CLINICAL STUDY PROTOCOL

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 8 mL [7.4 g]) of AMZ001 (Diclofenac Sodium Gel 3.06%) and One Dose Level (32 g) of Voltaren Arthritis Pain Gel (Diclofenac Sodium Gel 1%) in Healthy Subjects

Protocol No. AMZ001-008

IND Number:	116375
Study Product:	AMZ001
Indication:	Relief of the pain associated with arthritis
Phase:	1
Name and Address of Sponsor:	Amzell B.V. Siriusdreef 31 2132 WT Hoofddorp The Netherlands Telephone: +31.235.560.460 Fax: +31.235.560.461
Name and Address of Principal Investigator & Contract Research Organization:	Jonathan S. Dosik, M.D. TKL Research, Inc. One Promenade Blvd, Suite 1101/1201 Fair Lawn, NJ 07410 Telephone number: (201) 587-0500
Good Clinical Practice (GCP) Statement:	This study will be performed in compliance with ICH GCP guidelines.

SYNOPSIS

TITLE OF STUDY: 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 8 mL [7.4 g]) of AMZ001 (Diclofenac Sodium Gel 3.06%) and One Dose Level (32 g) of Voltaren Arthritis Pain Gel (Diclofenac Sodium Gel 1%) in Healthy Subjects

STUDY SITE(S): 1 site in the U.S.

PLANNED STUDY PERIOD: Approximately 18 weeks (study participation for a subject)	**CLINICAL PHASE:** 1

OBJECTIVES:

The primary objective of the study is to compare the bioavailability of two dose levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001 (diclofenac sodium gel 3.06%) applied once daily as 3 mL and 4 mL per knee on both knees, respectively, and one dose level of Voltaren Arthritis Pain Gel (32 g, diclofenac sodium gel 1%) applied four times daily as 4 g per knee on both knees after repeated topical administrations in healthy subjects for 7 days.

The secondary objectives of the study are to:

- Determine the pharmacokinetic (PK) profile of the two dose levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001 (diclofenac sodium gel 3.06%) and one dose level (32 g) of Voltaren Arthritis Pain Gel (diclofenac sodium gel 1%).
- Assess the safety and tolerability of the two different doses of AMZ001 (diclofenac sodium gel 3.06%) and Voltaren Arthritis Pain Gel (diclofenac sodium gel 1%).

DESIGN:

This is a randomized, open-label, three period crossover, multiple-dose study to investigate the bioavailability, safety, and tolerability of two dose levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001 applied once daily as 3 mL and 4 mL per knee on both knees, respectively, and one dose level of Voltaren Arthritis Pain Gel (32 g) applied four times daily as 4 g per knee on both knees after repeated dosing in healthy subjects for 7 days.

Before inclusion into the study, all subjects will undergo a physical examination, including vital signs, and blood samples will be collected for laboratory assessments (hematology, clinical chemistry, and urinalysis).

Subjects will be randomized to one of six dosing sequences:

Period 1

(Sequence 1) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD
(Sequence 2) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD
(Sequence 3) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily
(Sequence 4) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD

(Sequence 5) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD
(Sequence 6) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily

Period 2

(Sequence 1) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD
(Sequence 2) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily
(Sequence 3) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD
(Sequence 4) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD
(Sequence 5) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily
(Sequence 6) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD

Period 3

(Sequence 1) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily
(Sequence 2) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD
(Sequence 3) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD
(Sequence 4) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily
(Sequence 5) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD
(Sequence 6) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD

Note: Between Periods 1 and 2 and Periods 2 and 3, there will be a 21-day (+7-day) washout, and the subjects will not be confined to the study site.

The gel will be administered to the knee by subject under the supervision of 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 then spread the gel directly to the pre-defined treatment area of approximately 400 cm^2 (6 mL AMZ001 and 32 g Voltaren Arthritis Pain Gel) and 500 cm^2 (8 mL AMZ001) for each subject knee as per detailed application instructions. Subjects will be confined at the study site only for the duration of each treatment period (Days -1 to Day 8) of the study. Subjects are permitted to shower ≥ 1-hour pre-dose and after 3-hours or greater post-dose. Blood samples for PK analyses will be collected prior to (within 1 hour) application and at 2 (±15 min), 4 (±15 min), 6 (±15 min), 8 (±15 min), 10 (±15 min), 12 (±15 min), 14 (±15 min), 16 (±15 min), 18 (±15 min), 20 (±15 min), 24 (±30 min) hours after application on Days 1 and 7. One blood sample will be collected on Days 3, 4, 5, and 6 prior (within 1 hour) to the first daily application of gel to measure the trough level of each study drug.

At the end of Period 3, there will be three additional PK blood samples taken respectively at 3 weeks (21 days), 4 weeks (28 days), and 5 weeks (35 days) after the last application to compare elimination phase of AMZ001 vs. Voltaren Arthritis Pain Gel and to further describe the

elimination phase of the AMZ001 product. During this follow-up period, subjects will not be confined to the study site.

If a sampling time point and application time fall at the same time, the blood sample should be taken **immediately prior** to application.

NUMBER OF SUBJECTS (PLANNED): A sufficient number of subjects will be screened and approximately 75 subjects will be randomized to give 60 evaluable subjects across six dosing sequences.

CRITERIA FOR INCLUSION/EXCLUSION:

Inclusion criteria:

1. Healthy male or female subjects 18-65 years of age (e.g., in general good physical health, as judged by the Investigator and no clinically relevant abnormalities identified by a detailed medical history, full physical examination, including blood pressure and pulse rate measurement, 12-lead ECG or clinical laboratory tests)
2. Has a body mass index (BMI) between 18.0 and 35.0 kg/m^2 at Screening.
3. In the case of females of child-bearing potential ([FCBP) unless surgically sterilized [hysterectomy, bilateral oophorectomy, bilateral tubal ligation] or are postmenopausal for at least 12 months), are using two acceptable forms of birth control (hormonal contraceptives i.e., oral/implant/injectable/transdermal; intrauterine device (IUD) and/or barrier methods [female condom, male condom, diaphragm, cervical cap, spermicide]; note: 2 barrier methods are two acceptable forms of birth control)). Abstinence or partner's vasectomies are acceptable if the female subject agrees to implement two acceptable forms of birth control if her lifestyle/partner changes.
4. Females of child-bearing potential have a negative serum pregnancy test (SPT) at Screening and negative urine pregnancy test (UPT) on Day -1 of each period and at end of treatment (EOT) visit (Period 3, Day 8)
5. Are free of any systemic or dermatologic disorder and chronic or acute infections, which, in the opinion of the Principal Investigator (PI), will interfere with the study results or increase the risk of adverse events (AEs)
6. Read, understand, and provide signed informed consent before any assessment is performed.

Exclusion criteria:

The presence of any of the following excludes a subject from study enrollment:

1. Subject has any visible skin disease, skin lesions, wounds, or a significant amount of hair at the application site (knee)
2. Use of an investigational medicinal product (IMP) within 30 days or 5 half-lives (if known), whichever is longer, of enrollment or during the study.

3. Treated with systemic or local diclofenac within 30 days of enrollment or during the study (except for study IMP)
4. Known hypersensitivity to diclofenac, any excipients (ethanol, propylene glycol, diethylene glycol monoethyl ether, myristyl alcohol, hydroxypropyl cellulose, and water), aspirin, Xarelto, coumadin, or other non-steroidal anti-inflammatory drugs (NSAIDs), including Cyclooxygenase-2 (COX-2) inhibitors.
5. Any history of drug hypersensitivity, asthma, urticaria, or other significant allergic diathesis. Subjects with uncomplicated seasonal allergic rhinitis can be accepted only if the expected allergy season is clearly outside enrollment / treatment periods.
6. Females who are pregnant and/or lactating
7. Of child-bearing potential but not willing to use adequate contraception for the duration of the study
8. Subject is a current smoker and unable to abstain from smoking during the treatment periods.
9. Use of any topical medication, cosmetics, cream, ointments, lotions on the treatment site 1 week prior to enrollment through EOT visit.
10. Use of any medication (including over-the-counter medication, dietary supplements, and herbal remedies) within 2 weeks before first scheduled study drug administration or within less than 5 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. Consumption of any drug metabolizing enzyme (e.g., cytochrome P450 3A4 (CYP3A4) or other cytochrome P450 enzymes) inducing or inhibiting beverages or food (e.g., broccoli, Brussel sprouts, grapefruit, grapefruit juice, star fruit) within 3 days prior to and during each treatment period.

    Allowed treatments are:

    - Systemic contraceptives and hormone replacement therapy, as long as female subject is on stable treatment for at least 3 months and continues treatment throughout the study.
    - Occasional use of acetaminophen 500 mg (up to 1000 mg daily)
    - Single intake of other drugs only if judged by the investigator to have no clinical relevance and will not confound the interpretation of the study results.
11. Subject has a known or suspected malignancy, excluding basal cell cancer unless it is associated with the treatment area.
12. Subject has a positive blood screen for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), hepatitis C antibody (Anti-HCV)
13. Subject has any acute or chronic condition or is using medications, which, in the investigator's opinion, would make it unsafe for the subject to participate in this study,

including clinically significant abnormal laboratory values, vital signs, physical examination findings prior to randomization or during study participation.

14. History or current evidence of renal disease or impaired renal function at screening as indicated by abnormal levels of serum creatinine (> 1.43 mg/dL) or BUN (≥ 35 mg/dL) or the presence of clinical. significant abnormal urinary constituents (e.g., albuminuria)
15. History or current evidence of ongoing hepatic disease or impaired hepatic function at screening. A subject will be excluded if more than one of the following lab value deviations are found: 1) AST (≥ 1.5 ULN), ALT (≥ 1.5 ULN), 2) GGT (≥ 1.5 ULN), ALP (≥ 1.5 ULN), 3) total bilirubin (> 2.00 mg/dL) or creatine kinase (≥ 3 ULN). A single deviation from the above values is acceptable and will not exclude the candidate, unless specifically advised by the Investigator.
16. Subject has clinically relevant chronic or acute infectious illnesses or febrile infections within 2 weeks prior to the first scheduled study drug administration
17. Subject has gastrointestinal bleeding issues, e.g., Gastroesophageal Reflux Disease (GERD), Peptic Ulcer Disease (PUD)
18. Subject has a hospital admission or major surgery within 30 days prior to randomization.
19. Subject has a 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.
20. Subject has a history of alcohol abuse, prescription drug abuse, or illicit drug use within 6 months prior to Screening.
21. Subject meets eligibility criteria, but study is filled
22. Subject who is an investigational site staff member directly involved in the conduct of the study and his/her family members, site staff member otherwise supervised by the Investigator, or subject who is a Amzell B.V. employee directly involved in the conduct of the study

INVESTIGATIONAL PRODUCT: AMZ001 is provided in dispensing package. Each dispenser contains diclofenac sodium 3.06% in a gel base. AMZ001 is delivered in aliquots of either 1 mL (0.92 g containing 28.2 mg of diclofenac sodium) or 1.5 mL (1.38 g containing 42.2 mg of diclofenac sodium) per pump actuation. Voltaren Arthritis Pain Gel is provided in tubes containing 100 g of the topical gel in each tube. Each tube contains diclofenac sodium in a gel base (10 mg of diclofenac sodium per gram of gel or 1%).

DURATION OF STUDY: The study duration is approximately 18 weeks for each subject, inclusive of screening period.

ENDPOINTS:

Primary endpoint:

- Compare the exposure to diclofenac, as measured by plasma concentration, area under the curve from time zero to 24 hours (AUC_{0-24}), and maximum (peak) plasma drug concentration (C_{max}) at steady state between two different dose levels of AMZ001 and one dose level of Voltaren Arthritis Pain Gel in healthy subjects on Day 7.

