CLINICAL STUDY REPORT

Study Title:	A Randomized, Double-Blind, Placebo-Controlled, Multicenter, Proof-of-Concept, Phase 2a Study to Evaluate the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of Intravenous TAK-242 in Subjects With Acute Alcoholic Hepatitis Causing Decompensation of Alcohol-related Cirrhosis and Acute-on-Chronic Liver Failure
Investigational Product:	TAK-242
Indication Studied:	Acute-on-chronic liver failure
Description of Study:	This was a phase 2a double-blind, randomized, placebo-controlled, multicenter, proof-of-concept study to evaluate the efficacy, safety, pharmacokinetics, and pharmacodynamics of TAK-242 in subjects with acute decompensation of alcohol-related cirrhosis due to alcoholic hepatitis resulting in acute-on-chronic liver failure.
Name of Sponsor:	Akaza Bioscience Limited
Protocol Number:	TAK-242-2001
Trial Registry Number(s):	EudraCT Number: 2019-001969-33
Development Phase:	2a
First Subject Enrolled:	12 November 2020
Date of Early Termination:	31 March 2022
Last Subject Completed:	14 July 2021
Coordinating Investigator:	**United Kingdom**: Dr. Ryder, MRCP, FRCP, DM, CSST **France**: Dr. Bonadona, DM **Germany**: Dr. med. Lange (from 28 April 2020 till 03 August 2021), DM; Prof. Dr. med. Trebicka (from 03 August 2021), DM, DAEM/DGEM **Hungary**: N/A
Sponsor Signatory:	Someit Sidhu – Akaza Chief Executive Officer
GCP Compliance:	This study was performed in compliance with International Council for Harmonisation (ICH) Good Clinical Practices (GCP), including the archiving of essential documents as well as the ethical principles of the Declaration of Helsinki.
Report Version and Date:	Final, 07 July 2023

2.0 SYNOPSIS

Name of Sponsor/Company: Akaza Bioscience Limited

Name of Active Ingredient: TAK-242

Title of Study:

A Randomized, Double-Blind, Placebo-Controlled, Multicenter, Proof-of-Concept, Phase 2a Study to Evaluate the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of Intravenous TAK-242 in Subjects With Acute Alcoholic Hepatitis Causing Decompensation of Alcohol-related Cirrhosis and Acute-on-Chronic Liver Failure

Investigators:

2 investigators at study centers that consented at least 1 subject.

Study Centers:

2 study centers in 1 country (United Kingdom) consented at least 1 subject.

Publication (Reference):

None

Study Period:	**Phase of Development:**
First subject enrolled: 12 November 2020 Last subject completed: 14 July 2021 Date of early termination: 31 March 2022	2a

Background and Rationale for the Study:

Acute-on-chronic liver failure (ACLF) is a syndrome characterized by acute deterioration of existing chronic liver disease progressing to organ failure and is associated with a high mortality.[1]

Acute-on-chronic liver failure can be graded according to the severity of the condition and the number of organ failures and has an estimated prevalence in the European Union (EU) of 2.35 cases per 10,000 of the population. It usually results after a precipitating event that may directly exaggerate liver injury such as alcoholic hepatitis, drug-induced liver injury, superimposed viral hepatitis, portal vein thrombosis, and ischemic hepatitis; or liver decompensation may result after extra-hepatic insults such as trauma, surgery, variceal bleeding, or infection.[2] In one study, approximately 43% of patients had no identifiable precipitating event.[3]

There are no approved treatments for the condition. Management of ACLF focuses on rapid treatment of the precipitating event when this can be identified and the delivery of supportive care that addresses all aspects of evolving or established multiple organ systems failure. Clinical management includes rapid restoration of metabolic and hemodynamic stability and provision of nutritional support, in tandem with organ-specific supportive care.[4] In many patients, such an approach will result in resolution of organ failure and hepatic stabilization. Nevertheless, the 28-day mortality in patients presenting with ACLF has been estimated to be approximately 34%.[3]

