified food or beverages containing grapefruit, grapefruit juice, grapefruit hybrids, star fruit, Seville orange, tangelo, pomelos, exotic citrus fruits, as specified in Table 5 of the study protocol, within 14 days prior to (re-) admission and during both treatment periods.

38. Regular consumption of poppy seed containing food prior to screening.
39. Female subject who plans to become pregnant during the clinical study period and for 3 months after final study intervention administration.
40. Donation or blood collection of more than 1 unit (approximately 450 mL) of blood (or blood products) or acute loss of blood during the 30 days prior to randomization.
41. Employee of the Sponsor, the Nuvisan Group, or other Contract Research Organization (CRO) involved in the clinical study.
42. Legal incapacity or limited legal capacity, or incarceration.
43. Inability to understand or communicate reliably with the Investigator or considered by the Investigator to be unable to or unlikely to co-operate with the protocol requirements, instructions, and study-related restrictions.
44. Any other conditions or factors which in the opinion of the Investigator may interfere with study conduct.

5.2.2. Exclusion Criteria to be re-checked upon Admission and Re-Admission (Day -1 of Periods 1 and 2)

Medical Conditions

45. No changes in medical conditions compared to screening.

Prior/Concomitant Therapy

46. No changes in prior/concomitant therapy compared to screening.

Prior/Concurrent Clinical Study Experience

47. No changes in prior/concomitant study experience compared to screening.

Diagnostic assessments

48. Positive screen for alcohol, drugs of abuse, and cotinine test upon admission to each intervention period
49. Supine systolic blood pressure >140 mmHg or <90 mmHg; diastolic blood pressure >90 mmHg or <50 mmHg and pulse rate <50 bpm or >90 bpm upon admission.

Other Exclusions

50. No clinically relevant changes in other exclusion criteria compared to screening.

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

- During the in-house stay(s), the subjects may only consume food and beverages provided by the study site. The diet provided (maximum of 4 meals per day) will consider the imposed restrictions. On Days 1 to 7 of both study periods, subjects will receive standardized meals, served at customary times.
- Further dietary restrictions to be observed are displayed in Table 5.

5.3.2. Caffeine, Alcohol, and Tobacco

- Restrictions on caffeine, alcohol, and tobacco are described in Table 5.

5.3.3. Activity

- Restrictions on activity are described in Table 5.

5.3.4. Other Restrictions

- Other restrictions are described in Table 5.

Table 5: Restrictions

What?	Restriction
Dietary restrictions	
Caffeine- or xanthine-containing food and beverages (e.g., coffee, tea, cola, chocolate, and energy drinks)	Not allowed during both treatment periods (Day -1 until end of confinement [Day 8] of Periods 1 and 2); permitted during washout period and otherwise. Excessive consumption of caffeine- or xanthine-containing food or beverages not permitted from 48 hours prior to first planned administration of study intervention (see exclusion criterion 36).
Food and beverages containing grapefruit, grapefruit juice, grapefruit hybrids, star fruit, Seville orange, tangelo, pomelos, exotic citrus fruits.	Not permitted within 2 weeks prior to (re-)admission and during both treatment periods (Day -1 until end of confinement [Day 8] of Periods 1 and 2). See exclusion criterion 37.
Special diets	Preventing the subjects from eating the standard meals during the treatment periods, e.g. strict vegetarian or low caloric diet.
Caffeine, alcohol, and tobacco	
Smoking, use of tobacco / nicotine containing products (e.g., nicotine patch, chewing gum, or e-cigarettes)	Not permitted during the study. See inclusion criterion 6.
Alcohol	Not allowed from 48 hours prior to (re-) admission (Day -1) and during both treatment periods (Day 1 until end of confinement [Day 8] of Periods 1 and 2); permitted during washout period and otherwise. See exclusion criterion 35.
Activity	
Physical activity	Usual activities are permitted, but no strenuous physical exercise from 72 hours prior to admission until EOT. Subjects may participate in light recreational activities during hospitalization phase (e.g., watching television, reading).
Other restrictions	
Showering, bathing, swimming	Subjects are permitted to shower, bath, or swim 1 hour pre-dose or earlier, and after 3 hours post-dose or later.
Topical medication and cosmetics on the treatment site	Use of any topical medication, cosmetics, cream, ointments, lotions on the treatment site not permitted 2 weeks prior to enrollment through EOT visit (see exclusion criterion 27).
Exposure of the pre-defined application area to direct sunlight	To be avoided during both treatment periods (Day -1 to Day 8 of Periods 1 and 2). Permitted during washout period.

continued

Table 5 (continued): Restrictions

Other restrictions (continued)	
Solarium	Not permitted during both treatment periods (Day -1 to Day 8 of Periods 1 and 2). Not permitted during washout period.
Gel contact with eyes, nose, and mouth	To be avoided during the entire study. If gel contact occurs, rinse the affected area thoroughly with water.
Gel contact with hands	Subjects will be advised to wipe their hands e.g. with an absorbent paper and to wash them thoroughly after rubbing the gel into their knees, after each full administration of study intervention. The e.g. absorbent paper should be thrown in the trash after use.
Female subjects (WOCBP) only: Donation of eggs (ova, oocytes) for the purpose of reproduction.	Not permitted during trial participation and until 30 days after the last administration of study intervention.

ED = early discontinuation; EOT = end of trial; WOCBP = woman/women of childbearing potential

5.4. Screen Failures

Screen failures are defined as subjects who consented to participate in the clinical study but are not subsequently randomly assigned to study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened up to 2 times (see Section 5.5). Rescreened subjects will be assigned a new screening number. Subjects who are rescreened are required to sign a new ICF (see Section 10.1).

5.5. Criteria for Temporarily Delaying Randomization

In case of abnormal results caused by intercurrent diseases, short-term treatable conditions, or other temporary health disorders (e.g., acute infection, iron deficiency, blood pressure outside defined range), the Investigator may decide to repeat the respective screening parameter(s). As a rule, up to 2 repetitions are acceptable.

6. Study Interventions and Concomitant Therapy

Study interventions are all pre-specified investigational and non-investigational medicinal products, medicinal devices, and other interventions (e.g., surgical, and behavioral) including marketed products, or placebo, or a combination of the same as per study plan, intended to be administered / applied to a study subject during the study conduct.

6.1. Study Interventions Administered

Table 6: Study Interventions

Intervention Label	AMZ001	Voltaren Emulgel
Intervention Name	AMZ001 gel	Voltaren Emulgel 1.16% Gel (Name of marketed reference product in Germany: Voltaren Schmerzgel 11,6 mg/g Gel)
Intervention Description	Transdermal hydroalcoholic gel formulation for skin administration	White to off white gel for skin administration
Type	Drug	Drug
Dose Formulation	Gel for topical administration	Gel for topical administration
Unit Dose Strength(s)	CCI of AMZ001 gel contains CCI of diclofenac sodium.	One (1) g of Voltaren Emulgel 1.16% Gel contains 11.6 mg of diclofenac diethylammonium, which corresponds to 10 mg diclofenac sodium.
Dosage Levels and Dosing Instructions	CCI of AMZ001 will be applied once daily CCI CCI for 7 days, as per detailed application instruction.	CCI of Voltaren Emulgel 1.16% Gel will be applied 4 times daily (CCI at relative times 0h, 5h, 10h, 15h), for 7 days, as per detailed application instruction.
Route of Administration	Topical administration	Topical administration
Use	Experimental	Active comparator
Marketing Authorization Holder	NA	Haleon Germany GmbH 80258 Munich, Germany
IMP and NIMP/AxMP	IMP	IMP

continued

Table 6 (continued): Study Interventions

Intervention Label	AMZ001	Voltaren Emulgel
Sourcing	The Sponsor will provide Nuvisan GmbH with the medication, which will be released by the Sponsor QP according to Good Manufacturing Practice Annex 13 for use in the study. CTS Nuvisan will create a CoC for the labelling activities performed.	Nuvisan will source medication available on the local market.
Packaging and Labelling	Study intervention will be provided by the Sponsor in dispensing packages, measuring 1 mL per actuation. Each IMP dispenser will be labelled according to country requirement by Nuvisan.	Study intervention will be used as unchanged product with market authorization

AxMP=auxiliary medicinal product, NA=not applicable, NIMP=non-investigational medicinal product; IMP=investigational medicinal product.

In each period, subjects will receive multiple topically applied doses of AMZ001 gel or Voltaren Emulgel 1,16% Gel from Day 1 to Day 7, as outlined in Table 6 and in line with the detailed administration instructions.

Study interventions will be self-administered to both knees by the subject himself / herself under the supervision of a designated clinical site staff member for consistency in application and to ensure the correct amount is applied to each knee at the specified dose and frequency for 7 consecutive days. The subject will spread the gel directly to the pre-defined treatment area of approximately 500 cm^2 for AMZ001 and 400 cm^2 for Voltaren Emulgel 1.16% Gel, for each subject knee as per detailed application instructions. Further information on restrictions is provided in Table 5. Further instructions for use will be provided in an IMP handling manual.