Secondary endpoint(s):

- The PK parameters C_{max}, minimum plasma drug concentration (C_{min}), time to reach maximum (peak) plasma concentration following drug administration (T_{max}), time to reach minimum plasma concentration following drug administration (T_{min}), terminal disposition rate constant (λ_z), the time it takes for the plasma drug concentration to decrease by half ($T_{½}$), average plasma drug concentration (C_{av}) and peak-to-trough fluctuation (PTF) of the two different dose levels of AMZ001 and one dose of Voltaren Arthritis Pain Gel will be calculated. The PK parameters for Day 1 will also be calculated and considered as secondary endpoints.

Safety endpoint(s):

- Adverse Events (AEs); including serious, treatment emergent, adverse drug reactions (ADRs)
- Local and systemic tolerability

STATISTICAL METHODS:

Below is a summary of the statistical analysis methods. For more details on each analysis, please refer to Section 9.

Sample Size: Based on the assumption (averaging historical data and 000084 result), intra-subject coefficient of variation (CV) was 61%. A total of sixty (60) evaluable subjects will achieve 80% power using 95% confidence interval (CI), to account for multiplicity. The true ratio that was used in the sample size calculation was 1.05. Enough healthy adults will be screened to randomize approximately seventy-five (75) subjects to ensure sixty (60) evaluable subjects, assuming a 20% dropout and non-evaluable rate. All subjects will receive all 3 treatments over the course of the 3 study periods. Subjects will be randomized to one of the 6 possible sequences of 3 treatments, as specified in the design section above, to provide adequate information about the PK parameters and safety for the purposes of this study.

Statistical analysis: Descriptive statistics will be used to summarize the baseline demographic information.

Safety analysis: Descriptive statistics will be used to summarize AEs, laboratory data, physical examinations, and vital signs.

Pharmacokinetic (PK) Analysis: PK analysis will be performed using SAS® system.

PK parameters area under the plasma concentration-time curve from time zero to time t ($AUC_{0-\tau}$) and C_{max} will be compared across treatment groups using an analysis of variance (ANOVA) model. The ratio of the PK parameters will be estimated along with 90% confidence limits to assess comparable bioavailability.

SIGNATURE PAGE

AMZ001 (Diclofenac Sodium Gel 3.06%)

Sponsor Protocol Number: **AMZ001-008**

TKL Protocol Number: **P1980822**

The signatures of the following representatives constitute their approval of this protocol and provide the necessary assurances that this study will be conducted according to all stipulations stated in the protocol, including all statements as to confidentiality. It is also agreed that the study will not be initiated without the approval of an appropriate Institutional Review Board (IRB).

Approved by the following:

Amzell B.V.:
Dario Carrara, PhD
Chief Executive Officer

DocuSigned by:
Dario Carrara, PhD
Signer Name: Dario Carrara, PhD
Signing Reason: I approve this document
Signing Time: 3/24/2023 | 5:42:16 AM PDT
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Signature
Dario Carrara, PhD

3/24/2023
Date

TKL Research, Inc:
John C. Lyssikatos
Vice President, Clinical Operations

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John Lyssikatos
Signer Name: John Lyssikatos
Signing Reason: I approve this document
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Date

Michael Tuley, PhD
Vice President, Data Sciences and Quality Assurance

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3/23/2023
Date

Irina Krause, RN, MSN
Director, Phase 1 Operations

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3/23/2023
Date

SIGNATURE PAGE FOR INVESTIGATOR

AMZ001 (Diclofenac Sodium Gel 3.06%)

Sponsor Protocol Number: **AMZ001-008**

TKL Protocol Number: **P1980822**

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the study as described. I will conduct this study in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the study within the time designated. I will provide copies of this protocol and access to all information furnished by Amzell B.V. to study personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the study product and study procedures. I will let them know that this information is confidential and proprietary to Amzell B.V. and that it may not be further disclosed to third parties. I understand that the study may be terminated or enrollment suspended at any time by Amzell B.V., with or without cause, or by me if it becomes necessary to protect the best interests of the study patients.

I agree to conduct this study in full accordance with Food and Drug Administration Regulations, Institutional Review Board Regulations, and International Council for Harmonisation Guidelines for Good Clinical Practices.

Jonathan S. Dosik, M.D.
Principal Investigator

DocuSigned by:
Jonathan S. Dosik, MD
Signer Name: Jonathan S. Dosik, MD
Signing Reason: I approve this document
Signing Time: 3/23/2023 | 11:21:27 AM PDT
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Signature
Jonathan S. Dosik, M.D.

3/23/2023
Date

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LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
ADR	Adverse drug reaction
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of Variance
Anti-HCV	Hepatitis C antibody
AST	Aspartate aminotransferase
AUC_{0-24}	Area under the curve for 0-24 hours
AUC_{τ}	Area under the plasma concentration-time curve from time zero to time t
BUN	Blood urea nitrogen
Cav	Average plasma drug concentration
CDISC	Clinical Data Interchange Standards Consortium
CFR	Code of Federal Regulations
CI	Confidence interval
CIPT	Cumulative Irritant Patch Test
C_{max}	Maximum (peak) plasma drug concentration
C_{min}	Minimum plasma drug concentration
CMP	Clinical Monitoring Plan
COV	Closeout Visit
COX	Cyclooxygenase
CRO	Contract Research Organization
C_{trough}	Concentration of drug reached immediately before the next dose
CV	Cardiovascular
DDC	Direct data capture
DDE	Direct data entry
ECG	Electrocardiogram
eCRF	electronic Case Report Form
EDC	Electronic data capture
eICF	Electronic informed consent form
EOT	End of treatment
ET	Early termination
FCBP	Females of child-bearing potential
FDA	Food and Drug Administration
g	Gram
GI	Gastrointestinal
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen

HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product (refers to both AMZ001 and Voltaren Arthritis Pain Gel)
IMV	Interim Monitoring Visit
IRB	Institutional Review Board
IUD	Intrauterine device
λ_z	Terminal disposition rate constant
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MedDRA	Medical Dictionary for Regulatory Activities
mg/dL	Milligram per deciliter
mL	Milliliter
N	Total number of subjects/observations
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
pg	Picograms per milliliter
QD	Once daily
QID	Four times a day
PI	Principal Investigator
PK	Pharmacokinetic(s)
PKAS	Pharmacokinetic analysis set
PTF	Peak-to-trough fluctuation
RIPT	Repeat Insult Patch Test
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SDV	Source data verification
SIV	Site Initiation Visit
SMP	Safety Management Plan
SOC	System organ class
SOP	Standard Operating Procedure
SPT	Serum pregnancy test
$T_{1/2}$	The time it takes for the plasma drug concentration to decrease by half
TEAE	Treatment emergent adverse event
TKL	TKL Research, Inc.
T_{max}	Time to reach maximum (peak) plasma concentration following drug administration
T_{min}	Time to reach minimum plasma concentration following drug administration
ULN	Upper limit normal
UPT	Urine pregnancy test

1. 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 and Jordan, 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 and Hunter, 2014; Allen and Golightly, 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).

AMZ001 (diclofenac sodium 3.06%) - formerly FE999312 (diclofenac sodium 3%), is being developed for the relief of pain symptoms as a result of OA of the knee. The formulation was designed for rapid absorption through the skin after application to target sites.

The aim of this study is to evaluate the bioavailability, safety, and tolerability of two dose levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001, applied once daily, in comparison with one dose level (32 g) of Voltaren Arthritis Pain Gel, applied four times daily, after repeated dosing in healthy subjects for 7 days.

1.1 Scientific Justification for Conducting the Study

Non-steroidal anti-inflammatory drugs (NSAIDs) are often employed for symptomatic relief of mild-to moderate pain associated with OA. Traditional orally ingested NSAIDs have a high incidence of gastrointestinal (GI) and cardiovascular (CV) adverse effects (FitzGerald and Patrono, 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 (Massó 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 and Sawyer, 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 and Fuller, 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).

Amzell B.V. is introducing a novel transdermal hydroalcoholic gel formulation with diclofenac sodium as the active ingredient (AMZ001). This formulation is designed for rapid and efficient diclofenac absorption compared with other topical diclofenac products. To date, four Phase 1 studies have been completed: a 21-Day, randomized, controlled study to evaluate the skin irritation potential of AMZ001 (diclofenac sodium 3.06%), 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), two PK studies (000084 and AMZ001-005 [a Phase 1 PK and safety study of AMZ001 (diclofenac sodium gel 3.06%) in comparison with Voltaren® (diclofenac sodium gel 1%)] in healthy subjects.

Amzell B.V. has focused the development on once-a-day therapy. The aim of this additional PK study (AMZ001-008) is to characterize the rate and extent to which diclofenac is absorbed from two different doses of AMZ001 applied once-daily. The two doses have been selected from the outcomes of the previous Phase 1 PK and safety study (AMZ001-005) in an attempt to provide comparable systemic diclofenac exposure as Voltaren Arthritis Pain Gel.

1.2 Benefit / Risk Aspects

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 the product, 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.

The excipients used for the formulation of AMZ001 are well known and have been used in previously conducted studies as well as in already Food and Drug Administration (FDA)-approved topical products, such as Elestrin and Anturol. 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.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objectives

The primary objective of the study is to compare the bioavailability of two dose levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001 (diclofenac sodium gel 3.06%) applied once daily as 3 mL and 4 mL per knee on both knees and one dose level of Voltaren Arthritis Pain Gel (32 g, diclofenac sodium gel 1%) applied four times daily as 4 g per knee on both knees after repeated topical administrations in healthy subjects for 7 days.

2.1.2 Secondary Objectives

- Determine the PK profile of the two dose levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001 (diclofenac sodium gel 3.06%) and one dose level (32 g) of Voltaren Arthritis Pain Gel (diclofenac sodium gel 1%).
- Assess the safety and tolerability of the two different dose levels of AMZ001 (diclofenac sodium gel 3.06%) and one dose level of Voltaren Arthritis Pain Gel (diclofenac sodium gel 1%).

2.2 Endpoints

2.2.1 Primary Endpoint

- Compare the exposure to diclofenac, as measured by plasma concentration, area under the curve from time zero to 24 hours (AUC_{0-24}), and maximum (peak) plasma drug concentration (C_{max}) at steady state between two different dose levels of AMZ001 and one dose level of Voltaren Arthritis Pain Gel in healthy subjects on Day 7.

2.2.2 Secondary Endpoint(s)

- The PK parameters C_{max}, minimum plasma drug concentration (C_{min}), time to reach maximum (peak) plasma concentration following drug administration (T_{max}), time to reach minimum plasma concentration following drug administration (T_{min}), terminal disposition rate constant (λ_z), the time it takes for the plasma drug concentration to decrease by half ($T_{½}$), average plasma concentration (C_{av}) and peak-to-trough fluctuation (PTF) of the two different dose levels of AMZ001 and one dose level of Voltaren Arthritis Pain Gel will be calculated. The PK parameters for Day 1 will also be calculated and considered as secondary endpoints.

2.2.3 Safety Endpoint(s)

- Adverse Events (AEs); including serious, treatment emergent, ADRs
- Local systemic tolerability

3. INVESTIGATIONAL PLAN

3.1 Overall Study Design

See Figure 1 for the treatment schedule (Three Treatments, Three Period Crossover Design).

3.1.1 Study Design

This is a randomized, open-label, three period crossover, multiple-dose study to investigate the bioavailability, safety, and tolerability of two dose levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001 applied once daily on both knees and one dose level of Voltaren Arthritis Pain Gel (32 g) applied four-times daily on both knees after repeated dosing in healthy subjects for 7 days.

Before inclusion into the study, all subjects will undergo a physical examination, including vital signs, and blood samples will be collected for laboratory assessments (hematology, clinical chemistry, and urinalysis).