TAK-242 is a novel small molecule selective inhibitor of toll-like receptor (TLR)-4-mediated signaling that exerts its actions by covalently binding to a specific cysteine, Cys747, in the intracellular Toll/IL-1 receptor domain of TLR-4. A number of nonclinical studies and clinical studies in healthy subjects have been performed to evaluate TAK-242. These studies have shown that TAK-242 has an inhibitory effect on multiple pro-inflammatory cytokines and acts by inhibiting TLR-4-mediated signaling. As TLR-4 signaling is central to regulation of the inflammatory response in ACLF, it was anticipated that TAK-242 would provide clinical

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benefit to patients with ACLF. This was the first study involving administration of TAK-242 to subjects with alcoholic cirrhosis and either Grade 1 or 2 ACLF to investigate the efficacy, safety, pharmacokinetics (PK), and pharmacodynamics of TAK-242.

Objectives and Endpoints:

Primary Objective

The primary objective of this study was to investigate the effect of intravenous (IV) TAK-242 administered for 7 days in subjects with cirrhosis due to alcoholic liver disease and superimposed alcoholic hepatitis (AH) resulting in Grade 1 or 2 ACLF on the Chronic Liver Failure Consortium (CLIF-C) ACLF score.

Secondary Objectives

The secondary objectives of the study were:

- To investigate the safety of IV TAK-242 administered for 7 days in subjects with Grade 1 or 2 ACLF.
- To investigate the effects of IV TAK-242 administered for 7 days in subjects with Grade 1 or 2 ACLF on key biomarkers including interleukin (IL)-8, c-reactive protein (CRP), total bilirubin (TB), and urine neutrophil gelatinase-associated lipocalin (NGAL).
- To investigate the effect of IV TAK-242 administered for 7 days in subjects with Grade 1 or 2 ACLF on Day 28 survival.

Additional Objectives

The additional objectives of the study were:

- To investigate the effect of IV TAK-242 administered for 7 days in subjects with Grade 1 or 2 ACLF on organ function (hepatic, renal, brain, coagulation, respiratory, cardiovascular).
- To investigate the effect of IV TAK-242 administered for 7 days in subjects with Grade 1 or 2 ACLF on clinical outcomes.
- To investigate the effect of IV TAK-242 administered for 7 days in subjects with Grade 1 or 2 ACLF on biomarkers of immune function and inflammation.
- To characterize the plasma pharmacokinetics, and pharmacodynamics of IV TAK-242 administered for 7 days in subjects with Grade 1 or 2 ACLF.

Exploratory Objective

The exploratory objective of the study was to investigate the effect of IV TAK-242 administered for 7 days on additional pro-inflammatory biomarkers in whole blood, peripheral blood mononuclear cells, and neutrophils (optional).

Endpoints:

Primary Endpoint

The primary endpoint of the study was the change in CLIF-C ACLF score from baseline to Day 8.

Secondary Endpoints

The secondary endpoints of the study were:

- The percentage of subjects who experienced at least 1 treatment-emergent adverse event (TEAE) or serious adverse event that met the Akaza Bioscience Limited markedly abnormal criteria.

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- The percentage of subjects who experienced at least 1 treatment-emergent clinical laboratory test result or abnormal electrocardiogram that met the Akaza Bioscience Limited markedly abnormal criteria.
- The percentage of subjects who discontinued the study drug due to an adverse event (AE) (including methemoglobinemia).
- Change in naturally log-transformed key biomarkers (TB, IL-8, high sensitivity CRP, and urinary NGAL) from baseline to Day 8.
- Survival at Day 28 after the initiation of TAK-242 therapy versus placebo.