AMZ001 IMP dispensers and Voltaren Emulgel 1.16% Gel tubes will be weighed throughout the study before and after each administration of study intervention, as outlined in the SoA (Section 1.3).

6.2. Preparation, Handling, Storage, and Accountability

1. The Sponsor will supply enough study intervention AMZ001 to Nuvisan Clinical Trial Supplies (CTS) Department.

2. Nuvisan will source the commercial reference product Voltaren Emulgel 1.16% Gel from the German market.
3. Nuvisan CTS Department will document the receipt of the study intervention AMZ001 and must confirm appropriate conditions (e.g., temperature) have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention AMZ001. Nuvisan CTS Department will be responsible for transfer to the site.
4. Study intervention AMZ001 will be packed and labelled study specifically by Nuvisan CTS Department before the clinical part of the study starts. QP release of clinical supplies will be performed by the Sponsor as per the German clinical study labelling requirements.
5. Only subjects enrolled in the study may receive study intervention and only authorized site staff may supply, prepare, or supervise the administration of study intervention. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the Investigator and authorized site staff.
6. All study intervention administrations will be performed in accordance with the product specifications under supervision of site staff authorized by the Principal Investigator.
7. The Investigator is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).
8. The number of unused study intervention will be documented by Nuvisan CTS Department. According to the provisions of the Sponsor the unused study intervention will be destroyed once the final reconciliation has been completed and the authorization for destruction has been received from the Sponsor.
9. Further guidance and information for the final disposition of unused study interventions are provided in the applicable manual.

6.3. Assignment to Study Intervention

Subject identification and intervention assignment	After informed consent procedure, every subject is given a screening number. Within the afternoon of Day -1 of Period 1 until pre-dose of Day 1 of Period 1, before any Day 1 procedures, subjects will be assigned a unique 3-digit randomization number in ascending numerical order. The number encodes the subject's assignment to one of the 2 arms of the study, according to the randomization list provided by Nuvisan CTS Department. Each subject will be dispensed study intervention, labelled with their unique randomization number throughout the study. Replacement subjects of already randomized subjects prior to dosing will be assigned to the intervention as the original subject. Randomization number will be the number of the original subject plus 100. For example, the replacement for screening number 012 will receive the random number 112.

Only subjects who comply with all eligibility criteria can be included into the study. Subjects will be assigned to randomization numbers in accordance with Nuvisan standard operational procedures (SOPs).

The Investigator will keep a record relating the subject identifiers (screening and random number) and the names of all subjects who have given their informed consent, to allow easy checking of data in subject files, when required. This record will also include the date of subject's enrolment and completion, as well as subjects who could not be randomly assigned to study intervention for whatever reason.

6.4. Blinding, Masking

The study will be performed in a non-blinded design.

6.5. Study Intervention Compliance

The administration of the study intervention will be performed in the clinical unit by qualified clinical professionals of the Investigator's team. Deviation(s) from the prescribed dosage regimen should be recorded in the case report form (CRF).

6.6. Dose Modification

No dose modifications are envisaged during this study.

6.7. Continued Access to Study Intervention after the End of the Study

As this is a study in healthy volunteers, no intervention is planned at the end of the study. If there are findings that are unclear in the final examination, a detailed assessment, a follow-up and, if necessary, specific medical treatment may be required.

6.8. Treatment of Overdose

For this study, any dose of study intervention greater than the protocol-defined dose will be considered an overdose.

The low systemic absorption of study interventions renders overdose very unlikely. However, according to the SmPC of Voltaren Emulgel 1.16% Gel, undesirable effects, similar to those observed following an overdose of diclofenac tablets, can be expected if Voltaren Emulgel 1.16% Gel is inadvertently ingested (e.g. 1 tube of 100g contains the equivalent of 1000 mg of diclofenac sodium).

Management of overdosage with NSAIDs essentially consists of supportive and symptomatic measures. There is no typical clinical picture resulting from Voltaren Emulgel 1.16% Gel overdosage. Supportive and symptomatic treatment should be given for complications such as hypotension, renal failure, convulsions, gastro- intestinal irritation, and respiratory depression; specific therapies such as forced diuresis, dialysis or hemoperfusion are probably of no help in eliminating NSAIDs due to their high rate of protein binding and extensive metabolism.

In the event of accidental ingestion, resulting in significant systemic adverse effects, general therapeutic measures normally adopted to treat poisoning with non- steroidal anti-inflammatory medicines should be used. The use of activated charcoal should be considered, especially within a short time (within one hour) of ingestion of a toxic dose (see SmPC).

According to the current IB of AMZ001, no incidents of overdose have been reported with AMZ001 (diclofenac sodium gel [redacted]). Possible effects of overdose include those observed after an overdose of oral diclofenac. Symptoms following excessive ingestion of an oral NSAID may include lethargy, drowsiness, nausea, vomiting, and epigastric pain. In severe cases, GI bleeding, hypertension, acute renal failure, respiratory depression, coma, and anaphylaxis may occur.

The Sponsor does not recommend specific treatment for an overdose with AMZ001, and treatment should be symptomatic and supportive as appropriate.

In the event of an overdose, the Investigator should:

1. Evaluate the subject to determine, in consultation with the responsible PhV expert, if possible, whether study intervention should be interrupted or whether the dose should be reduced.
2. Closely monitor the subject for any AE/SAE and laboratory abnormalities until study intervention can no longer be detected systemically (at least 1 day).

3. If requested by the responsible PhV expert (determined on a case-by-case basis), obtain a plasma sample for PK analysis within 1 day from the date of the last dose of study intervention.
4. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

Decisions regarding dose interruptions or modifications will be made by the Investigator in consultation with the Sponsor based on the clinical evaluation of the subject.

6.9. Prior and Concomitant Therapy

Any medication taken at or after the time of first study intervention, regardless of whether it had started prior to the study or not, is to be recorded as concomitant medication. Prior medications are defined as any medication taken which had been stopped prior to the first study intervention.

Subjects must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements) within 2 weeks or 10 half-lives (whichever is longer) before the start of study intervention until completion of the EOT visit. Information on non-permitted prior or concomitant therapy including drugs and herbal remedies is provided in the list of exclusion criteria and meals and dietary restrictions. See Sections 5.2 and Table 5 for further details.

In addition, subjects must abstain from using any topical medication, cosmetics, cream, ointments, lotions on the treatment site 2 weeks prior to enrollment through EOT visit, as indicated in the list of exclusion criteria (see Section 5.2 and Table 5).

Except when necessary to treat an AE, subjects are not allowed to use those medications, starting from the specified timepoints until completion of the study.

Paracetamol (acetaminophen) at doses of 500 mg (≤ 2 gram/day, if needed), is permitted for use any time during the study. Contraceptives as outlined in Section 10.4 are also permitted. Hormone replacement therapy is permitted, as long as the female subject is on stable treatment for at least 3 months and continues treatment throughout the study (see Section 5.2).

Single intake of other medication is only permitted, if it is judged by the Investigator to have no clinical relevance and if it is not expected to confound the interpretation of the study results.

Other concomitant medication may be considered on a case-by-case basis to avoid immediate hazard to the subjects by the Investigator who may wish to consult with the Medical Monitor or Sponsor.

For any concomitant therapy used, the name of the drug, the reason for use, dates of administration including start and end dates and dosage information including dose and frequency will be recorded in the CRF. In consultation with the Sponsor, a decision may be taken by the Investigator to withdraw a subject from the study when other concomitant medication is required or has been taken without consulting the Investigator.

6.9.1. Rescue Medicine

Not applicable.

7. Discontinuation of Study Intervention and Subject Discontinuation/Withdrawal

7.1. Discontinuation of Study Intervention

In rare instances, it may be necessary for a subject to permanently discontinue study intervention. If study intervention is permanently discontinued, the subject will remain in the study. Safety, and tolerability data as well as PK data will be collected to the furthest possible extent and the subject will be asked to participate in the early discontinuation visit.

See the SoA (Section 1.3) for data to be collected at the time of discontinuation of study intervention and ED / EOT visit and for any further evaluations that need to be completed.

7.1.1. Discontinuation Criteria for Individual Subjects

The Investigator will withdraw a subject from receiving further study intervention in the following cases:

- Occurrence of an AE of severe severity in case of causal relationship to study intervention.
- Relevant signs or symptoms affecting subject safety. Any events that unacceptably endanger the safety of the subject.
- If, in the Investigator's opinion, continuation of the study would be harmful to the subject's well-being.
- Hypersensitivity reactions classified as severe.
- Impossibility to obtain blood samples in general.
- Abnormal blood pressure including hypotension defined as systolic <70 mmHg and/or diastolic <40 mmHg, or hypertension defined as systolic >160 mmHg and/or diastolic >110 mmHg (evaluated with the subject in supine position for a minimum of 5 minutes and confirmed by 2 repeat measurements). In case of repeat abnormal findings, see above, this should lead to direct study withdrawal.
- Creatinine >1.1xULN
- Use of nonpermitted concomitant medications. However, any medications considered necessary for the subject's wellbeing may be given at the discretion of the Investigator.
- Occurrence of pregnancy. See Section 8.4.5 and Section 10.4.