Subjects will be randomized to one of six dosing sequences, see Figure 1 for an overall summary of the study design:

Period 1

(Sequence 1) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD

(Sequence 2) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD

(Sequence 3) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily

(Sequence 4) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD

(Sequence 5) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD

(Sequence 6) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily

Period 2

(Sequence 1) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD

(Sequence 2) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily

(Sequence 3) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD

(Sequence 4) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD

(Sequence 5) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily

(Sequence 6) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD

Period 3

(Sequence 1) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily

(Sequence 2) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD

(Sequence 3) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD

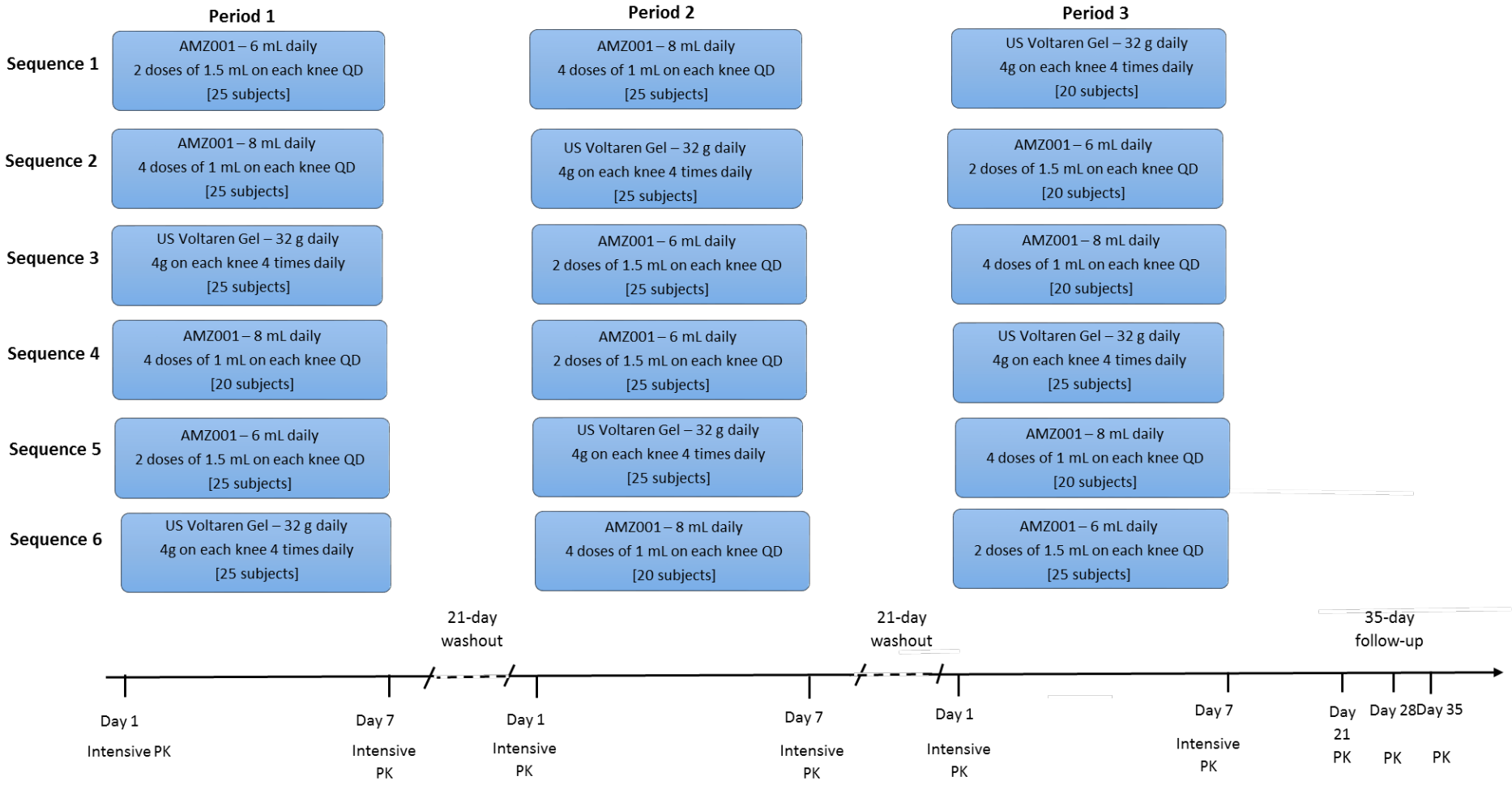
(Sequence 4) Voltaren Arthritis Pain Gel – 32 g daily: 4 g on each knee 4 times daily

(Sequence 5) AMZ001 – 8 mL (7.4 g) daily: 4 doses of 1 mL on each knee QD

(Sequence 6) AMZ001 – 6 mL (5.5 g) daily: 2 doses of 1.5 mL on each knee QD

Note: Between Periods 1 and 2 and Periods 2 and 3, there will be a 21-day (+ 7-day) washout, and the subjects will not be confined to the study site.

Figure 1: Study Design: Three Treatments, Three Period Crossover Design



The gel will be administered to the knee by subject under supervision of 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 then spread the gel directly to the pre-defined treatment area of approximately 400 cm^2 (6 mL AMZ001 and 32 g Voltaren Arthritis Pain Gel) and 500 cm^2 (8 mL AMZ001) for each subject's knee as per detailed application instructions.

Subjects will be confined at the study site only for the duration of each treatment period (Days -1 to Day 8) of the study. Subjects are permitted to shower ≥ 1-hour pre-dose and after 3-hours or greater post-dose. Blood samples for PK analyses will be collected prior to (within 1 hour) application and at 2 (±15 min), 4 (±15 min), 6 (±15 min), 8 (±15 min), 10 (±15 min), 12 (±15 min), 14 (±15 min), 16 (±15 min), 18 (±15 min), 20 (±15 min), and 24 (±30 min) hours after initial application on Days 1 and 7. One blood sample will be collected on Days 3, 4, 5, and 6 prior to (within 1 hour) the first application of gel to measure the trough level of each study drug.

At the end of Period 3, there will be three additional PK blood samples taken respectively at 3 weeks (21 days), 4 weeks (28 days), and 5 weeks (35 days) after the last application to compare elimination phase of AMZ001 vs. Voltaren Arthritis Pain Gel and to further describe the elimination phase of the AMZ001 product. During this follow-up period, subjects will not be confined to the study site.

If a sampling time point and application time fall at the same time, the blood sample should be taken **immediately prior** to application.

3.1.2 Application Instructions

The specified Investigational Medicinal Product (IMP) will be administered by subject under supervision of 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, as per the AMZ001 and Voltaren Application Instructions.

3.1.3 Study Schedule

The study duration is approximately 18 weeks for each subject.

3.2 Planned Number of Study Sites and Subjects

The study will be conducted at 1 site in the U.S. A sufficient number of subjects will be screened and approximately 75 subjects will be randomized to give 60 evaluable subjects across the six dosing sequences.

3.3 Interim Analysis

Not applicable.

3.4 Data Monitoring Committee

No Data Monitoring Committee is planned.

3.5 Discussion of Overall Study Design and Choice of Control Groups

3.5.1 Study Design

A randomized, open-label, three period crossover, multiple-dose design will evaluate the PKs and compare the bioavailability of two different dose levels of AMZ001 and one dose level of Voltaren Arthritis Pain Gel.

Note: Between Periods 1 and 2, Periods 2 and3, there will be a 21-day (+7-day) washout. At the end of Period 3 there will be a 35-day follow-up period, during which time there will be 3 scheduled PK follow-up outpatient visits (see Table 2).

Safety and tolerability will also be assessed.

3.5.2 Selection of Endpoints

The PK parameter levels (AUC_{0-24} and C_{max}) of AMZ001 will be used to compare the bioavailability of diclofenac sodium gel 3.06% to the marketed Voltaren Arthritis Pain Gel product.

3.5.3 Blinding

The clinical conduct of this study will be open-label. The bioanalytical lab will not be provided with the randomization schedule and therefore will be blinded to the treatments administered.

3.5.4 Selection of Doses in the Study

Higher concentration and absorption characteristics of the AMZ001 formulation will provide a delivery of diclofenac that allows for application once daily (QD) compared to dosing four times a day (QID) recommended for the reference product, Voltaren Arthritis Pain Gel. Comparative efficacy trials support the proposal of QD dosing for AMZ001 (Bihlet et al. 2020). Using already conducted PK studies, PK modelling and simulation have been conducted to estimate appropriate doses that should provide same exposure as Voltaren Arthritis Pain Gel. Two doses have been selected: 6mL and 8mL of AMZ001. Overall, the objective is to identify one dose of the AMZ001 formulation.

See Section 3.1.1 for doses selected.

3.5.5 Selection and Timing of Dose for Each Subject

3.5.5.1 On-Site Application during PK Sampling

The IMP should be applied at the specified times:

- AMZ001 will be administered to the knee by the subject under supervision of designated clinical site staff member QD in the morning.
- Voltaren Arthritis Pain Gel will be applied QID, approximately every 4-5 hours while subjects are awake (e.g., 7:00 am, 12:00 pm, 5:00 pm, and 10:00 pm)

Each application time is subject to a ±30-minute window based on Day 1 dosing times.

If a sampling time point and application time fall at the same time, the blood sample should be taken **immediately prior** to application.

4. SELECTION OF STUDY POPULATION

4.1 Study Population

Every effort will be made to enroll approximately equivalent numbers of male and female subjects.

4.1.1 Inclusion Criteria

1. Healthy male or female subjects 18-65 years of age (e.g., in general good physical health, as judged by the Investigator and no clinically relevant abnormalities identified by a detailed medical history, full physical examination, including blood pressure and pulse rate measurement, 12-lead ECG or clinical laboratory tests)
2. Has a body mass index (BMI) between 18.0 and 35.0 kg/m^2 at Screening
3. In the case of females of child-bearing potential ([FCBP] unless surgically sterilized [hysterectomy, bilateral oophorectomy, bilateral tubal ligation] or are postmenopausal for at least 12 months), are using two acceptable forms of birth control (hormonal contraceptives i.e., oral/implant/injectable/transdermal; intrauterine device (IUD) and/or barrier methods [female condom, male condom, diaphragm, cervical cap, spermicide]; note: 2 barrier methods are two acceptable forms of birth control)). Abstinence or partner's vasectomies are acceptable if the female subject agrees to implement two acceptable forms of birth control if her lifestyle/partner changes.
4. Females of child-bearing potential have a negative serum pregnancy test (SPT) at Screening and negative urine pregnancy test (UPT) on Day -1 of each period and at end of treatment (EOT) visit (Period 3, Day 8)
5. Are free of any systemic or dermatologic disorder and chronic or acute infections, which, in the opinion of the Principal Investigator (PI), will interfere with the study results or increase the risk of adverse events (AEs)
6. Read, understand, and provide signed informed consent before any assessment is performed.