Additional Endpoints

The additional endpoints of the study were:

- Plasma maximum concentration and average concentration of TAK-242 and metabolites.
- Daily assessments of organ failure, systemic inflammation, and ACLF including CLIF-C organ failure score (CLIF-C OF), CLIF-C acute decompensation score (CLIF-C AD), CLIF-C ACLF score and Systemic Inflammatory Response Score.
- Liver: daily assessment of liver scores including model for end-stage liver disease (MELD) score, MELD-sodium, Child Turcotte Pugh, and Maddrey Discriminant Function score.
- Brain: clinical grading (daily; West Haven criteria); electroencephalogram could be used to resolve uncertainty about the presence of covert encephalopathy if available.
- Kidney: Change in acute kidney injury (AKI) stage from baseline to Day 8.
- Number of days in intensive care/intensive therapy unit.
- Liver histopathology and immunohistochemistry, when available.
- Change in inflammatory markers and ACLF-related panel including, but not limited to, IL-6, TNF-α, IL-10, and M65 from baseline to Day 8.

Exploratory Endpoints

The exploratory endpoint of the study was the change in ex-vivo whole blood responses (whole blood lipopolysaccharide-triggered pro-inflammatory responses) from baseline to Day 8.

Study Design:

This was a phase 2a double-blind, randomized, placebo-controlled, multicenter, proof-of-concept study to evaluate the efficacy, safety, PK, and pharmacodynamics of TAK-242 in subjects with acute decompensation of alcohol-related cirrhosis due to AH resulting in ACLF.

The study population was planned to consist of up to 100 subjects with Grade 1 or 2 ACLF. Men and women ≥18 and <75 years of age with AH and continued heavy alcohol use for >6 months (defined as >40 g alcohol/day in women and >60 g/day in men);[5,6] with <60 days abstinence before the onset of jaundice; and recent history (within 6 weeks before screening) of acute decompensation (including, but not limited to, jaundice, ascites, gastrointestinal bleeding, hepatic encephalopathy and/or acute bacterial infections); with or without a defined precipitating illness, were recruited. A liver biopsy was not required before entry into the study, but when conducted as part of a site's usual care, subject liver biopsy data were collected in study records.

Subjects were hospitalized for at least 8 days during screening and the treatment period. After admission to the hospital and during a 2-day screening period, subject assessment included diagnostic tests for infection and response to supportive care. The study consisted of a 2-day (48 hours) screening period (Days -2 to -1) with

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baseline assessment of inclusion/exclusion criteria after which eligible subjects were randomized (Day 1) to receive study drugs by constant IV infusion for 7 days (Days 1 to 7) and assessed on Day 8. Follow-up assessments were performed at approximately 4, 8, and 12 weeks (Days 28±5, 56±6, and 84±7). The total duration of subject participation in the study was to be 86±7 days.

Number of Subjects (Planned and Analyzed):

A total of 60 to 100 subjects were to be enrolled, 4 subjects consented to participate, and 3 subjects were randomized.

A total of 3 subjects were treated, and 1 subject completed the study.

Diagnosis and Main Criteria for Inclusion and Exclusion:

Inclusion

Adult men and women subjects ≥18 and <75 years of age with a history of alcohol-related cirrhosis who continued to drink heavily, had history of an acute decompensating event with a clinical and/or liver biopsy diagnosis of alcoholic hepatitis. Eligible subjects had to meet the definition of Grade 1 or 2 ACLF using the CLIF-C OF score; or bilirubin criteria (>20 mg/dL at diagnosis); or criteria of AKI Stage 1b or 2 after initial supportive treatment with fluids, albumin, or terlipressin; and the CLIF-C ACLF score was >35 and <64, calculated within 16 hours prior to randomization.