7.1.1.1. Liver Chemistry Stopping Criteria

Discontinuation of study intervention for abnormal liver tests is required by the Investigator when a subject meets one of the following conditions or if the Investigator believes that it is in best interest of the subject:

- Increase in ALT or AST ≥3xULN and/or total bilirubin >2xULN considered by the Investigator to be at least possibly related to the study intervention. ALT ≥3xULN and bilirubin ≥2 x ULN (>35% direct bilirubin) has to be reported as SAE (refer to Section 10.5).

7.2. Subject Discontinuation / Withdrawal from the Study

7.2.1. Discontinuation / Withdrawal Criteria for Individual Subjects

- A subject may withdraw from the study at any time at their own request without giving reasons. The subject will not suffer any disadvantage as a result. A subject may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon.
- At the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted. See SoA in Section 1.3 for data to be collected at the time of study discontinuation and ED and for any further evaluations that need to be completed.
- The subject will be permanently discontinued from both the study intervention and the study at that time.
- If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a subject withdraws from the study, they may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.
- Subjects who discontinue may not re-enter the study.
- Depending on the timepoint of discontinuation, a withdrawn subject is referred to as either “screening failure” or “dropout” as specified in Sections 5.4 and Section 7.2.2.

The Investigator will withdraw a subject from the study in the following cases:

- Request by the subject to discontinue (withdrawal of consent).
- At the specific request of the Sponsor and in liaison with the Investigator (e.g., obvious non-compliance).
- Any clinically relevant symptom or sign which in the opinion of the Investigator and/or Sponsor warrants subject withdrawal.
- Impossibility to obtain samples.
- Positive results from pregnancy testing.
- Protocol deviation judged as significant by the Investigator, including non-compliance to the required study considerations (e.g., food/diet requirements).

- Depending on the timepoint of withdrawal, a withdrawn subject will be referred to as either "screening failure" or "dropout" as specified in Section 5.4 and Section 7.2.2.

7.2.2. Replacement of Subjects

A subject who discontinues the study prematurely for any reason is defined as a 'dropout' if the subject has already been randomly assigned to study intervention.

Dropouts will be replaced only if the number of subjects completing the study becomes less than 34 to ensure a total number of 26 evaluable subjects. For details regarding intervention assignment of replacement subjects, please refer to Section 6.3.

The data obtained from dropouts will be used in the evaluation to the largest possible extent.

7.3. Further Stopping Rules

7.3.1. Premature Discontinuation of the Complete Study

The Sponsor may discontinue the complete study at any time, for ethical or scientific reasons. The Principal Investigator is entitled to stop the study at any time due to medical reasons. In such a case, the Principal Investigator should consult the Sponsor at the earliest opportunity.

The study may be terminated prematurely or temporarily halted if any unacceptable findings are identified. The occurrence of 1 of the following stopping criteria shall result in an immediate stop of dosing and a temporary halt of the study:

- A serious adverse reaction (i.e., an SAE considered at least possibly related to the study intervention administration) in 1 subject.
- AEs of at least moderate severity in ≥50 % of the subjects for which a causal relationship to the study intervention or study related procedures cannot be excluded.
- Severe non-serious adverse reaction (i.e., severe non-serious AEs considered as, at least, possibly related to the study intervention administration) in 2 subjects, independent of within or not within the same system organ class.
- Unacceptable risks, any relevant toxicity, or a negative change in the risk/benefit assessment is identified. This might include the occurrence of AEs which character, severity or frequency is new in comparison to the existing risk profile.
- Any data derived from other clinical studies or toxicological studies become available which negatively influence the risk/benefit assessment.

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable

regulatory requirements. The Investigator shall promptly inform the subject and should assure appropriate subject therapy and/or follow-up.

Further information on study and site closure is provided in Section 10.1.11.

7.4. Lost to Follow-up

A subject will be considered lost to follow-up if they repeatedly fail to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether the subject wishes to and/or should continue in the study or not.
- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, contact via email along with 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.

Should the subject continue to be unreachable, they will be considered to have withdrawn from the study.

8. Study Assessments and Procedures

- Prior to performing any study assessments, the Investigator will obtain written informed consent as specified in Section 10.1.4.
- Study procedures and their timing are summarized in the SoA (Section 1.3). Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study intervention.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.
- In the event of a significant study-continuity issue (e.g., caused by a pandemic), alternate strategies for subject visits, assessments, medication distribution and monitoring may be implemented by the Sponsor or the Investigator, as per local health authority/ethics requirements. All procedures adapted to the situation must be submitted, if required as per local regulations, through a protocol amendment to the Regulatory Authority and IEC for approval prior to implementation of mitigation procedures.
- The maximum amount of blood collected from each subject over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

8.1. Administrative and Baseline Procedures

8.1.1. Demographic

For demographic assessment the following parameters will be recorded: age (incl. year of birth), sex, race / ethnicity.

8.1.2. Body Weight and Height, BMI

Body weight will be measured by a member of the Investigator's team under the following conditions:

- Subject in underwear and without shoes after having emptied their bladder
- Electronic physician (column) scale with digital display, measurement units 0.1 kg

The subject's height (without shoes) will be measured to calculate the BMI. The BMI will be calculated by data management directly in the electronic CRF. The study site might calculate in addition for eligibility check.

8.1.3. Medical History

Medical history findings (i.e., previous diagnoses, diseases, or surgeries) considered relevant to the study will be collected:

Any relevant findings from the past that occurred prior to signing the informed consent form or started prior to signing the informed consent form, are still ongoing but resolve before start of first study intervention administration will be recorded in the Medical History section of the CRF.

Occurrences after obtaining informed consent or presently occurring and worsening after signing the informed consent form will be recorded in the AE section of the CRF.

8.1.4. Baseline Treatment Area Assessment

A treatment area of approximately 500 cm^2 for AMZ001 and 400 cm^2 for Voltaren Emulgel 1.16% Gel will be pre-defined for each subject knee on Day -1 of each study period, as per detailed application instructions.

A baseline treatment area assessment will be performed for each knee during screening, and on Days-1 of Period 1 and Period 2, as outlined in Section 8.3.8 for the assessment of local tolerability.

8.1.5. Other Baseline Characteristics

Information on smoking and alcohol consumption will be collected.

8.2. Efficacy and/or Immunogenicity Assessments

This section is not applicable as efficacy / effectiveness and/or immunogenicity is not assessed in this study.

8.3. Safety Assessments

The safety profile of the study interventions will be assessed through the recording, reporting and analysis of baseline medical conditions, AEs, physical examination findings, vital signs, ECGs, and clinical laboratory tests.

Planned timepoints for all safety assessments are provided in the SoA (Section 1.3). Additional assessments during the study will be conducted at the discretion of the Investigator.

8.3.1. Physical Examinations

- A comprehensive physical examination will be performed by a physician and will include at a minimum, assessment of the eyes, ears, nose, and throat as well as assessment of the cardiac,

peripheral vascular, pulmonary, musculoskeletal, neurologic, abdominal, lymphatic, and dermatologic system.

- An abbreviated physical examination will be performed by a physician and will include, at a minimum, assessments of the skin, abdomen (liver and spleen), lungs, and cardiovascular system.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.
- All pre-existing and relevant medical events must be recorded and described in the source documents. All efforts to attach support documentation (i.e., reports, results, etc.) must be done.

8.3.2. Vital Signs

- Vital signs will be measured after at least 5 minutes rest in a supine position and will include body temperature, pulse rate, and systolic and diastolic blood pressure at the timepoints specified in the SoA (Section 1.3).
- Blood pressure and pulse should not be measured on the same arm where blood samples are taken from.
- Body temperature will be measured auricularly with a calibrated device.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only in case of doubt or if an automated device is not available.
- Further vital signs measurements during the clinical study are at the discretion of the Investigator.

8.3.3. Electrocardiograms

- The ECG is for screening purposes only. A single 12-lead ECGs will be obtained as outlined in the SoA (Section 1.3) during using an ECG machine that automatically calculates the heart rate and measures PR(Q), QRS, QT, and QTcF intervals.
- Subjects should rest for at least 5 minutes in supine position before ECG collection is performed.
- ECG results will be stored electronically, printed, and timely reviewed by the Investigator. The original printout will be stored with the subject's source data.
- The ECG should be interpreted (normal/abnormal) by the Investigator. For abnormal ECG, the clinical significance (yes/no) should be judged by the Investigator and the abnormality is to be specified. ECG recording will be repeated as appropriate.