4.1.2 Exclusion Criteria

The presence of any of the following excludes a subject from study enrollment:

1. Subject has any visible skin disease, skin lesions, wounds, or a significant amount of hair at the application site (knee).
2. Use of an investigational medicinal product (IMP) within 30 days or 5 half-lives (if known), whichever is longer, of enrollment or during the study

3. Treated with systemic or local diclofenac within 30 days of enrollment or during the study (except for study IMP)
4. Known hypersensitivity to diclofenac, any excipients (ethanol, propylene glycol, diethylene glycol monoethyl ether, myristyl alcohol, hydroxypropyl cellulose, and water), aspirin, Xarelto, coumadin, or other NSAIDs, including Cyclooxygenase-2 (COX-2) inhibitors
5. Any history of drug hypersensitivity, asthma, urticaria, or other significant allergic diathesis. Subjects with uncomplicated seasonal allergic rhinitis can be accepted only if expected allergy season is clearly outside enrolment/ treatment periods.
6. Females who are pregnant and/or lactating
7. Of child-bearing potential but not willing to use adequate contraception for the duration of the study
8. Subject is a current smoker and unable to abstain from smoking during the treatment periods.
9. Use of any topical medication, cosmetics, cream, ointments, lotions on the treatment site 1 week prior to enrollment through EOT visit.
10. Use of any medication (including over-the-counter medication, dietary supplements, and herbal remedies) within 2 weeks before first scheduled study drug administration or within less than 5 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. Consumption of any drug metabolizing enzyme (e.g., cytochrome P450 3A4 (CYP3A4) or other cytochrome P450 enzymes) inducing or inhibiting beverages or food (e.g., broccoli, Brussels sprouts, grapefruit, grapefruit juice, star fruit) within 3 days prior to and during each treatment period.

    Allowed treatments are:

    - Systemic contraceptives and hormone replacement therapy, as long as female subject is on stable treatment for at least 3 months and continues treatment throughout the study.
    - Occasional use of acetaminophen 500 mg (up to 1000 mg daily)
    - Single intake of other drugs only if judged by the investigator to have no clinical relevance and will not confound the interpretation of the study results.
11. Subject has a known or suspected malignancy, excluding basal cell cancer unless it is associated with the treatment area.
12. Subject has a positive blood screen for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), hepatitis C antibody (Anti-HCV)
13. Subject has any acute or chronic condition or is using medications, which, in the investigator's opinion, would make it unsafe for the subject to participate in this study,

including clinically significant abnormal laboratory values, vital signs, physical examination findings prior to randomization or during study participation.

14. History or current evidence of renal disease or impaired renal function at screening as indicated by abnormal levels of serum creatinine (> 1.43 mg/dL) or BUN (≥ 35 mg/dL) or the presence of clinically significant abnormal urinary constituents (e.g., albuminuria)
15. History or current evidence of ongoing hepatic disease or impaired hepatic function at screening. A subject will be excluded if more than one of the following lab value deviations are found: 1) AST (≥ 1.5 ULN), ALT (≥ 1.5 ULN), 2) GGT (≥ 1.5 ULN), ALP (≥ 1.5 ULN), 3) total bilirubin (> 2.00 mg/dL) or creatine kinase (≥ 3 ULN). A single deviation from the above values is acceptable and will not exclude the candidate, unless specifically advised by the Investigator.
16. Subject has clinically relevant chronic or acute infectious illnesses or febrile infections within 2 weeks prior to the first scheduled study drug administration
17. Subject has gastrointestinal bleeding issues, e.g., Gastroesophageal Reflux Disease (GERD), Peptic Ulcer Disease (PUD)
18. Subject has a hospital admission or major surgery within 30 days prior to randomization.
19. Subject has a 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.
20. Subject has a history of alcohol abuse, prescription drug abuse, or illicit drug use within 6 months prior to Screening.
21. Subject meets eligibility criteria, but study is filled
22. Subject who is an investigational site staff member directly involved in the conduct of the study and his/her family members, site staff member otherwise supervised by the Investigator, or subject who is a Amzell B.V. employee directly involved in the conduct of the study

Reasons for permanent discontinuation from the study may include the following:

1. Subject request
2. Adverse events that the Investigator believes may compromise the safety of the subject or the interpretation of study results.

4.2 Method of Assigning Subjects to Treatment Groups

4.2.1 Recruitment

The study site will recruit only healthy subjects. COVID-19 screening, testing, and prevention procedures will be followed as per local guidelines and study plan.

4.2.2 Randomization

The treatment given to individual subjects is determined by a randomization schedule.

4.3 Restrictions

4.3.1 Prior and Concomitant Therapies

This study is being performed in normal healthy subjects. No planned consumption of concomitant medications, dietary supplements, and herbal remedies (other than hormonal contraceptives and occasional use of acetaminophen) will be allowed 2 weeks prior to start of the study and throughout study completion.

4.3.2 Other Restrictions

- Subjects will be advised to avoid exposing the pre-defined application area to direct sunlight during the treatment periods.
- Subjects should avoid gel contact with the eyes, nose, and mouth; if gel contact occurs, rinse the affected area thoroughly with water.
- Subjects will be advised to wash their hands thoroughly after rubbing the gel into their knees, after each full IMP administration.
- No smoking throughout the treatment period of the study (permitted during washout periods and follow-up)
- No consumption of alcohol containing products throughout treatment period (permitted during washout periods and follow-up)
- No consumption of caffeine/theophylline (xanthine) – containing products (e.g., coffee, green tea, black tea, coco-cola, cocoa) throughout treatment period (permitted during washout periods and follow-up)
- Acceptance of standardized food and beverages throughout each treatment period
- No performance of highly strenuous physical exercise (body building, high performance sports) from 72 hours prior to start of the study and throughout the study until completion
- No consumption of food or beverages that have an influence on IMP pharmacokinetics such as, star fruit, grapefruit or grapefruit containing food or beverages from 3 days prior to and during each treatment period (permitted during washout periods and follow-up)

4.4 Withdrawal Criteria

The following medical and other reasons justify a premature termination (by subject or Investigator) of any of the IMPs:

- Withdrawal of informed consent,

- Serious adverse event (SAE)/AE,
- Allergic reaction to the study materials,
- Subject's request,
- Occurrence of one of the safety criteria for exclusion **after** treatment has been instituted,
- Subject is lost to follow-up, and/or
- Investigator's judgment.

The subjects have the right to withdraw from the study at any time for any reason, without the need to justify their decision. However, the Investigator should record the reason for the subject's withdrawal, if possible. The Investigator also has the right to withdraw subjects if the Investigator considers that the study treatment may compromise the safety of the subject or the subjects' non-compliance may impact the interpretation of the study results.

For any discontinuation, the Investigator will perform the follow-up examinations, obtain all the required details, and document the date of the premature termination and the main reason in the electronic Case Report Form (eCRF).

Data obtained after the subject has withdrawn his/her consent cannot be entered into the database. This also applies to biological samples drawn prior to the withdrawal of the consent but not analyzed at the time of the withdrawal, unless the subject consents to the use of these data.

4.5 Subject Replacement

Subjects that are withdrawn during the study after the first administration of IMP will be replaced so there are 60 evaluable subjects across the six dosing sequences.

5. TREATMENTS

5.1 Treatments Administered

5.1.1 Investigational Medicinal Product (IMP)

Both AMZ001 and Voltaren Arthritis Pain Gel will be referred to as study IMPs.

5.1.1.1 AMZ001

AMZ001 is provided in dispensing package containing 3.06% diclofenac sodium in a gel base. AMZ001 is delivered in aliquots of either 1 mL (0.92 g containing 28.2 mg of diclofenac sodium) or 1.5 mL (1.38 g containing 42.2 mg of diclofenac sodium) per pump actuation. For the purpose of this study, AMZ001 will be administered using the dispensers measuring 1 mL/actuation (for the 8 mL (7.4 g) daily dose) or 1.5mL/actuation (for the 6 mL (5.5 g) daily dose).

A detailed application instruction will be provided to the dedicated clinical site staff member and subjects.

5.1.1.2 Voltaren Arthritis Pain Gel

Voltaren Arthritis Pain Gel is provided in tubes containing 100 g of the topical gel in each tube. Each tube contains diclofenac sodium in a gel base (10 mg of diclofenac sodium per gram of gel

or 1%). A detailed application instruction will be provided to the dedicated site study member and subjects.

5.2 Medicinal Product(s)

5.2.1 Characteristics and Source of Supply

AMZ001 is manufactured according to the principles of Good Manufacturing Practice (GMP) and is a 3.06% diclofenac sodium topical gel administered from a dispensing package measuring either 1 mL per actuation or 1.5 mL per actuation. The amount of gel contained in each delivery dispenser is sufficient to cover the duration of the 7-day treatment period.

Voltaren Arthritis Pain Gel is sourced within the US from GlaxoSmithKline plc and is a 1% diclofenac sodium topical gel administered from 100g tubes.

5.2.2 Packaging and Labeling

5.2.2.1 Investigational Medicinal Product (IMP)

Labelling of the AMZ001 dispensing package will be performed under the responsibility of TKL Research, Inc. (TKL) in accordance with GMP and Good Clinical Practice (GCP) guidelines, as well as any national regulatory requirements. Each IMP dispenser will be labeled with study specific labels. The verbiage on the labels will be in accordance with US federal regulation 21 CFR § 312.6:

- The immediate package of an investigational new drug intended for human use shall bear a label with the statement “Caution: New Drug – Limited by Federal (or US) law to investigational use”.

- The label or labelling of an investigational new drug shall not bear any statement that is false or misleading in any particular and shall not represent that the investigational new drug is safe or effective for the purposes for which it is being investigated.

The dispensing packages are packaged in boxes according to their administration volume. The dispenser label will have a pre-printed protocol number and a dedicated space for filling in the subject number. The site will record the subject number on the dispenser label, and batch/lot number in the pharmacy records prior to use. The dispenser will also be weighed to assure compliance.

Each Voltaren Arthritis Pain Gel tube will be labelled. The label will contain a dedicated space for filling in the subject number and protocol information by clinical site staff prior to dispensing to subjects. The tube label will have a pre-printed protocol number and a dedicated space for filling in the subject number. The site will record the subject number on the tube label and batch/lot number in the pharmacy records prior to use. The tube(s) will also be weighed to assure compliance.

The study site will, under the responsibility of the Investigator, complete and return the Drug Delivery Note (packing slip) to Amzell B.V. verifying the receipt of the drug.

5.2.3 Conditions for Storage and Use

The Investigator will ensure that both IMPs will be stored in appropriate conditions at room temperature – AMZ001: STORE UPRIGHT 20-25°C (68-77°F) with a permitted excursion of 15-30°C (59º-86ºF); Voltaren Arthritis Pain Gel: 20-25°C (68-77°F) with a permitted excursion of 15-30°C (59º-86ºF); in a secure location with controlled access. The temperature in the storage area shall be monitored daily on-site when the office is open, and the values shall be documented in a temperature log. Storage and/or transit temperature deviations must be reported to Amzell B.V. without delay. All affected IMP will be quarantined on-site until a determination of acceptability is completed, and approved for use by Amzell B.V.

5.3 Treatment Compliance

5.3.1 Dispensing and Accountability

All handling of medicinal products will be done by trained and authorized personnel at the site. The IMP will only be applied to subjects who meet the eligibility criteria and are allocated as applicable in the study.

The study site will document the dates and quantities (IMPs to be weighed before and after dose application) of IMP applied to each subject and will maintain the batch/lot numbers of the IMP. Subject numbers will be marked on each delivery dispenser (AMZ001) and tube (Arthritis Pain Gel) by the study site and recorded. The monitor will verify the drug accountability during the study and document any discrepancies. Used dispensing packages and tubes will be saved for drug accountability.

5.3.2 Assessment of Compliance

Compliance will be assessed by weighing the dispensing package or tube before and after use daily on-site.

5.4 Prior and Concomitant Therapy

Any prior or concomitant medication will be recorded in the electronic Case Report Form (eCRF) with the main reason for its use.

5.5 Return and Destruction of Medicinal Products

IMP samples assigned as reserve (retention) IMP samples will be maintained at study site until the end of the required retention period, as per US federal regulations 21 CFR § 320.38 and 320.63. Reserve IMP samples will be randomly selected and set aside prior to the beginning of the study enrollment.

When drug accountability has been finalized, verified by the monitor, and signed off by the Investigator, all used and unused IMP (except for the reserve IMP samples retained on site, as per US federal regulations 21 CFR § 320.38 and 320.63) will be sent by study site for destruction in accordance with national requirements.