Exclusion

Subjects were not eligible if they had received certain previous therapies (any investigational drug within 30 days of randomization, corticosteroids for alcohol-induced liver failure within 4 weeks of randomization, or received TAK-242 in any previous study) or if they had any of the following conditions:

- Liver cirrhosis from other chronic diseases, liver failure from other causes, a history of liver transplantation, post-operative decompensation after partial hepatectomy, acute or subacute liver failure without underlying cirrhosis.
- Any untreated infections including gram-positive infections, or active or latent tuberculosis, sepsis or septic shock, or coinfection with hepatitis B virus, hepatitis C virus, hepatitis E virus, or human immunodeficiency virus.
- Chronic or pre-existing kidney failure, uncontrolled medical disorder that might confound study results or compromise subject safety, oxygen saturation <90%, or requires mechanical ventilation.
- Uncorrected anemia, methemoglobinemia, disseminated intravascular coagulation, significant or uncontrolled bleeding, atypical laboratory screening tests.
- Uncontrolled seizures, Grade 3 or 4 hepatic encephalopathy, Creutzfeldt-Jakob disease, glucose-6-phosphate dehydrogenase deficiency.
- Active extra-hepatic malignancy or survival prognosis of <6 months.

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Test Product, Dose, and Mode of Administration, Batch Number:

Study drugs (TAK-242 or matching placebo) were administered as a continuous IV infusion with standard care starting with a loading dose of 0.9 mg/kg administered over 30 minutes, followed by a continuous, constant rate infusion of 1.8 mg/kg/day for 7 days.

Batch number(s):

- TAK-242 FGU305172
- GLUCOSE FGU305456/ FGU305249
- INTRALIPID FGU305457/ FGU305250

Duration of Treatment:

The total duration of subject participation in the study was to be 86±7 days.
This study was terminated early after a study hold period due to the shelf life of the clinical trial batch of TAK-242. It was extended based on the data available at that time from the 28 February 2021 to the 30 August 2021. Routine testing of the drug in Japan discovered an out of trend analysis, although the IMP was still within specification. The Sponsor was made aware of these findings on 18 May 2021 by telephone. After further discussion with the manufacturer, the decision was taken to put the study on temporary hold and to quarantine the clinical trial batch on 20 May 2021.

Subsequently, the manufacturer has assigned an expiry date of 30 April 2021 to the clinical trial batch of IMP. The decision to terminate the study was taken by sponsor on 24th March 2022 based on collective inability to complete this trial without significant changes to the previously jointly pre agreed protocol.

Statistical Methods:

Sample Size Justification:

The planned sample size estimation was based on a Bayesian decision method with predefined criteria.

Statistical Considerations:

No statistical analyses were performed due to the early termination of this study. For a description of planned analyses, please refer to Section 16.1.1.

Summary – Conclusions:

Safety Results:

Incidence of TEAEs by Relationship to Study Drug

For the 3 subjects in the study, there was a total of 34 AEs reported of which only 1 AE was considered by the investigator to be related to study drug. For subject UK001-001, the infusion caused an adverse reaction that forced the subject to have to utilize the commode in between the loading and maintenance dose causing an IMP dose 23-minute interruption.

Incidence of TEAEs by Severity

There was a total of 34 AEs reported for the 3 subjects. Most AEs were reported as mild or moderate in severity. There was a total of 8 severe AEs reported. Details of the severe AEs reported for the study subjects are as follows:

- Subject UK001-001: Severe AEs (2) consisted of acute kidney injury 3 and multi-organ failure.
- Subject UK004-001: Severe AEs (6) consisted of gastrointestinal bleeding, myocardial infarction, multi-organ failure, hypoxia, fluid overload, and worsening acute kidney injury.

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- No severe AEs were reported for Subject UK004-003.

Deaths

Two subjects died during the treatment period. No death was considered to be related to study drug. Details of the two subject deaths are as follows:

- Subject UK001-001 experienced 1 severe AE (multi-organ failure), which was not related to study treatment, reported on 22Apr2021. This AE was ultimately fatal, and the subject's death was reported on 25Apr2021.
- Subject UK004-001 experienced 2 severe AEs (worsening acute kidney injury and multi-organ failure), which were not related to study treatment, reported on 14Nov2020 and 25Dec2020, respectively. Both AEs were fatal, and the subject's death was reported on 25Dec2020.

Conclusion:

Due to the small sample size and early termination of the study, no conclusions can be drawn regarding safety of TAK-242.

Date and Version of Report:

Final, 07 July 2023