8.3.4. Clinical Safety Laboratory Tests

- Clinical laboratory assessments will be performed by Nuvisan GmbH.
- All protocol-required laboratory assessments, as defined in Section 10.2, must be conducted in accordance with the laboratory manual and the SoA (Section 1.3).
- Any abnormalities in any of the laboratory parameters will be judged by an Investigator individually in relation to the reference ranges.
- The Investigator must review the laboratory results, document this review, and record any clinically significant changes occurring during the study after first dosing as an AE. The laboratory results must be retained with source documents.
- All laboratory tests with values considered clinically significantly abnormal, with major deviation and/or possible pathological relevance during participation in the study should be repeated until the values return to normal or baseline or the absence of clinical relevance can be confirmed. If clinically significant values do not return to normal/baseline within a period judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

8.3.5. Pregnancy Testing

- WOCBP subjects should only be included after a negative highly sensitive serum pregnancy test at screening and highly sensitive urine pregnancy test on Days -1 of each study period, within 24 hours before the first dose of study intervention. If a urine test cannot be confirmed as negative (e.g., an ambiguous result), a serum pregnancy test is required. In such cases, the subject must be excluded from participation if the serum pregnancy result is positive.
- Additional urine pregnancy testing will be performed on Day 8 of Period 2 (EOT) and during the early discontinuation visit.
- Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected during the time from ICF signature until follow-up visit/early discontinuation visit.

8.3.6. Suicidal Ideation and Behavior Risk Monitoring

Neither the test nor the reference product is considered a CNS-active drug. Therefore, there is no increased risk of suicidal ideation or behavior.

8.3.7. Other Assessments

Alcohol breath tests, and urine drug screens and urine cotinine tests will be performed as outlined in the SoA (Section 1.3).

8.3.8. Local tolerability

Local skin irritation will be assessed by the Principal Investigator or designee at 30 minutes after application of the gel (first morning dose) in each study period using the modified Lanman scoring system (Berger, 1982), i.e., the numerical 'dermal response score', as outlined in Table 7 at the timepoints specified in the SoA (Section 1.3). Each application site will receive a separate dermal response score. Dermal response scores require that at least 25% or more of the area demonstrate an observable response. Any dermal response score ≥2 will be documented as a TEAE.

Table 7: Dermal response score

Dermal Response Score	
Score	**Definition**
0	No evidence of irritation
1	Minimal erythema, barely perceptible
2	Definite erythema, readily visible; minimal oedema or minimal papular response
3	Erythema and papules
4	Definite oedema
5	Erythema, oedema, and papules
6	Vesicular eruption
7	Strong reaction spreading beyond test site

Source: EMA/CHMP/EWP/280/96 Rev1.

8.4. Adverse Events and Serious Adverse Events

- The definitions of AEs and SAEs can be found in Section 10.3.
- An AE will be reported by the subject or observed by members of the study team elicited by general questioning or by the Investigator/designee. The Investigator will review this data and determine the seriousness, the severity, the causality, the action to be taken, and the relationship to an IMP.
- AEs will be documented, and the following information will be given for each AE: description of the AE, onset date and time, end date and time, maximum severity, action taken, outcome, seriousness, and relationship to an IMP.

- The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up unresolved AEs.
- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.3.

8.4.1. Time Period and Frequency for Collecting AE and SAE Information

- All AEs and SAEs will be collected from the signing of the informed consent until EOT / early discontinuation visit at the timepoints specified in the SoA (Section 1.3).
- All SAEs will be recorded and reported to the Sponsor or designee within the time frames indicated in Section 10.3.4.
- Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, or death as outcome of an SAE, at any time after a subject has been discharged from the study, and they consider the event to be reasonably related to an IMP, or study participation, the Investigator must promptly notify the Sponsor.

8.4.2. Method of Detecting AEs and SAEs

During the reporting period unfavorable changes in the subject's condition will be recorded as AEs, regardless, if reported by the subjects or observed by the investigative team. Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences (e.g., "How do you feel?" or "How have you been feeling since the last questioning?").

8.4.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs and SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is given in Section 10.3.

8.4.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study intervention under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the competent authority (CA) and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authorities, Independent Ethics Committees (IEC), and Investigators.

- The Sponsor is responsible for assessing whether an SAE is expected or not (see also Section 10.3). Section 7 of the current IB of AMZ001, and the SmPC of Voltaren Emulgel 1.16% Gel will be used as reference safety information (RSI) for this clinical study.
- Suspected unexpected serious adverse reactions (SUSARs) will be expedited by the Sponsor's designee to IECs, CA, and Investigator following pertinent national legislation.
- Within the EU, all relevant information about any SUSAR will be reported electronically and without delay to the EudraVigilance database. The reporting period for SUSARs is determined as follows:
    - Fatal or life-threatening SUSARs, as soon as possible, and in any event not later than 7 days after the Sponsor became aware of the reaction
    - Non-fatal or non-life-threatening SUSARs, not later than 15 days after the Sponsor became aware of the reaction
    - SUSARs which were initially considered to be non-fatal or non-life-threatening, but which turn out to be fatal or life-threatening, as soon as possible and in any event not later than 7 days after the Sponsor became aware of the reaction being fatal or life-threatening.
- To ensure timely reporting, the Sponsor may submit an initial incomplete report followed up by a complete report.
- If Investigators become aware of an SAE with a suspected causal relationship to the study intervention and/or study procedure occurring after the end of the clinical trial, Investigators shall, without undue delay, report the SAE to the Sponsor.
- Unexpected events which affect the benefit-risk balance of the clinical trial but are not SUSARs must be notified in the EU without undue delay, but no later than 15 days from the date the Sponsor became aware of this event.
- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IEC, if appropriate according to local requirements.
- Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.
- Further information on SAE reporting is provided in Section 10.3.4.

8.4.5. Pregnancy

Females are only allowed to participate in this study, if they are women of non-childbearing potential or if they agree to use highly effective contraceptive measures (in combination with a

barrier method, if applicable), see Section 5.1 and Section 10.4. Therefore, pregnancies can be largely excluded.

In case, a pregnancy occurs until 30 days after the last administration of study intervention, the following applies:

- The Investigator will attempt to collect pregnancy information on any pregnant female subject.
- After obtaining the necessary signed informed consent, the pregnant female subject will be followed to determine the outcome of the pregnancy.
- Details of all pregnancies in female subjects beginning in the period from signing of the ICF / start of study intervention and until 30 days after the last administration of study intervention will be collected.
- Any pregnancy that occurs during trial participation in any female participant must be recorded by the Investigator on the appropriate pregnancy form and reported **within 24 hours** of the first awareness of the event to the safety department of the Sponsor's designee and the Sponsor by e-mail: PPD
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- Whenever possible, a pregnancy should be followed to term, any premature terminations should be reported, and the status of the mother and child should be reported to the Sponsor after delivery. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such.
- Elective abortions without complications should not be handled as AEs.
- The pregnant female subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the pregnant female subject and the neonate, and the information will be forwarded to the Sponsor. This follow-up will be recorded on the pregnancy outcome form and will include an assessment of the possible relationship of the IMP to any pregnancy outcome. The pregnancy outcome form should be reported within **4 weeks** after delivery to the safety department of the Sponsor's designee by e-mail PPD.
- Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date for a healthy newborn. In case of a congenital anomaly or other illness of the newborn, follow-up will continue until the illness has resolved or there is a definite outcome of the event.

- Any poststudy pregnancy-related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor as described in Section 8.4.4. While the Investigator is not obligated to actively seek this information in former pregnant study subjects, he may learn of an SAE through spontaneous reporting.
- Any female subject who becomes pregnant while participating in the study will discontinue study intervention or be withdrawn from the study.

8.4.6. Adverse Events of Special Interest

No AEs of special interest have been defined for this study.

8.5. Pharmacokinetics

- Bioanalytical analyses will be performed at Nuvisan GmbH Bioanalytical Department.
- Detailed information on sample collection, handling, storage, and shipment will be provided in a laboratory manual.
- All sample handling procedures, including the date/time of each sample collection, the date/time of placement into frozen storage (at the end of the sample workup), and the date/time of transfer or shipment of the samples to the responsible analyst will be documented in detail.
- Plasma samples will be collected for measurement of plasma concentrations of diclofenac as specified in the SoA (Section 1.3).
- The actual date and time (24-hour clock time) of each sample will be recorded. In case the actual sampling time deviates from the scheduled sampling time, a comment must be given in the e-source. Samples collected within the time windows defined in the deviation manual (see Section 1.3), will not be considered a protocol deviation. Deviations will be considered when calculating the PK parameters.

  Samples will be used to evaluate the PK of study intervention. Samples collected for analyses of diclofenac plasma concentration may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.
- Genetic analyses will not be performed on these plasma samples.