6. STUDY PROCEDURES

6.1 Study Flow Chart

This same flow chart will be used for all 3 periods; all dosing sequences have the same procedures.

Note: Between Periods 1 and 2 and Periods 2 and 3, there will be a 21-day (+ 7 day) washout

Table 1 - Study Flow Chart for Periods 1, 2 and 3[3]

Procedures (Days)	-30 to -2	Day -1[2]	1	2	3	4	5	6	7	8[E]
	Screening[1]									
Pre-Treatment Assessments										
Informed consent	X[A]									
Medical history	X									
Demographics	X									
Inclusion/Exclusion	X	X	X							
Sample Collection										
Clinical chemistry, hematology, and urinalysis	X									X
HIV and HBsAg, Anti-HCV	X									
Blood samples for PK			X[BG]	X[H]	X[F]	X[F]	X[F]	X[F]	X[BG]	X
SPT (FCBP)	X									
UPT (FCBP)		X								X[I]
Investigational Medicinal Product (IMP)										
Weigh IMP			X[C]	X[C]	X[C]	X[C]	X[C]	X[C]	X[C]	
IMP application			X[D]	X[D]	X[D]	X[D]	X[D]	X[D]	X[D]	
Other Assessments/Procedures										
Vital signs	X	X								X
Physical examination	X	X								X
ECG	X									
Baseline Treatment area assessment	X	X								
Assessment of skin reaction			X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X
Admit / Discharge		X								X

[1]Note, only 1 screening will be done before Period 1.
[2]Day -1 will be repeated after each washout period and before starting Periods 2 and 3 of treatment. [3]Use Table 2 at the completion of Period 3.

A. Subject must sign informed consent prior to any study procedures
B. On Day 1 and Day 7, Pre-dose (within 1 hour pre-dose) and 2 (±15 min), 4 (±15 min), 6 (±15 min), 8 (±15 min), 10 (±15 min), 12 (±15 min), 14 (±15 min), 16 (±15 min), 18 (±15 min), 20 (±15 min), and 24 (±30 min) hours (on Days 2 and 8) post initial application
C. Before and after dosing
D. As specified for the assigned treatment regimen
E. If a subject prematurely withdraws from the study at any time, all attempts should be made to complete the following: reason for withdrawal; assessment of skin reaction; weigh IMP container; physical examination; vital signs; clinical laboratory safety tests; AEs and concomitant medication.
F. Collect PK blood sample prior to morning dosing (within 1 hour pre-dose).
G. On Day 1 and 7, if Voltaren Arthritis Pain Gel applications overlap with PK collections, blood samples will be obtained prior to each Gel application.
H. Day 2 pre-dose will be the same collection as 24 hours post application on Day 1.
I. Only conducted on Day 8 of Period 3 (EOT visit) or Early Termination (ET) visit.

At the end of Period 3, there will be three additional PK blood samples taken respectively at 3 weeks (21 days), 4 weeks (28 days), and 5 weeks (35 days) after the last application to compare elimination phase of AMZ001 vs. Voltaren Arthritis Pain Gel and to further describe the elimination phase of the AMZ001 product. During this follow-up period, subjects will not be confined to the study site. Subjects will be reminded on the restrictions to be followed as part of concomitant medications prior to being discharged on Day 8 of this 3rd period.

Table 2 - PK Outpatient Visits

PK Outpatient Visits (Post Period 3)	3 Weeks (21 days) ±2 days		4 Weeks (28 days) ±2 days		5 Weeks (35 days) ±2 days
Sample Collection					
Blood samples for PK	X		X		X
Other Assessments/Procedures					
Adverse events	X		X		X
Concomitant medication	X		X		X

6.2 Study Procedures

All subjects **must** provide written informed consent prior to any study procedures.

6.2.1 Screening (Days -30 to -2)

The following procedures will be performed or reviewed at this visit:

- Informed consent
- Medical history
- Demographics
- Inclusion/Exclusion criteria
- Treatment area assessment
- Physical examination
- Vital signs
- Clinical laboratory safety tests (clinical chemistry, hematology, and urinalysis)
- HIV, HBsAg and Anti-HCV
- SPT (for FCBP)
- 12-lead ECG
- Concomitant medication

6.2.2 Day -1

Day -1 will be repeated after each washout period and before starting Periods 2 and 3 of treatment.

The following procedures will be performed or reviewed:

- Inclusion/Exclusion criteria
- Vital signs
- Physical exam
- UPT (for FCBP)
- Baseline treatment area assessment
- AEs
- Concomitant medication
- Subjects will then be admitted into the study site

6.2.3 Days 1, 7, and 8

Subjects in each dosing sequence (1-6) will begin treatment at this visit and 24-hour PK samples will be collected.

The following procedures will be performed or reviewed:

- Assessment of treatment area prior to Randomization (Period 1, Day 1 only)
- Inclusion/Exclusion criteria
- Randomization (Period 1, Day 1 only)
- Weigh Voltaren Arthritis Pain Gel container or AMZ001 dispenser – before and after dosing (Day 1 and Day 7)
- IMP application according to respective treatment period and dosing sequence (Day 1 and Day 7)
- Vital signs (Day 8)
- AEs
- Concomitant medication

- Assessment of skin reaction prior to the first application on Day 7 and on Day 8 (approximately 24 hours after the first application on Day 7)
- Physical examination (Day 8)
- Blood samples for PK on Day 1 and Day 7: Pre-dose (within 1 hour pre-dose) and at 2 (±15 min), 4 (±15 min), 6 (±15 min), 8 (±15 min), 10 (±15 min), 12 (±15 min), 14 (±15 min), 16 (±15 min), 18 (±15 min), 20 (±15 min), and on Day 8 approximately 24 (±30 min) post initial application on Day 7. Day 2 pre-dose will be the same collection as 24 hours post application on Day 1.
- UPT (for FCBP) (Day 8)
- Clinical chemistry, hematology, and urinalysis (Day 8)
- Then discharged from the study site (Day 8)

6.2.3.1 Day 2, 3, 4, 5, and 6

Subjects in each dosing sequence (1-6) will continue treatment and one PK sample will be collected prior to dosing.

The following procedures will be performed:

- Weigh Voltaren Arthritis Pain Gel container or AMZ001 dispenser – before and after dosing
- Assessment of skin reaction prior to the first application of the day
- IMP application according to respective treatment period and dosing sequence (Day 2, 3, 4, 5 and 6)
- AEs
- PK blood sample – within 1 hour pre-dose in the morning. The Day 2 pre-dose will be the same collection as 24 hours post application on Day 1.
- Concomitant medication

Note: Between Periods 1 and 2 and Periods 2 and 3, there will be a 21-day (+ 7-day) washout, and the subjects will not be confined to the study site.

At the end of Period 3, there will be three additional PK blood samples taken respectively at 3 weeks (21 days), 4 weeks (28 days), and 5 weeks (35 days) (see Table 2) after the last application to compare elimination phase of AMZ001 vs. Voltaren Arthritis Pain Gel and to further describe the elimination phase of the AMZ001 product.

7. STUDY ASSESSMENTS

Laboratories will provide Laboratory Manuals that will cover all blood and urine tests and describe sampling and shipment procedures including contact details, storage conditions, equipment, volume, analytical method, reference ranges, etc. The laboratory manuals will be provided to the clinical investigational unit before the start of the study.

7.1 Assessments Related to the Primary Endpoint

7.1.1 Pharmacokinetics

Blood samples for measurement of plasma concentration of diclofenac will be collected on the 1st and 7th day of treatment (Days 1 and 7). Samples will be collected pre-dose (within 1 hour) and 2 (±15 min), 4 (±15 min), 6 (±15 min), 8 (±15 min), 10 (±15 min), 12 (±15 min), 14 (±15 min), 16 (±15 min), 18 (±15 min), 20 (±15 min), and 24 (±30 min) hours post initial application (Day 8). One blood sample will be collected on the 3rd, 4th, 5th, and 6th day of treatment (Days 3, 4, 5, and 6). These samples will be collected within 1 hour prior to dosing in the morning. At the end of Period 3, there will be three additional PK blood samples taken respectively at 3 weeks (21 days), 4 weeks (28 days), and 5 weeks (35 days) after the last application of IMP (see Table 2).

Specific instructions on sample handling will be described in the laboratory manual provided by the bioanalytical laboratory. Samples will be split into two aliquots: one aliquot for analysis (with sufficient material to allow for a repeat analysis at the bioanalytical laboratory) and one aliquot remaining at the clinical site to serve as a backup sample. Samples will be shipped frozen to the bioanalytical laboratory for analysis. A validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with an LLOQ of 50 pg/mL for the analysis of diclofenac will be employed for sample analysis, as described in a sample analysis plan.

7.2 Other Assessments

7.2.1 Vital Signs

Vital signs comprising systolic and diastolic arterial blood pressure, pulse and body temperature will be assessed at screening, Day -1, and Day 8 visits. Systolic and diastolic blood pressure will be measured after the subject has been in seated or supine position for at least 1 minute. All recordings will be performed using standard equipment.

Clinically significant abnormal findings will be reported as adverse events (AEs).

7.2.2 Laboratory Parameters

7.2.2.1 Clinical Safety Laboratory Tests

Blood samples for clinical chemistry, hematology and urinalysis will be collected at screening and on Day 8. Laboratory safety evaluations will be performed according to Table 3 below. The clinical chemistry, hematology and urinalysis will be performed by a diagnostic laboratory.

Clinically significant abnormal findings will be reported as AEs.

Table 3 - Safety Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
Alanine aminotransferase	Hematocrit	Protein
Alkaline phosphatase	Hemoglobin	Glucose
Aspartate aminotransferase	Platelet count	Bilirubin
Calcium	Red blood cell count	pH
Cholesterol	Reticulocytes	Nitrite
Creatinine	White blood cell count with differential	Ketone
Gamma-glutamyl transferase	Monocytes, large unclassified cells	Urobilinogen
Glucose		Blood
Potassium		Leukocytes
Sodium		
Total bilirubin		
Triglycerides		
Urea (blood urea nitrogen)		
SPT (screening only)		
Serology: HIV, HBsAG, and HCV test (screening only)		

7.2.3 Physical examination

Physical examination will be performed at the screening, Day -1, and Day 8. Clinically significant abnormal findings at follow-up will be reported as AEs.

A full physical examination will include an evaluation of the head, eyes, ears, nose, throat, neck, heart, chest, lungs, abdomen, extremities, skin, and any other notable conditions.

7.2.4 12-Lead Electrocardiogram (ECG)

A standard bedside 12-lead ECG will be recorded using the study site's own ECG equipment and will be evaluated by the Investigator.

7.2.5 Administration Site Reactions

The skin at the application site will be examined by the Investigator or designee (see Section 6.2 for when examinations will take place). The skin tolerability will be documented in the eCRF according to the terminology of the International Contact Dermatitis Research Group:

0 no reaction (negative documentation)

1 doubtful reaction

2 mild erythema

3 strong erythema

4 very strong erythema and edema

These administration site reactions will be recorded in an eCRF page dedicated to dermal findings. Mild, strong, and very strong erythema and edema (skin tolerability score ≥2) will be reported as AEs.

7.3 Handling of Biological Samples

A detailed description of all sample collections and shipment procedures will be included in a separate laboratory manual.

7.4 Laboratory Values

The Investigator will review the laboratory test results, evaluate, and document whether the results are non-clinically or clinically significant.