8.5.1. Bioanalytical Methods

- Bioanalytical analyses will be performed in accordance with the principles of Good Laboratory Practice (GLP), GCP, and ICH M10. The procedure for determination of concentrations of diclofenac in plasma samples will be described in the bioanalytical protocol.
- Information relating to the responsible persons, specification of communication rules, handling of the informed consent and confidentiality of personal data, specification of the analytical method (matrix, calibration range, samples volume, etc.), sample storage, “Incurred Samples”

(ISR), specification of the applicable regulatory guidelines and archiving process will be described in the bioanalytical protocol.

- Samples will be analyzed using a validated LC-MS/MS method.

8.5.2. Pharmacokinetic Evaluation

- The following PK parameters will be calculated for Day 1 and Day 7 from the individual plasma concentration-time data of diclofenac for each intervention by non-compartmental analysis using Phoenix® WinNonlin® Version 7.0 or higher (applying linear trapezoidal linear/log interpolation method for calculation of AUC), see Table 8.
- or definition of PK parametersSection 10.6.

Table 8: Pharmacokinetic Parameters for diclofenac in plasma

	Day 1	**Day 7**
Main PK Parameters		
	-	AUC_{0-24}
Additional PK Parameters		
	AUC_{0-24}	-
	C_{max}	C_{max}
	-	C_{min}
		Swing = $(C_{max} - C_{min}) / C_{min}$
	C_{ave}	C_{ave}
	t_{max}	t_{max}
	-	t_{min}
	-	PTF
	$Racc_{(AUC\ 0-24h)}$ Day 7 versus Day 1	

- Individual PK parameters will be calculated using actual sampling times. A pre-dose sample will always be considered as if it had been taken simultaneously with the intervention administration. If there should have been any deviations in post-dose sampling, the actual sampling times relative to drug administration will be used, unless stated otherwise. Missing data will not be replaced or imputed in any way.

- For calculation of PK parameters, concentrations below the lower limit of quantification (LLOQ) will be treated as zero.
- AUC will be regarded as unreliable if more than two consecutive results are missing or if the concentrations were quantifiable for fewer than 5 timepoints. C_{max} and t_{max} will be regarded as unreliable if the maximum was observed preceding or following a sample with missing data. In case of multiple peaks, C_{max} and t_{max} refer to the highest measured concentration even if there should be earlier peaks. In case of two or more samples with the same concentration (as supplied by the analyst), t_{max} refers to the earlier of these.
- Unreliable parameters will be listed and flagged accordingly and set to missing for calculation of descriptive statistics and statistical analysis. If a PK parameter is unreliable for more than 20% of the subjects, this parameter will additionally be evaluated as sensitivity analysis including the reliable cases.
- More details will be provided in the SAP.

8.6. Pharmacodynamics

PD parameters are not evaluated in this study.

8.7. Genetics

Pharmacogenetics are not evaluated in this study.

8.8. Biomarkers

Biomarkers are not evaluated in this study.

8.9. Immunogenicity Assessments

Not applicable.

8.10. Health Economics or Medical Resource Utilization and Health Economics

Health economics or medical resource utilization and health economics parameters are not evaluated in this study.

9. Statistical Considerations

The statistical analyses will be performed by Nuvisan.

9.1. Statistical Hypotheses

No formal statistical hypotheses have been defined for this systemic bioavailability study.

9.2. Analysis Sets

For purposes of analysis, the following analysis sets are defined:

Analysis Set	Description
Screening Set	All subjects screened.
Randomized Set	All subjects randomly assigned to study intervention.
Safety Set	All subjects randomly assigned to study intervention and who receive at least 1 dose of study intervention. Subjects will be analyzed according to the intervention they actually received. This analysis set will be used for the analysis of safety.
PK Set	This analysis set is a subset of the Safety Set and includes all subjects who complete at least one period without any findings/events likely affecting PK. This analysis set will be used for the analysis of PK.

Finding/events potentially affecting PK will be pre-specified in a deviation manual.

The final decision to exclude complete subjects or single periods of subjects from the PK analysis will be made during a DRM prior to database lock. The respective meeting minutes will be signed together with the Sponsor.

9.3. Statistical Analyses

Statistical analysis will be performed using SAS® and the version used will be specified in the SAP, which will be finalized before database lock. The SAP will contain a more comprehensive explanation than described below of the methodology used in the statistical analyses. The SAP will also contain the rules and data handling conventions to be used to perform the analyses.

Objectives and endpoints are described in Section 3 (Table 4).

9.3.1. Efficacy Analyses

This section is not applicable as efficacy is not assessed in this study.

9.3.2. Safety Analyses

All safety analyses will be performed on the Safety Set.

- Safety data will be listed by subject.
- AEs will be encoded using the MedDRA dictionary, latest version. Summary tables by intervention will be generated for TEAEs (as defined in Section 10.3.1), sorted by system organ class and preferred term, in which the number and percentages of subjects with treatment-emergent AEs and frequency of the events are reported.
- Any AEs that continue but change in severity within a study intervention will be counted as one and only one AE considering the worst severity in frequency tables whereas a recurrent AE (e.g. a headache for a couple of hours each day) will be counted as several AEs.
- AE occurring after administration of any study intervention will be counted towards the last intervention received before the onset, even if the event is not resolved at the beginning of the following intervention. An AE that worsens after a later intervention period will be counted towards both study interventions.
- Laboratory values outside the normal ranges will be flagged. A listing of abnormal laboratory values and clinically relevant abnormal laboratory values will also be provided.
- Descriptive statistics of vital signs and ECG data will be presented for each timepoint by intervention.
- The ECG interpretation of the Investigator will be tabulated as the number and percentage of subjects with “normal”, “abnormal, not clinically significant”, or “abnormal, clinically significant” results by intervention and timepoint.
- The skin irritation scores (i.e., the ‘dermal response score’, see Table 7) will be summarized by application site and treatment. Furthermore, the proportion of subjects with each irritation score will be presented by application site and treatment.

9.3.3. Pharmacokinetic Analyses

- All PK analyses will be performed on the PK Analysis Set, if not stated otherwise.
- Concentration-time courses and PK parameters will be tabulated by intervention.
- The following statistics will be calculated for each of the sampling timepoints: number of cases/ measurements (denoted as “n”), geometric mean and geometric coefficient of variation (CV, in %), arithmetic mean, standard deviation (SD) and CV (in %), minimum, median, maximum.
- Individual and arithmetic mean plasma concentration versus time curves (using the actual sampling times for individual plots and the scheduled sampling times for mean plots) will be plotted by intervention using both linear and log-linear scale.

Further analysis for the primary PK parameter AUC_{0-24}:

- In order to achieve a better approximation to a normal distribution, the pharmacokinetic parameters AUC_{0-24} on Day 1 and Day 7 will be logarithmically transformed. Afterwards, they will be evaluated statistically using ANOVA with INTERVENTION, PERIOD, SEQUENCE and SUBJECT(SEQUENCE) as fixed effects. Least-squares (LS) means, LS intervention difference, and 90% confidence intervals (CI) for the intervention differences on the log-scale will be obtained for AUC_{0-24} on Day 1 and on Day 7. The results will be back-transformed to the original scale by exponentiation to provide geometric least-square means, point estimate of the geometric mean ratio (Test/Reference) and its corresponding 90% CI.
- Only subjects that have a valid AUC_{0-24} in both periods will be included in the above-mentioned model.

Further analysis for the secondary PK parameter C_{max}:

- The same ANOVA model as described for AUC_{0-24} above will be provided for the secondary parameter C_{max}.

9.3.4. Other Analyses

Not applicable.

9.4. Interim Analysis

No formal interim analyses will be performed within Nuvisan Biostatistical Department.

9.5. Sample Size Determination

It is planned to assign 34 subjects to study intervention to have at least 26 evaluable subjects completing the study. If deemed necessary to meet the target number of 26 evaluable subjects, dropouts may be replaced (refer to Section 7.2.2).

Based on the assumption (average CV of 3 historical studies), the intra-subject coefficient of variation (CV) was about 43%. A total of 26 evaluable subjects will achieve 80% power, that the 90% confidence interval (CI) is included in the pre-defined acceptance range of 0.7-1.43, which is deemed to be appropriate. The true ratio that was used in the sample size calculation was 1.05. Enough healthy adults will be screened to randomize approximately 34 subjects to ensure 26 evaluable subjects, assuming an approximate 20% dropout and non-evaluable rate.

9.6. Protocol Deviations

- A protocol deviation is any change, divergence, or departure from the study design, or procedures defined in the protocol. Protocol deviations will be identified prior to database lock.

- Important protocol deviations are a subset of protocol deviations that may significantly affect the completeness, accuracy and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being.
- Protocol deviations will be pre-specified in a deviation manual, discussed on an ongoing basis and finally categorized as important/non-important during the DRM. A protocol deviation may also be declared as finding/event that leads to an exclusion of data or complete subjects from an Analysis Set (see Section 9.2).
- Important protocol deviations will also be described in the CSR.

9.7. Data Management

Nuvisan will be responsible for clinical data management activities for this study. The full details of procedures for data handling will be documented in the Data Management Plan.

Medical coding will be done using the latest version of the coding dictionaries for MedDRA for AEs and medical history to the primary SOC and WHO Drug Global Dictionary for concomitant medications. Coding will be performed by Data Management and needs to be approved by the Investigator and Sponsor.

9.7.1. Database Lock

Study database must be soft and hard locked to ensure their integrity for the generation of results, analysis and submissions.

9.7.2. Soft Lock

When validation (including external data reconciliation), SAE reconciliation and all coding activities, as well as medical review activities, QC activities have been completed and there are no further open issues or open queries the database will be soft locked.

9.7.3. Data Review Meeting

A DRM will be held for the study by the biostatistician to check the data status, discuss any open issues and provide proposals for resolution, as well as finally agree on the categorization of the protocol deviations and assignment of subjects to analysis sets.

9.7.4. Hard Lock

After finalization and approval of the DRM minutes, and after all actions resulting from the DRM minutes are completed, e.g., additional solving of queries, the database will be hard locked. Hard lock needs to be authorized by the study team.

9.7.5. SDTM and ADaM Conversion

The database and the electronic external data will be converted to CDISC / SDTM and ADaM.

10. Supporting Documentation and Operational Considerations

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

Before the start of the study, Nuvisan on behalf of the Sponsor will apply for approval for the performance of the study at the CA (German Federal Institute for Drugs and Medical Devices [BfArM]) and the IEC.

10.1.1. Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
    - Applicable ICH GCP Guidelines
    - Applicable laws and regulations
- The protocol, modifications to the protocol (modifications), ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IEC and reviewed and approved by the IEC before the study is initiated.
- Any substantial modification to the protocol (substantial modification) will require CA and IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.
- The Investigator will be responsible for the following:
    - Notifying the IEC of SAEs or other significant safety findings as required by IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to ICH guidelines, the IEC, European Regulation 536/2014, and all other applicable local regulations
    - Performing a benefit-risk assessment on an ongoing basis and informing the IEC about any changes. Moreover, making sure that any substantial modifications to the protocol (substantial modifications) will be submitted and approved prior to implementation

10.1.2. Financial Disclosure

- Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities.
- Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

10.1.3. Clinical Study Insurance and Subject Compensation

- In accordance with local law, insurance coverage will be provided for all subjects participating in this study.
- Subjects will be paid compensation for participation and will be reimbursed for travel-related costs.

10.1.4. Informed Consent Process

- The Investigators or their representative will explain the nature of the study to the subject and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects will be required to sign a statement of informed consent that meets the requirements of local regulations, ICH guidelines, and the IEC.
- The written ICF must be signed and personally dated by the subject and by the Investigator who conducted the informed consent discussion.
- Subjects should be informed of the possibility to withdraw consent without giving any reason and to require that all previously retained identifiable samples will be destroyed to prevent future analyses, according to national provisions. The information should include a statement that the consequence of the subject's withdrawal of consent will be that no new information will be collected from the subject and added to existing data or database.
- All subjects who sign the ICF will be assigned a screening number.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study. Any revised ICF must receive the IEC approval / favorable opinion in advance of use.
- One original of the signed and dated ICF will be provided to the subject. A second original will be retained in the Investigator site file.
- The Investigator should maintain a log of all subjects who signed the ICF.
- Subjects who are rescreened are required to sign a new ICF.

10.1.5. Recruitment

The study will be performed in adult subjects fully capable of giving informed consent.

Detailed description of the recruitment strategy will be provided in country- and site-specific documentation, as applicable.

10.1.6. Data Protection

Personal and sensitive personal data will be treated as confidential. Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing subject data.

Authorized access only is assured by strict rules on Nuvisan firewall. Nuvisan uses strict rules to separate the networks within the company. User groups with various permission sets are maintained within Nuvisan network to ensure confidentiality of records. Connections to the Nuvisan network from the off-site access point have to use a virtual private network. The Nuvisan network is constantly being monitored for potential threats, viruses, and other security related issues by a separate security operation center. To prevent a security breach, all Nuvisan employees are trained on how to proceed in case of receiving suspicious email.

- Subjects will be assigned a unique identifier. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred. Only the Investigator and the clinical team will be able to link the subjects' trial data to the subjects via an identification list kept at the site. The subjects' original medical data that are reviewed at the site during source data verification by the monitor, audits and during health authority inspections will be kept strictly confidential.
- The subject must be informed that their personal study-related data will be used by the Sponsor in accordance with the EU General Data Protection Regulation. The level of disclosure must also be explained to the subject.
- The subject must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor and by inspectors from regulatory authorities without violating the confidentiality of the subject to the extent permitted by law and regulations.
- All personal data collected and processed for the purpose of this study should be managed by the Investigator and their staff with adequate precautions to ensure confidentiality of those data, as per national and/or local laws and regulations on personal data.
- Measures are in place to mitigate the possible adverse effects of a data security breach and are in line with the EU General Data Protection Regulation and relevant national legislations. These measures are defined in the CRO's SOPs regarding IT Security and Serious Breaches.

10.1.7. Committees Structure

Not applicable.

10.1.8. Dissemination of Clinical Study Data

This clinical study will be registered in a clinical study database (e.g., ClinicalTrials.gov) before enrolment of the first subject. All data and results and all intellectual property rights in the data

and results derived from the study will be the property of the Sponsor who may utilize them in various ways, such as for submission to government regulatory authorities or disclosure to other Investigators.

10.1.9. Data Quality Assurance

- All subject data relating to the study will be recorded and transmitted to the Sponsor or designee electronically. The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The Investigator must permit study-related monitoring, audits, IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- Nuvisan GmbH is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 25 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

10.1.10. Source Documents

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected.
- CRF data must be consistent with the source documents, or the discrepancies must be explained.
- Definition of what constitutes source data can be found in the source data location form.

10.1.11. Study and Site Closure

- The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor.
- Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.
- The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.
- Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:
    - Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines;
    - Inadequate recruitment of subjects by the Investigator;
    - Discontinuation of further study intervention development.

10.1.12. Publication Policy

- A summary of the results of the clinical trial according to Annex IV of the EU Clinical Trials Regulation (Regulation (EU) No 536/2014) will be uploaded to CTIS within 1 year and published within 30 months after the end of the clinical trial.
- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.13. Clinical Study Report

- After completion of the study, a clinical study report covering clinical and statistical aspects of the study will be prepared by Nuvisan GmbH in line with ICH E3 in consultation with the Sponsor.

10.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 9 will be performed by the local laboratory at the timepoints specified in the SoA (Section 1.3).
- Details of all methodology and reference ranges will be provided in the TMF.
- Investigators must document their review of each laboratory safety report.
- The results of each test will be transferred electronically to the clinical database.
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.
- Sampling for scheduled laboratory examinations will be done with subjects in a fasted state, i.e. an overnight fast (= nothing to eat or drink except water) of ≥10 h has to be adhered to.

Table 9: Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters		
Hematology	Platelet count		White Blood Cell Count with Differential (absolute and %): Neutrophils Lymphocytes Monocytes Eosinophils Basophils
	Reticulocytes		
	Hemoglobin		
	Hematocrit		
	Red blood cell count		
Clinical Chemistry	Alanine aminotransferase[a]	Calcium	Glucose
	Alkaline phosphatase	Cholesterol	Magnesium
	Aspartate aminotransferase	Creatinine	Potassium
	Bilirubin (total)[a,b]	Creatine phosphokinase[c]	Sodium
	Blood urea nitrogen	γ-Glutamyl-transferase	Triglycerides
Urinalysis	• Specific gravity • pH, hemoglobin, urobilinogen, bilirubin, protein, glucose, ketones, nitrite, leukocyte esterase by dipstick • In case of positive results for protein, leukocyte esterase, hemoglobin or nitrite on the dip stick, flow cytometry count and classification will be performed.		
Other Tests	• Urine drug screen: Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methamphetamines, methylenedioxymethamphetamine, methadone, opiates, tricyclic antidepressants, phencyclidine • Urine cotinine test • Serum (at screening only) and highly sensitive urine human chorionic gonadotropin (hCG) pregnancy test (female subjects only) • At screening only: o Thyroid stimulating hormone (TSH)[d] o Follicle Stimulating Hormone (FSH) (to confirm postmenopausal status, female subjects only)		

Laboratory Assessments	Parameters
	• Serology: Hepatitis B surface antigen [HBsAg], Hepatitis C antibody [anti-HCV], anti-human immunodeficiency virus antibodies (anti-HIV 1 + 2) and HIV p24 antigen combined

Notes:

[a] Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7 and Section 10.5. All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE.

[b] In case of increased bilirubin (total), the direct bilirubin will be determined.