8. ADVERSE EVENTS

8.1 Adverse Event (AE) Definition

An AE is any untoward medical occurrence in a subject participating in a clinical study. It includes:

- Any unfavorable and unintended sign, symptom or disease temporally associated with the use of the IMP, whether or not considered to be caused by the IMP.
- AEs commonly observed, and AEs anticipated based on the pharmacological effect of the IMP.
- Any laboratory abnormality, vital sign or finding from physical or other examinations assessed as clinically significant by the Investigator [Note: findings from assessments and examinations done during screening are not AEs but are recorded as medical history.]
- Accidental injuries, reasons for any change in medication (drug and/or dose), reasons for any medical, nursing or pharmacy consultation, or reasons for admission to hospital or surgical procedures.

- Overdoses and medication errors with and without clinical consequences.

All pre-treatment events will be captured as medical history.

A treatment emergent AE (TEAE) is defined as any AE occurring after start of IMP administration and within the time of residual drug effect (5 days), or a pre-treatment AE or pre-existing medical condition that worsens in intensity after treatment with the IMP and within the time of residual drug effect.

A post-treatment AE is defined as any AE occurring after the first dose of study drug and until the last study visit (end-of-study visit) or until 3 weeks post dose (whichever is longer).

8.2 Collection and Recording of Adverse Events

8.2.1 Collection of Adverse Events

The Investigator must monitor the health of the subject throughout the study from the time of enrolment until the last study visit.

The sources of AEs cover:

- The subject's response to questions about his or her health (a standard non-leading question such as "How have you been feeling since your last visit?" is asked at each visit).
- Symptoms spontaneously reported by the subject.
- Investigations and examinations where the findings are assessed by the Investigator to be clinically significant changes or abnormalities.
- Other information relating to the subject's health becoming known to the Investigator (e.g., hospitalization).

8.2.2 Recording of Adverse Events

The Investigator must record all AEs in the AE Log provided in each subject's eCRF with information about:

- AE
- Date and time of onset (time can be omitted, if applicable)
- Intensity
- Causal relationship to IMP
- Action taken with IMP.
- Other action taken.

- Date and time of outcome (time can be omitted, if applicable)
- Outcome
- Seriousness

Each of the items in the AE Log is described in detail in the following sections.

Adverse Event

Adverse events should be recorded as diagnoses, if available. If not, separate signs and symptoms should be recorded. One diagnosis/symptom should be entered per record.

If a subject suffers from the same AE more than once and the subject recovers in between the events, the AEs should be recorded separately. If an AE changes in intensity, a worst-case approach should be used when recording the event, i.e., the highest intensity and the longest duration of the event. Exception: *If a symptom or diagnosis with onset before the first IMP administration (i.e., a pre-treatment event) increases in intensity, this must be recorded as AE. The initial symptom or diagnosis should be recorded as medical history. While AE should be reported with date/time of onset when the intensity increased.*

Note: A procedure is not an AE; the reason for conducting the procedure is. Hospitalization is not an AE; the reason for hospitalization is. Death is not an AE, but the cause of death is (an exception is sudden death of unknown cause, which is an AE).

Date and Time of Onset

The date of onset is the date when the first sign(s) or symptom(s) were first noted. If the AE is an abnormal clinically significant laboratory test or outcome of an examination, the onset date is the date the sample was taken, or the examination was performed.

Intensity

The intensity of an AE must be classified using the following 3-point scale:

Mild:	Awareness of signs or symptoms, but no disruption of usual activity.
Moderate:	Event sufficient to affect usual activity (disturbing).
Severe:	Inability to work or perform usual activities (unacceptable).

Causal Relationship to IMP

The possibility of whether the IMP caused the AE must be classified as one of the following:

Reasonable possibility:

There is evidence or argument to suggest a causal relationship between the IMP and the AE. The AE may occur as part of the pharmacological action of the IMP or may be unpredictable in its occurrence.

Examples:

- adverse events that are uncommon but are known to be strongly associated with IMP exposure.
- adverse events that are not commonly associated with IMP exposure, but the event occurs in association with other factors strongly suggesting causation, such as a strong temporal association or the event recurs on rechallenge.

No reasonable possibility:

- There is no reasonable evidence or argument to suggest a causal relationship between the IMP and the AE.

Examples:

- known consequences of the underlying disease or condition under investigation.
- adverse events common in the study population, which are also anticipated to occur with some frequency during the course of the study, regardless of IMP exposure.

Action Taken with IMP

The action taken with the IMP in response to an AE must be classified as one of the following:

- No change (medication schedule maintained or no action taken)
- Withdrawn
- Interrupted

Other Action Taken

Adverse events requiring therapy must be treated with recognized standards of medical care to protect the health and well-being of the subject. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment for an emergency situation.

If medication is administered to treat the AE, this medication should be entered in the eCRF.

Date and Time of Outcome

The date and time (time can be omitted, if applicable) the subject recovered or died. If outcome is "Not recovered" or "Recovering" no date of outcome can be recorded.

Outcome

The outcome of an AE must be classified as one of the following:

- Recovered (fully recovered or the condition has returned to the level observed at initiation of study treatment)
- Recovered with sequelae (resulted in persistent or significant disability/incapacity)
- Recovering
- Not recovered
- Fatal

8.3 Pregnancy and Pregnancy Outcome

If a pregnancy occurs in female subjects, the IMP should be immediately stopped, and the subject must be withdrawn from the study (as pregnancy is an exclusion criteria). TKL will inform Amzell B.V. including information about contact details for the follow-up on the course and outcome of the pregnancy. The pregnancy must be followed-up at least until the birth of the infant and one month after the birth of the infant. In general, the follow-up will include the course, duration, and the outcome of the pregnancy as well as neonatal health. If a pregnancy results in an abnormal outcome (birth defect/congenital anomaly) TKL will report this as a serious AE to Amzell B.V.

8.4 Serious Adverse Events

8.4.1 Serious Adverse Event (SAE) Definition

An event is defined a serious adverse event if it:	**Guidance**
results in **death**	Any event resulting in a fatal outcome must be fully documented and reported, including deaths occurring within four weeks after the treatment ends and irrespective of the causal relationship to the IMP. The death of a subject enrolled in a study is *per se* not an event, but an outcome.
is **life-threatening**	The term life-threatening refers to an AE in which the subject was at immediate risk of death at the time of the event. It does not refer to an event, which may have caused death if it were more severe.
requires **in-patient hospitalization** or prolongation of existing hospitalization	The term hospitalization means that the subject was admitted to hospital, or that existing hospitalization was extended as a result of an event. Hospitalization describes a period of at least 24 hours. An overnight stay for observation, stay at emergency room or treatment on an out-patient basis do not constitute a hospitalization. However, medical judgment must always be exercised and when in doubt the case should be considered serious (i.e., if the case fulfills the criterion for a medically important event). Hospitalizations for administrative or social purposes do not constitute an SAE. Hospital admissions and/or surgical operations planned before study inclusion are not considered AEs, if the illness or disease existed before the subject was enrolled in the study, provided that the condition did not deteriorate during the study.
results in persistent or significant **disability/incapacity**	Disability/incapacity means a substantial disruption of a person's ability to conduct normal life functions. In doubt, the decision should be left to medical judgment by the Investigator.
is a **congenital anomaly/birth defect**	Congenital anomaly/birth defect observed in any offspring of the subject conceived during treatment with the IMP.

An event is defined a serious adverse event if it:	Guidance
is an **important medical event**	Important medical events are events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of important medical events include AEs that suggest a significant hazard, contraindication or precaution, occurrence of malignancy or development of drug dependency or drug abuse. Medical and scientific judgment should be exercised in deciding whether events qualify as medically important. Important medical events include any suspected transmission of an infectious agent via a medicinal product. Any organism virus or infectious particle (e.g., prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings indicating an infection in a subject exposed to a medicinal product.

8.4.2 Collection, Recording and Reporting of Serious Adverse Events

SAE Reporting by the Investigator

All SAEs must be reported **immediately** as soon as it becomes known to the Investigator and not later than within 24 hours of their knowledge of the occurrence of an SAE.

TKL Pharmacovigilance Group
Allyson Hudson
TKL Research, Inc.
Tel: (201) 587-0500, ext. 2522
Cell: (973) 897-7152
Fax: (201) 296-6660
pharmacovigilance@tklresearch.com

The Investigator will complete the eCRF SAE report Form with the fullest possible details **within 3 calendar days** of the Investigator's knowledge of the SAE.

Additional information relevant to the SAE such as hospital records, results from investigations, e.g., laboratory parameters (that are not already uploaded in the eCRF), invasive procedures, scans and x-rays, and autopsy results can be faxed or scanned and e-mailed to TKL using the contact details in the section above. In any case this information must be supplied by the Investigator upon

request from TKL. On any copies provided, such details such as subject's name, address, and hospital ID number should be redacted, and the subject number provided, instead.

The Investigator will supply TKL and the institutional review board (IRB) with any additional requested information such as results of post-mortem examinations and hospital records.

Completion of the Demographics, AE Log, Medical History Log and Concomitant Medication Log are mandatory for initial reports and for follow-up reports if any relevant changes have been made since the initial report.

Expedited Reporting

TKL will report all SAEs to Amzell B.V. as per the protocol-specific SAE flow procedures.

Any AEs that are **serious, unexpected, and with a reasonable possible causality to the IMP,** as judged by either the Investigator or Amzell B.V., will be reported to regulatory authorities in US and to IRB within the stipulated timelines. Full details on the reporting procedures, responsibilities, and personnel are presented in the Safety Management Plan (SMP).

The expectedness is assessed by Amzell B.V.

SAEs will be considered reportable regardless of whether or not the IMP was used in accordance with the provisions in the protocol.

8.5 Follow-up of Adverse Events and Serious Adverse Events

8.5.1 Follow-up of Adverse Events with Onset during the Study

During the study, the Investigator must follow-up on each AE until it is resolved or until the medical condition of the subject is stable.

After the subject's last visit, the Investigator must follow-up on any AEs that occurred during the study classified as serious or considered to have a reasonable possible causality to the IMP with the outcome "Not recovered" until resolved or until the medical condition of the subject is stable. All such relevant follow-up information must be reported to Amzell B.V. If the event is a chronic condition, the Investigator and Amzell B.V. may agree that further follow-up is not required.

Collection of SAEs with Onset after Last Visit

If an Investigator becomes aware of a SAE after the subject's last visit and assesses the SAE to have a reasonable possible causality to the IMP, the case will have to be reported to Amzell B.V., regardless how long after the end of the study this takes place. Such reports will be considered for expedited reporting and managed as such.

9. STATISTICAL METHODS

Additional details of the proposed statistical analysis methods will be documented in the Statistical Analysis Plan (SAP), which will be written following finalization of the protocol and prior to study unblinding/analysis (as appropriate). This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints. The SAP creation and statistical analysis will be performed by Lead Statistician (Guidance for Industry Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs-General Considerations, March 2014).

All concentration and PK data will be listed. This includes any data for subjects who are not included in the analysis (e.g., subjects withdrawn from the study due to AEs).

9.1 Determination of Sample Size

Based on the assumption (averaging historical data and Phase 1 Study 000084 study results), intra-subject coefficient of variation (CV) was 61%. A total of sixty (60) evaluable subjects will achieve 80% power using 95% confidence interval (CI), to account for multiplicity. The true ratio that was used in the sample size calculation was 1.05. Enough healthy adults will be screened to randomize approximately seventy-five (75) subjects to ensure sixty (60) evaluable subjects, assuming a 20% dropout and non-evaluable rate. All subjects will receive all 3 treatments over the course of the 3 study periods. Subjects will be randomized to one of the 6 possible sequences of 3 treatments, as specified in the design in Figure 1, to provide adequate information about the PK parameters and safety for the purposes of this study.

9.2 Subject Disposition

All healthy subjects screened and enrolled will be followed to completion. All subjects should be included in the analysis unless prespecified in the protocol e.g., PK parameters can be derived or major deviations that impact PK as described below. The subjects screened but not found eligible will be included in the study report but not study analysis.

9.3 Protocol Deviations

Major protocol violations, such as significant non-compliance or other serious unforeseen violations deemed to invalidate the data collected for the purpose of the study will lead to exclusion of the data from analysis. In case of minor protocol violations, data will not be excluded from the data analysis. The rating of protocol violations as either ‘minor’ or ‘major’ will be decided on the basis of a review of the data before declaration of the ‘clean file’ and lock of database.

9.4 Statistical Analysis Sets (or Populations)

9.4.1 Safety Population

The safety population will include randomized subjects who received at least one dose of AMZ001 or Voltaren Arthritis Pain Gel. The randomized population will be used for safety analysis,

presentation of compliance and all baseline characteristics (demographics, medical history, prior and concomitant medication, and physical examination).

9.4.2 Pharmacokinetic (PK) Population

The PK population is defined as all randomized subjects who have at least one post-dose PK value, and who have no major protocol deviations concerning PKs.

9.4.3 PK Analysis Set

Two pharmacokinetic analysis sets (PKAS1 and PKAS2) will be considered for this clinical trial.

PKAS1 includes all subjects of the PK population, for which the relevant PK parameters (at least area under the plasma concentration-time curve from time zero to time to [AUC_{0-t}] or C_{max}) can be derived for the reference treatment along with at least one of AMZ001 test products, 6 mL or 8 mL, regardless of baseline diclofenac concentration of the individual C_{max} in the relevant periods. This analysis set will be used in PK summaries and the primary analysis and the secondary PK analysis.

PKAS2 includes all subjects of the PK population, for which the relevant PK parameters (at least AUC_{0-t} or C_{max}) can be derived for the reference treatment along with at least one of AMZ001 test products, 6 mL or 8 mL, and with baseline concentration ≤ 5% of the individual C_{max} in the relevant periods. This analysis set will be used for sensitivity analysis (and perform same analysis as PKAS1).

9.5 Study Population

9.5.1 Demographics and other Baseline Characteristics

Descriptive statistics from the randomized population will be used to summarize the baseline demographic information. For continuous variables, data will be presented by descriptive statistics (e.g., number, mean, median, standard deviation, medians, and range). For categorical variables, these data will be presented as counts and percentages.

Missing values will not be imputed.

9.5.2 Medical History, Concomitant Medication and Physical Examination

Medical history and concomitant medication will be presented in by-subject listings for the randomized population.

9.6 Treatment Compliance

Treatment compliance will be presented from the safety population.

9.7 Endpoint Assessments

9.7.1 General Considerations

Descriptive statistics will be used to summarize these study data. For continuous variables, data will be presented by descriptive statistics (e.g., number, mean, median, standard deviation,

medians, and range). For categorical variables, these data will be presented as counts and percentages.

Missing values will not be imputed.

9.7.2 Pharmacokinetics (PK)

PK analysis will be performed using the SAS® system. PK parameters assessed at Day 1 and Day 7 will include $AUC_{0-\tau}$, C_{max}, C_{min}, C_{av}, PTF, T_{max}, T_{min}, λ_z, and $T_{½}$, and will be presented in listings for individual subjects and summarized in tables and figures.

PK parameters $AUC_{0-\tau}$ and C_{max} will be compared across treatment groups using an analysis of variance (ANOVA) model. The ratio of the PK parameters will be estimated along with 90% confidence limits to assess comparable bioavailability.

Please refer to the SAP for detailed analysis method.

9.7.3 Primary Endpoint

Compare the exposure to diclofenac sodium, as measured by plasma concentration, AUC_{0-24} and C_{max} at steady state between two different dose levels of AMZ001 and one dose level of Voltaren Arthritis Pain Gel in healthy subjects on Day 7.

9.7.4 Secondary Endpoint(s)

The PK parameters C_{max}, C_{min}, T_{max}, T_{min}, λ_z, $T_{½}$, C_{av} and PTF of the two different dose levels of AMZ001 and the one dose level of Voltaren Arthritis Pain Gel will be calculated. The PK parameters for Day 1 will also be calculated and considered as secondary endpoints.

9.7.5 Safety Endpoint(s)

Adverse events (AEs) including serious, treatment emergent, adverse drug reactions (ADRs)

Local and systemic tolerability

9.7.5.1 Vital signs

Vital signs (blood pressure, pulse rate, and body temperature) will be summarized.

9.7.5.2 Clinical chemistry, hematology and urinalysis

Change from baseline of clinical chemistry, hematology and urinalysis parameters will be summarized.

9.7.6 Adverse Events (AEs)

A SMP is created to specifically identify how safety of the subjects will be monitored and follow-up will be performed for the study. The SMP is summarized below.

AEs will be coded according to the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) and grouped by system organ class (SOC). All data will be listed by subject.

Only TEAEs will be presented in summary tables. Separate data listing will be provided for AEs that are defined as pre-treatment or post-treatment emergent.

9.7.6.1 Overview of Treatment Emergent Adverse Events (TEAEs)

A TEAE summary table will be presented including for each treatment the number of subjects reporting an AE, the percentage of subjects with an AE, and the number of events reported, for the following categories:

- All AEs
- Severe adverse events
- SAEs
- ADRs
- AEs leading to subject withdrawal.
- Death

AEs classified as a reasonable possibility are categorized as related to the IMP and are defined as ADRs.

9.7.6.2 Incidence of TEAEs

Summary tables will be prepared for the incidence of TEAEs per treatment by MedDRA SOC and preferred term, presenting number of subjects reporting an AE, the percentage of subjects (%) with an AE, and the number of events reported. Summary tables will be prepared for:

- All TEAEs
- AEs by causality
- AEs by intensity
- ADRs by intensity

Missing values will be treated as missing except for causality, intensity, seriousness, and outcome of an AE, at which occurrence a "worst case" approach will be taken. Thus, if causality is missing the AE will be regarded as related to the IMP, if the intensity is missing the intensity of the AE will be regarded as severe, if seriousness is missing the AE will be regarded as an SAE, and if the outcome is missing and no date of outcome is present the outcome is regarded as "not recovered".

9.7.6.3 Serious adverse events and other significant adverse events

Separate listings will be provided for SAEs and other significant AEs.

9.7.6.4 Application site reactions

Assessments of application site reactions will be summarized by dose over the assessment time.

9.7.6.5 Physical examination

Physical examination will be listed by subject.

9.8 Interim Analyses

No interim analyses are planned.

9.9 Exclusion of Data from Analysis

Refer to Statistical Analysis Plan for details.

9.10 Handling of Dropouts and Missing Data

Refer to Statistical Analysis Plan for details.

10. DATA HANDLING AND RECORD KEEPING

10.1 Recording of Data

Data will be recorded via electronic data capture (EDC) and e-source direct data capture (DDC). Electronic Case Report Forms (eCRFs), which will be designed to identify each subject by randomization number and, where appropriate, subject's initials, the product being evaluated, study visit parameters to be measured or collected and the results observed. All entries to the eCRFs must be made as instructed by Amzell B.V. at study initiation.

The PI must sign the designated page(s) of the eCRFs, thereby stating that he/she takes responsibility for the accuracy of the data in the entire eCRF. All records will be kept in conformance to applicable national laws and regulations.

The copy of signed electronic informed consent form (eICF) will be included in each subject's file. When the study treatment is completed, the eICF will be kept in the appropriate file folder; otherwise, a note indicating where the records can be located will be made.

In addition to the eCRFs, individual subject e-source DDC files will be maintained. These documents may include the visit dates, the clinical study number, the name or initials of the subject, medical history or previous physical examinations, demographic and medical information, including laboratory data, skin assessments, concomitant medications/treatments, etc., any AEs encountered, and other notes, as appropriate.

10.2 Data Management

Data will be double-keyed and validated using ClinPlus (DZS Software Solutions), which directly generates SAS® data sets, Clinical Data Interchange Standards Consortium (CDISC) format. After resolution of double-key discrepancies and a combination of manual and automated data review

procedures, the final data sets will be subject to a Quality Assurance audit. SAS® programs for data analysis and presentation will be applied to secure validated data sets.

10.3 Retention of Documents

Essential documents, as listed below, must be retained in a secure place by the Investigator for a minimum of 5 years from completion of the study, or as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). Amzell B.V. will notify the Investigator(s)/institution(s) when the study-related records are no longer required. The Investigator agrees to adhere to the document retention procedures by signing the protocol.

Essential documents include:

- The study protocol and any amendments,
- IRB approvals for the study protocol and all amendments,
- All e-source documents and laboratory records,
- eCRF originals or copies,
- Subjects' eICFs (with study number and title of study),
- FDA Financial Disclosure Forms
- Site Delegation of Duties Log
- IP Accountability Documentation
- FDA form 1572 (as required)
- Any other pertinent study document (i.e., correspondence, study report[s]).

All study related documents will be stored at either the study site at 1201 Promenade Blvd, Fair Lawn, NJ 07410 in a secured room accessible only to TKL employees, or at a specified offsite location that provides a secure environment with burglar/fire alarm systems, camera detection, and controlled temperature and humidity. Originals or copies of the eCRFs, source documents, correspondence, IRB documents, study reports, etc., will be available for the Sponsor's review on the premises of TKL. Amzell B.V. must be informed of the offsite location at the time the documents are transferred to long-term storage.

No study site document may be destroyed without prior written agreement between the Investigator and Amzell B.V. Should TKL choose to assign the study documents to another party or move them to another location, Amzell B.V. must be notified.

10.4 Confidentiality of Subject Data

The Investigator will preserve the confidentiality of the subjects' data. eCRFs and other documents submitted to Amzell B.V. will reference subjects only by an anonymized subject ID, which uniquely identifies the subject in the context of the study. The Investigator will maintain

documents not meant for submission to the sponsor, e.g., the confidential subject identification code and the signed informed consent forms, in strict confidence. All data are subject to monitoring, audits and inspection. If, as an exception, it is necessary for safety or regulatory reasons to identify the subject, both Amzell B.V. and the Investigator are bound to keep this information confidential.

10.5 Clinical Monitoring Plan (CMP)

The CMP identifies the monitoring schedule and the rationale for the frequency and type of monitoring visits. Since this study is using Direct Data Entry (DDE), in addition to agreed-upon source data verification (SDV), on-site monitoring visits will be limited to assuring that the site understands and is following the protocol, is adequately monitoring subject safety, and is managing the drug supply. The CMP also provides details on the use of risk-based monitoring and source document verification. If the monitor is not allowed access to any e-source records during source document verification, certified printouts provided by the site can be used. In addition to on-site monitoring, monitors will perform remote monitoring, electronically reviewing data in near real-time.

Communication	Timeframe
Site initiation visit (SIV)	The site must have a SIV (if SSV not performed).
First monitoring visit	See CMP.
Interim monitoring visits (IMV)	As specified in the CMP
Closeout visit (COV)	The site must have a COV.
Site Update and Monitoring Calls	Monitors will contact the site as needed via email or telephone, based on review of site activity and the quality of data entry and DDC.
Teleconferences between the site and Contract Research Organization (CRO)	Monitors will schedule teleconferences as appropriate to discuss the overall study status and to discuss study-wide related issues.

Communication	Timeframe
Initiation, Monitoring and Closeout Visit Reports	Interim monitoring visits can be performed on-site or online. On-site visits are preceded by a confirmation letter sent to the site. The confirmation letter must outline the date, time and purpose of the monitoring visit Monitors will route all online, Initiation, Monitoring and COV Reports for signature to their supervisor(s). The TKL Project Manager will review the report and enter comments if needed. Following the completion of an on-site monitoring visit report, the monitor provides feedback to the site, identifying any outstanding issues from the visit.
Study updates, Protocol Amendments, etc.	Will be forwarded to site during study.
Adverse Events (AE) and Serious Adverse Events (SAE)	The primary method for reporting the event consists of entering data into the AE and SAE forms and completing the MedWatch FDA Form 3500A.