[c] If increased, creatine kinase (muscle-brain type) and Troponin I (if CK-MB/CK-total x 100 >6.0% or CK-MB >25 U/L) will be determined.

[d] If TSH is out of range, additionally free triiodothyronine and free thyroxine will be determined.

10.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Investigators are responsible for monitoring the safety of subjects who have entered this study. Each subject will be carefully monitored by the Investigator or a delegate for adverse events (AEs). All adverse events will be reported and documented as stated below.

The Investigator is responsible for appropriate medical care of subjects during the study.

The Investigator remains responsible for following through an appropriate healthcare option with study subjects who experienced AEs until resolution or until the AE is recognized as stabilized.

10.3.1. Definitions

Adverse Event
An AE is any untoward medical occurrence in a clinical study subject, temporally associated with the use of study intervention, whether or not considered related to the study intervention. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.
Treatment-Emergent Adverse Event
All AEs occurring prior to the initiation of study intervention will be referred to as pre-treatment-AEs, which include any unintended sign, symptom, or disease that occurs between the screening and the first administration of study intervention. All AEs that emerge during treatment and having been absent pre-treatment or worsening relative to the pre-treatment state are referred to as treatment emergent AEs (TEAEs).
Adverse Drug Reaction
In the pre-approval phase of a new medicinal product or its new usages, particularly when the therapeutic dose(s) may still be established, all noxious and unintended responses to a medicinal product related to any dose should be considered an adverse drug reaction (ADR). The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. An adverse reaction, the nature or severity of which is not consistent with the applicable product Reference Safety information is called an unexpected ADR.

Serious Adverse Reaction
• A serious adverse reaction (SAR) is a SAE that the Investigator or Sponsor assesses to have a reasonable possibility of a causal relationship to the IMP.
Suspected Unexpected Serious Adverse Reaction
• A suspected unexpected serious adverse reaction (SUSAR) is a SAR, the nature, severity or outcome of which is not consistent with the reference safety information (RSI) (IB for an unapproved investigational medicinal product). All SUSARs are subject to expedited regulatory reporting, including electronic reporting without delay to the EudraVigilance database.
Events <u>Meeting</u> the AE Definition
• Any abnormal laboratory test results (e.g., hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator. • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or severity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose *per se* will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
Events <u>NOT</u> Meeting the AE Definition
• Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2. Definition of SAE

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event, after medical and scientific judgment in the view of either the Investigator or Sponsor. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Procedures done in or visits to a clinic or outpatient facility are not considered SAEs. Admission to a rehabilitation facility, transitional care unit, or nursing home is not considered a hospitalization. A hospitalization for an elective treatment of a pre-existing condition that did not worsen from baseline, or a routinely scheduled treatment is not considered an SAE because a "procedure" or "treatment" is not an untoward medical occurrence. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
d. Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect Intrauterine development of an organ or structure that is abnormal in form, structure, or position.
f. Other situations Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may

require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the CRF, as outlined in Section 8.4. For SAEs, the SAE form must also be completed.
- In case an existing AE changes in severity all AEs should be recorded separately in the CRF.
- It is **not** acceptable for the Investigator to send photocopies of the subject's medical records to the Sponsor in lieu of completion of the AE/SAE CRF.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all subject identifiers, with the exception of the screening number, will be redacted on the copies of the medical records before submission to the Sponsor.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Severity

The Investigator will assess the severity for each AE and SAE reported during the study and assign it to 1 of the following categories (severity is a clinical observation and describes the intensity of the event):

Mild:

A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate:

A type of adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe:

A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the severity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE according to the following:
  - **Not related**: Not reasonably related to the study intervention(s). AE could not medically (pharmacologically/clinically) be attributed to the study intervention(s). A reasonable alternative explanation will be available.
  - **Related**: Reasonably related to the study intervention(s). AE could medically (pharmacologically/clinically) be attributed to the study intervention(s).
- In addition, the Investigator will assess the relationship between protocol required procedure(s) and each occurrence of each AE/SAE according to the following:
  - **Not related**: Not reasonably related to protocol required procedure(s).
  - **Related**: Reasonably related to protocol required procedure(s).
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- For causality assessment, the Investigator will also consult the IB (IB) and/or Product Information, for marketed products.
- The Investigator **must** review and provide an assessment of causality for each AE/SAE and document this in the medical notes.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor. However, **it is very important that the Investigator always makes an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor.**
- The Investigator may change their opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.

- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Outcome

The outcome of the AE should be classified according to the following definitions:

Recovered / resolved: the event has resolved (no further symptoms are present, and no treatment is being received by the subject).

Recovering / resolving: the condition is improving, and the subject is expected to recover from the event.

Not recovered/ Not resolved: the event is not yet resolved and is ongoing.

Recovered / resolved with sequelae: the event has resolved but there may be lingering effects present (e.g., a scar following a cut or abrasion).

Fatal: the subject died as a result of the event. This code should only be used for the event that caused the death, not any event that was present at the time of the subject's death. Fatal events require immediately reporting to the Sponsor (or an authorized representative).

If outcome is not known or not reported:

Unknown: may only be used in the event that the subject is lost to follow-up and no reliable data can be obtained.

All efforts should be made to classify the AE according to the above categories.

Follow-up of AEs and SAEs

- All (S)AEs must be followed by the Investigator until resolved, stabilized, or judged no longer clinically significant. Thus, follow-up visits may be required even after the administration of the study intervention has been discontinued.
- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally completed CRF.
- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

Action Taken with Study Intervention

The action taken with the study intervention will be assigned to one of the following categories:

- **Dose not changed**: No action taken regarding the dosing scheme of the study intervention
- **Drug withdrawn**: Discontinuation of the study intervention.
- **Not applicable**: This category should be used in circumstances such as when the subject has died, or the treatment had been completed before reaction(s) or event(s) or the study intervention had not been administered.
- **Unknown**: The information is unknown or implausible and it cannot be supplemented or verified.

Medication Error, Overdose, Abuse, and Misuse

Medication errors, overdose, abuse, and misuse must be documented in the CRF and are defined as follows:

Medication error:

Any unintentional error in the prescribing, dispensing or administration of study intervention while in the control of the healthcare professional, patient, or consumer. Examples for medication errors include administration of expired study intervention, administration of study intervention that has undergone temperature excursion from the specified storage range, use of study intervention outside of what is foreseen in the protocol, administration of study intervention to subjects not involved in the trial or foreseen to receive another study intervention.

Overdose:

Administration of a quantity of the study intervention given per administration or cumulatively which is above the maximum dose (see Section 6.8).

Abuse:

Persistent or sporadic, intentional excessive use of the study intervention which is accompanied by desired physical or psychological effects.

Misuse:

Situation where the study intervention is intentionally and inappropriately used not in accordance with the protocol.

Stop and start date as well as the action with the study intervention due to medication errors, overdose, abuse, or misuse must be documented as follows:

- **Dose not changed**
- **Drug interrupted**
- **Drug withdrawn**
- **Unknown**
- **Not applicable**

If a medication error, overdose, abuse, and misuse is accompanied by an AE, as determined by the Investigator, the AE should be recorded on the CRF.

10.3.4. Reporting of SAEs

SAE Reporting via SAE Report Form

- SAEs will be reported from signing of the ICF up to the EOT / ED visit. Additionally, any SAEs that occur after this time frame and are considered related to a medicinal (investigational) product by the Investigator must be reported.
- The Investigator must record all SAEs on the SAE report form and submit it to the Sponsor without undue delay but not later than 24 hours after obtaining knowledge of the event. SAEs considered related to the medicinal (investigational) product occurring after the end of the clinical study must be reported to the Sponsor without undue delay.
- The initial report should contain as much information as possible, but at least the following information:
  - Subject number
  - IMP information (date of administration)
  - Event term (only one term should be entered)
  - Date of onset (and time of onset if on the day of IMP administration)
  - Name of the Investigator
  - Causality assessment (relationship to study intervention, or trial procedure)
  - Severity
- SAE reporting contact:
  e-mail: PPD
  e-fax: PPD
- Facsimile transmission of the SAE report form is the preferred method to transmit this information to the Sponsor.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE form within the designated reporting time frames.
- Contacts and details for SAE reporting can be found in the Safety Management Plan.

10.4. Appendix 4: Contraceptive Guidance

10.4.1. Definitions

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are considered WOCBP (fertile):

1. Following menarche
2. From the time of menarche until becoming postmenopausal unless permanently sterile (see below)

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT).
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.
- Permanent sterilization methods (for the purpose of this study) include:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., Mullerian agenesis, androgen insensitivity, gonadal dysgenesis), Investigator discretion should be applied to determining study entry.

Note: Documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.

If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Woman of Nonchildbearing Potential (WONCBP)

Women in the following categories are considered WONCBP:

1. Premenopausal female with permanent infertility due to one of the following (for the purpose of this study):

   a. Documented hysterectomy

   b. Documented bilateral salpingectomy

   c. Documented bilateral oophorectomy

   For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., Mullerian agenesis, androgen insensitivity, gonadal dysgenesis), Investigator discretion should be applied to determining study entry.

Note: Documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.

2. Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH (>40 IU/L or mIU/mL) is required.
    - Females on HRT and whose menopausal status is in doubt must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

10.4.2. Contraception Guidance

• **CONTRACEPTIVES[a] ALLOWED DURING THE STUDY INCLUDE:**
Highly Effective Methods[b] That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
Implantable progestogen-only hormone contraception associated with inhibition of ovulation[c]
Intrauterine device (IUD)
Intrauterine hormone-releasing system (IUS)[c]
Bilateral tubal occlusion
Azoospermic partner (vasectomized or due to a to medical cause) *Azoospermia is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.* Note: documentation of azoospermia for a male subject can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.
Highly Effective Methods[b] That Are User Dependent *Failure rate of <1% per year when used consistently and correctly.*
Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[c] – oral – intravaginal – transdermal – injectable
Progestogen-only hormone contraception associated with inhibition of ovulation[c] – oral – injectable
Sexual abstinence *(Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.)*
a) Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for those participating in clinical studies. b) Failure rate of <1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly. c) Male condoms must be used in addition to hormonal contraception. If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action. **Note**: Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM) are not acceptable methods of contraception. Male condom and female condom should not be used together (due to risk of failure from friction).

10.5. Appendix 5: Liver Safety: Suggested Actions and Follow-up Assessments

Phase 1 liver chemistry stopping criteria are designed to assure subject safety and to evaluate liver event etiology.

Phase 1 Liver Chemistry Stopping Criteria and Follow-Up Assessments

<table>
<tr><th colspan="3">Liver Chemistry Stopping Criteria - Liver Stopping Event</th></tr>
<tr><td>ALT/AST-absolute</td><td colspan="2">ALT or AST ≥3 x ULN
ALT or AST ≥3 x ULN AND total bilirubin ≥ 2 x ULN (for subjects with known Gilbert’s syndrome these criteria only apply if total bilirubin ≥2xULN, and direct bilirubin >2xULN and at least doubled from baseline value) OR international normalized ratio (INR) >1.5, report to the Sponsor in expedited manner and as an SAE if SAE criteria are met [a,b].</td></tr>
<tr><th colspan="3">Suggested Actions, Monitoring, and Follow up Assessments</th></tr>
<tr><th colspan="2">Actions</th><th>Follow Up Assessments</th></tr>
<tr><td colspan="2">• Immediately discontinue study intervention.
• Report the event to the Sponsor within 24 hours.
• Complete the liver event eSource and complete an SAE data collection tool if the event also met the criteria for an SAE[b]
• Perform liver follow-up assessments as described in the Follow Up Assessment column.
• Do not restart/rechallenge subject with study intervention
• Monitor the subject until liver chemistry test abnormalities resolve, stabilize, or return to baseline (see MONITORING).

MONITORING:
If ALT or AST ≥3 x ULN AND total bilirubin ≥2 x ULN or INR >1.5:
• Repeat liver chemistry tests (include ALT, AST, alkaline phosphatase, total bilirubin, and INR) and perform liver event follow-up assessments within 24 hours.</td><td>• Viral hepatitis serology[c]
• Obtain blood sample for PK analysis after the most recent dose[d].
• Obtain CPK, LDH, GGT, glutamate dehydrogenase (GLDH) and serum albumin.
• Fractionate bilirubin if total bilirubin ≥2 x ULN.
• Obtain complete blood count with differential to assess eosinophilia.
• Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE CRF.
• Record use of concomitant medications (including acetaminophen, recreational drugs, herbal remedies, and other over-the-counter medications) on the concomitant medications CRF.
• Record alcohol use on the liver event alcohol intake CRF.</td></tr>
</table>

• Monitor subject twice weekly until liver chemistry test abnormalities resolve, stabilize, or return to baseline. • A specialist or hepatology consultation is recommended. **If ALT or AST ≥3 x ULN AND total bilirubin <2 x ULN and INR ≤ 1.5:** • Perform liver chemistry tests (include ALT, AST, alkaline phosphatase, total bilirubin, and INR) and liver chemistry follow-up assessments within **24 to 72 hours.** • Monitor subjects weekly until liver chemistry abnormalities resolve, stabilize, or return to baseline.	**If ALT or AST ≥3 x ULN AND total bilirubin ≥ 2 x ULN or INR >1.5** obtain the following in addition to the assessments listed above**:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Liver imaging (ultrasound, magnetic resonance, or computed tomography) to evaluate liver disease; complete liver imaging CRF. • Liver biopsy may be considered and discussed with local specialist if available, for instance: o In subjects when serology raises the possibility of autoimmune hepatitis o In subjects when suspected DILI progresses or fails to resolve on withdrawal of study intervention o In subjects with acute or chronic atypical presentation • If liver biopsy is conducted, then complete liver biopsy CRF.

Notes:

[a] Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study intervention if ALT or AST ≥ 3 x ULN **and** total bilirubin ≥ 2 x ULN. Additionally, if serum bilirubin fractionation testing is unavailable, **record the absence/presence of detectable urinary bilirubin on dipstick** which is indicative of direct bilirubin elevations suggesting liver injury.

[b] All events of ALT or AST ≥ 3 x ULN **and** total bilirubin ≥ 2 x ULN (for subjects with known Gilbert's syndrome these criteria only apply if total bilirubin ≥2xULN, and direct bilirubin >2xULN and at least doubled from baseline value) or ALT or AST ≥ 3 x ULN **and** INR >1.5 may indicate severe liver injury **(possible 'Hy's Law') and must be reported to Sponsor in an expedited manner and as an SAE if SAE criteria met (excluding studies of hepatic impairment or cirrhosis)**. The INR stated threshold value will not apply to subjects receiving anticoagulants.

[c] Includes: Hepatitis A immunoglobulin M (IgM) antibody; HBsAg and HBcAb; hepatitis C RNA; cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, heterophile antibody or monospot testing); and hepatitis E IgM antibody.

[d] PK sample may not be required for subjects known to be receiving placebo or non-comparator interventions. Record the date/time of the PK blood sample draw and the date/time of the last dose of study intervention prior to the PK blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the Laboratory Manual.

10.6. Appendix 6: Pharmacokinetic Parameters

Symbol	Definition
AUC_{0-24}	The partial area under the concentration-time curve (AUC) from time zero (= dosing time) to 24 hours. In cases where the actual observation time is not equal to the scheduled observation time AUC_{0-24} will be calculated according to the following rules: • using the actual study time at scheduled 24 hours if the sample was taken earlier and within the allowed time window defined separately in a deviation manual • by interpolation if the sample was taken later than the scheduled 24 hours • in all other cases, AUC_{0-24} will be set to missing
C_{ave}	The average concentration on Day 7. $C_{ave} = AUC_{0–24}/24$.
C_{max}	Maximum observed concentration.
C_{min}	The minimum observed concentration on Day 7.
PTF	The peak trough fluctuation on Day 7. $PTF = 100*(C_{max}-C_{min})/C_{ave.}$
$R_{acc(AUC\ 0-24)}$ Day 7 versus Day 1	The accumulation ratio after repeated administration calculated as $R_{acc(AUC\ 0–24)}$ = (AUC_{0-24} after multiple dose on Day 7) / (AUC_{0-24} on Day 1).
Swing	(C_{max} - C_{min}) / C_{min} on Day 7
t_{max}	Time of observed maximal concentration (time to reach C_{max}).
t_{min}	The time to reach the minimum observed concentration.

10.7. Appendix 7: Sponsor Signature Page

The people signing hereby declare that they have read this protocol and agree to its contents.

_____ _____

Signature Date of Signature

Name, academic degree:	PPD
Function/Title:	PPD
Institution:	AMZELL B.V
Address:	Siriusdreef 31, 2132 WT Hoofddorp, The Netherlands
Telephone number:	PPD
Fax number:	PPD
E-mail address:	PPD

10.8. Appendix 8: Principal Investigator Signature Page

The people signing hereby declare that they have read this protocol and agree to its contents. They confirm that the study will be conducted and documented in full accordance with the protocol (and modifications), International Conference for Harmonization (ICH) guidelines for current Good Clinical Practice (GCP) specified herein, the national drug law, and applicable regulatory requirements. They will also ensure that sub-Investigator(s) and other relevant members of their staff have access to copies of this clinical study protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

_____ _____

Signature Date of Signature

Name, academic degree:	Steffen Haffner, Dr. med.
Function/Title:	Principal Investigator
Institution:	Nuvisan GmbH
Address:	Wegenerstraße 13, 89231 Neu-Ulm, Germany
Telephone number:	PPD
Fax number:	Not applicable
E-mail address:	PPD

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