10.6 Audit Procedures

In addition to the routine monitoring procedures, the study site will be audited in depth for study Quality Assurance by Amzell B.V., an external auditor on behalf of the Sponsor, and maybe by a regulatory authority. This audit may include a review of all source documents, drug records, reserve sample retention records and original eCRFs at the study unit used in the study. Subject confidentiality will be maintained at all times and consent for this will be obtained before entry of the subject into the clinical study. If an inspection is requested by a regulatory authority, the Investigator must immediately inform Amzell B.V. that this request has been made.

10.7 Investigational Medicinal Product (IMP)

Monitors will verify that the Investigator maintains accurate and adequate records including dates of treatment, duration of treatment, and appropriate follow-up. Monitors will perform IMP and reserve sample accountability and verify storage conditions of the IMP and reserve samples (secure location, temperature logs, etc.) in accordance with manufacturers' instructions. Monitors will verify that the site has an adequate supply for ongoing and new subjects.

During the course of the study, the monitor is responsible for assuring the accountability and reconciliation of the IMP. To assist with this, the monitor will review or perform the following, as appropriate:

- IMP Receipt and return of delivery note(s)
- Reserve Sample Request/Log
- All used and unused IMP (except for the reserve IMP samples retained on site) will be sent by the study site for destruction in accordance with national requirements:
    - Certificate of destruction to be provided to Amzell once done.

10.8 Laboratory Samples and Analysis

Laboratory samples, both for clinical safety and PK analysis, will be processed through a local laboratory (LabCorp for safety analysis and Sannova for PK analysis) and the results will be sent to TKL.

Specific details for PK laboratory sample processing are described in the Sannova Sample Analysis Plan. Specific details for clinical laboratory sample handling (clinical chemistry, hematology, serology and urinalysis samples) are detailed in the LabCorp Laboratory Manual.

11. CHANGES IN CONDUCT OF THE STUDY

11.1 Protocol Amendments

Any changes or additions to this clinical study protocol require a written protocol amendment that must be approved by Amzell B.V. and the Investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study, require additional approval by the appropriate IRB for the study site. A copy of the written approval of the IRB, which becomes part of the protocol, must be given to the Sponsor. Examples of amendments requiring such approval are:

1. An increase in study product dosage or duration of product exposure of subjects,
2. A significant change in the study design,
3. An increase in the number of invasive procedures to which subjects are exposed, and
4. Addition or deletion of a test procedure for safety monitoring.

These requirements for approval should in no way prevent any immediate action from being taken by the Investigator or Amzell B.V. in the interests of preserving the safety of all subjects included in the trial. If an immediate change to the protocol is felt to be necessary by the Investigator and is implemented by him/her for safety reasons, Amzell B.V. should be notified immediately and the IRB at the center should be informed within 10 working days.

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval, but the IRB of each center must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB approval that can be treated as administrative amendments include:

1. Changes in the staff used to monitor studies (e.g., Amzell B.V. staff versus TKL), and
2. Minor changes in the packaging or labeling of the IP.

11.2 Deviations from the Protocol

Deviations from the planned study conduct are not permitted; any deviations and changes in study conduct must be reported to Amzell B.V., the IRB, and noted in the final clinical study report.

11.3 Premature Study Termination

Both Amzell B.V. and the Investigator (after consultation with Amzell B.V.) reserve the right to terminate or suspend the study at any time. Should this be necessary, the procedures will be arranged on an individual study basis after review and consultation by both parties. It is the responsibility of the Investigator to notify the IRB of the termination/suspension and the reason(s). In terminating the study, Amzell B.V. and the Investigator will ensure that adequate consideration is given to the protection of the subjects' interests.

12. REPORTING AND PUBLICATION

12.1 Clinical Study Report

The data and information collected during this study will be reported in a Clinical Study Report prepared by the TKL.

12.2 Confidentiality and Ownership of Study Data

Any confidential information relating to the IMP or the Study, including any data and results from the study will be the exclusive property of Amzell B.V. The Investigator and any other persons involved in the study will protect the confidentiality of this proprietary information belonging to Amzell B.V.

12.3 Publication Policy

No presentation or publication of data from this study can be initiated without the explicit written consent and in direct collaboration with Amzell B.V.

13. ETHICAL AND REGULATORY ASPECTS

13.1 Institutional Review Board (IRB)

Before implementing this study, the protocol, the proposed ICF and other information to subjects, must be reviewed by a properly constituted IRB. A signed and dated statement that the protocol and informed consent have been approved by the IRB must be given to TKL and Amzell B.V. before study initiation. The name and occupation of the chairman and the members of the IRB must also be supplied to TKL and Amzell B.V. This committee must also approve any amendments to the protocol, other than administrative ones, and a signed and dated statement of approval must be sent to Amzell B.V. prior to initiation of the amendment procedures.

13.2 Ethical Conduct of the Study

This study must be carried out in compliance with the protocol and in accordance with TKL's standard operating procedures (SOPs). These are designed to ensure adherence to good clinical practice (GCP) guidelines, as described in:

- International Conference on Harmonisation (ICH) Tripartite Guidelines for GCP 1996. Directive 91/507/, The Rules Governing Medicinal Products in the European Community.
- United States 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB.
- Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964 and amendments).

The Investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of GCP that it conforms to.

13.3 Subject Information and Consent

The Investigator or designee must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved, and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary, that he/she may withdraw from the study at any time, and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and he/she should be given a copy of the signed document. If written consent is not possible, oral consent (assent) can be obtained, if witnessed by a signed statement from one or more people not involved in the study, mentioning why the subject was unable to sign the form.

No subject can enter the study before a dated informed consent has been obtained from him/her, or his/her legally authorized representative.

The ICF is considered to be part of the protocol and must be submitted by the Investigator with it for IRB approval. TKL will supply a proposed ICF to be approved by Amzell B.V. TKL's ICF contains all elements (information or statements), as per ICH E6(R2) section 4.8.10 and 21 CFR § 50.25[c], and is presented in a non-technical language that is understandable to the subject. Any changes to the proposed consent form suggested by the Investigator must be agreed to by TKL and Amzell B.V. before submission to the IRB, and a copy of the approved version must be provided to Amzell B.V. after IRB approval.

14. ARCHIVING

14.1 Trial Master File

TKL will archive the trial master file in accordance with ICH-GCP, applicable regulatory requirements and TKL SOP.

15. REFERENCES

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PROTOCOL CLARIFICATION LETTER (06-June-2023)

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 8 mL [7.4 g]) of AMZ001 (Diclofenac Sodium Gel 3.06%) and One Dose Level (32 g) of Voltaren Arthritis Pain Gel (Diclofenac Sodium Gel 1%) in Healthy Subjects

Amzell B.V. Protocol No. AMZ001-008
TKL STUDY NO. P1980822
Protocol Version: Final v1.0
Protocol Date: 23-March-2023

The purpose of this protocol clarification letter is to correct an error in Figure 1 of the protocol.

Dario Carrara, PhD
Chief Executive Officer

DocuSigned by:
Dario Carrara, PhD
Signature

6/9/2023
Date

Signer Name: Dario Carrara, PhD
Signing Reason: I approve this document
Signing Time: 6/9/2023 | 5:40:18 AM PDT
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Irina Krause, RN, MSN
Senior Director, Phase I Operations

DocuSigned by:
Irina Krause, RN, MSN
Signature

6/6/2023
Date

Signer Name: Irina Krause, RN, MSN
Signing Reason: I approve this document
Signing Time: 6/6/2023 | 11:23:04 AM EDT
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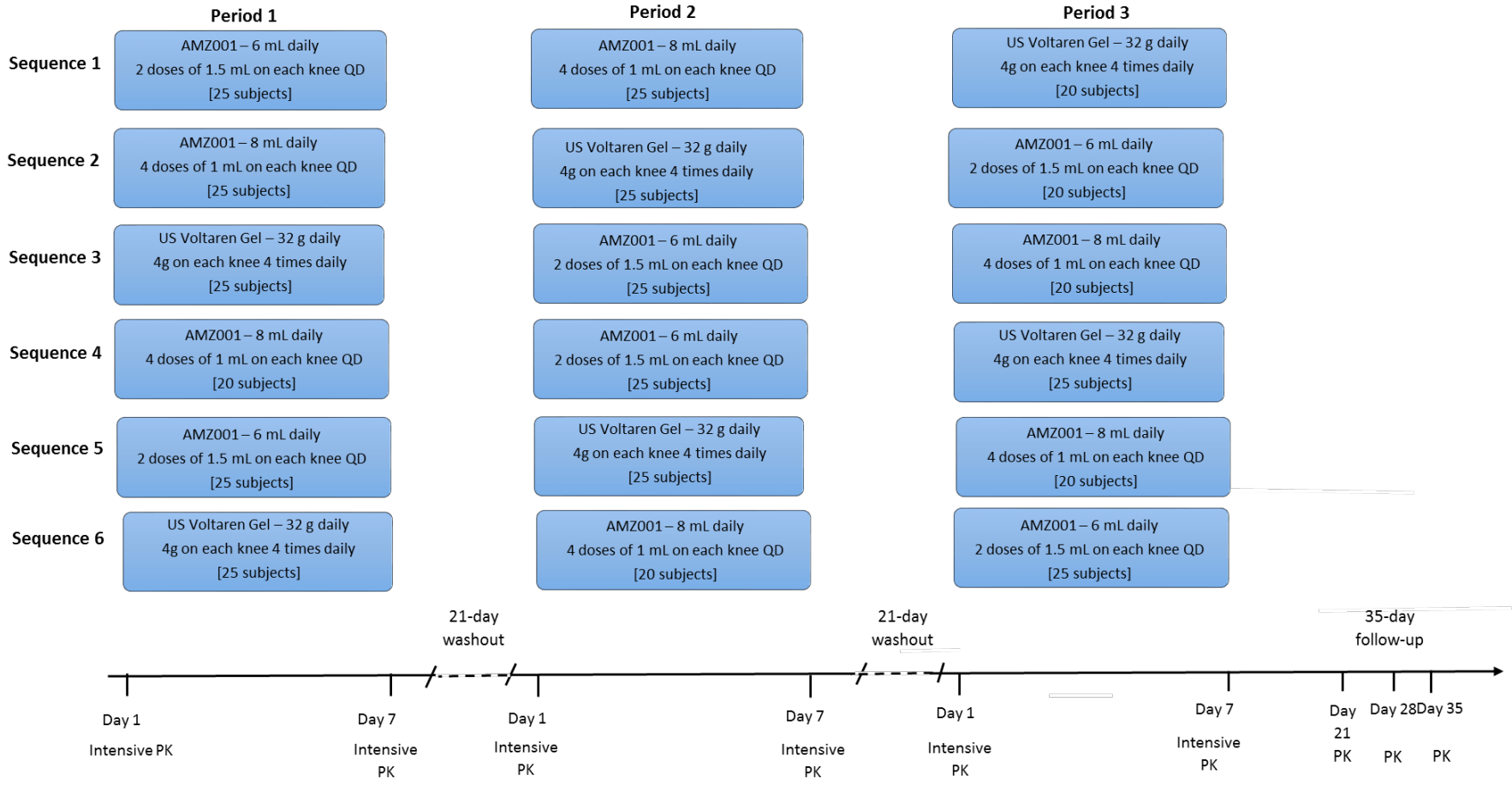
1. **To remove the subject numbers from Figure 1**

Figure 1: Study Design: Three Treatments, Three Period Crossover Design

Current text:



Revised text: