CLINICAL TRIAL PROTOCOL

Document Number:		**c33415518-04**
BI Trial No.	1123-0040	
BI Investigational Medicinal Product	Metalyse®, tenecteplase	
Title	A phase III multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group trial to assess efficacy and safety of tenecteplase versus alteplase in Chinese patients with acute ischaemic stroke within 4.5 hours after stroke onset	
Lay Title	A study in Chinese patients to compare how tenecteplase and alteplase given after a stroke improve recovering of physical activity	
Clinical Phase	III	
Clinical Trial Leader	[redacted] Phone : [redacted] Fax : [redacted]	
[redacted] **Investigator**	[redacted] Tel: [redacted] Fax: [redacted]	
Current Version and Date	**Version 4.0**	**23 Feb 2023**
Original Protocol Date	**Version 1.0**	**09 Sep 2020**

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Original Protocol date	09 Sep 2020
Revision date	23 Feb 2023
BI trial number	1123-0040
Title of trial	A phase III multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group trial to assess efficacy and safety of tenecteplase versus alteplase in Chinese patients with acute ischaemic stroke within 4.5 hours after stroke onset
[redacted] **investigator**	[redacted]
Trial sites	Multi-centre study conducted in China (at neurology clinics or stroke units and with 24 hours [h] access to adequate imaging scanner and laboratory testing) Approximately 55 sites
Clinical phase	III
Trial rationale	This trial is a phase III study to determine whether tenecteplase 0.25 mg/kg is non-inferior to alteplase 0.9 mg/kg for thrombolysis in AIS patients within 4.5 h after stroke onset in the Chinese population, thus providing more treatment options for acute ischaemic stroke (AIS) patients in China.
Trial objectives	• To assess whether tenecteplase is non-inferior to alteplase in favourable outcome (modified Rankin scale achieving 0 or 1 [mRS0-1]) in Chinese patients with AIS who are eligible for intravenous (iv) thrombolysis within 4.5 h of symptom onset • To assess the safety of tenecteplase in AIS patients • To assess the efficacy of tenecteplase in other key functional outcomes
Trial endpoints	Primary Efficacy Endpoint • mRS score of 0 or 1 at day 90 Secondary Efficacy Endpoint • Major neurological improvement at 24 h (National Institute of Health Stroke Scale [NIHSS] score of 0 or improvement of at least 4 points compared with baseline) • mRS score of 0-2 at day 90

	• Change from baseline of NIHSS score at day 90 • Distribution of mRS at day 90 • Barthel Index score ≥95 at day 90 Secondary Safety Endpoint • Symptomatic intracranial haemorrhage (sICH) per ECASS III definition • 90-day mortality • mRS score of 5 or 6 at day 90
Trial design	Multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group phase III trial
Total number of patients randomised	Approximately 1478
Number of patients on each treatment	Approximately 739
Diagnosis	AIS within 4.5 h after stroke onset
Main in- and exclusion criteria	Main inclusion criteria: 1. Age ≥18 years old 2. Diagnosis of ischaemic stroke with a measurable neurological deficit on NIHSS (0< NIHSS ≤25); if the total NIHSS <4, patients have to be with at least a measurable deficit on motor function (upper or lower limbs ≥1). 3. Stroke symptoms should have been presented for at least 30 min without significant improvement prior to screening 4. Thrombolytic therapy can be initiated within 4.5 h of AIS onset 5. Patients with premorbid mRS 0 or 1 6. Signed and dated written informed consent in accordance with good clinical practice (GCP) and local legislation prior to admission to the trial Main exclusion criteria: 1. Evidence of intracranial haemorrhage on the computed tomography (CT) scan or symptoms suggestive of subarachnoid haemorrhage, even if the CT scan is normal 2. Use restricted medications or any drug considered likely to interfere with the safe conduct of the trial 3. Acute bleeding diathesis 4. Bacterial endocarditis, pericarditis 5. Acute pancreatitis

	6. Significant trauma or major surgery (according to the investigator's assessment) in the past 3 months (m) 7. Imaging demonstrates multi-lobar infarction 8. Severe uncontrolled arterial hypertension 9. Blood glucose <50 mg/dL at screening 10. Seizure at stroke onset 11. Known hypersensitivity to active substance alteplase or tenecteplase, gentamicin or to any of the excipients 12. Currently enrolled in another investigational device or drug study, or <30 days since ending another investigational device or drug study(s), or receiving other investigational treatment(s) 13. Women who are pregnant 14. Patient has a confirmed active infection with SARS-CoV-2 in medical history within the prior 3 m, based on which investigator considers likely to interfere with the safe conduct or the trial evaluation, procedures, or completion 15. Any other conditions that, in the opinion of the investigator or BI physician, if consulted, would pose a risk to subject safety or interfere with the trial evaluation, procedures or completion
Test product	Metalyse®, tenecteplase (TNK-tPA)
dose	Total single dose will be 0.25 mg/kg. The upper dose limit is set to 25 mg/patient
mode of administration	iv bolus over 5-10 seconds
Comparator product	Actilyse®, alteplase (rt-PA)
dose	Total single dose will be 0.9 mg/kg. The upper dose limit is set to 90 mg.
mode of administration	10% of the total dose administered as an initial iv bolus and remaining 90% of the total dose administered as an iv infusion over 1 h
Duration of treatment	Tenecteplase: single bolus over 5-10 seconds Alteplase: single bolus immediately followed by 1 h infusion
Statistical methods	Non-inferiority between tenecteplase and alteplase will be declared if the relevant confidence interval (CI) for risk ratio in mRS 0-1 response rate at day 90 is within the predefined regions of (0.937, $+\infty$). Based on the log binominal regression model, the risk ratio with corresponding 95% CI adjusted for the continuous covariates of

	NIHSS at baseline, age and time to drug administration will be calculated as the primary analysis. After the demonstration of non-inferiority, the superiority between tenecteplase and alteplase will be declared in a hierarchical manner with overall type I error control if the relevant CI for risk ratio in mRS0-1 response rate at day 90 is within the predefined regions of $(1, +\infty)$. All safety data in this study will be descriptively summarised.

FLOW CHART

Trial periods	**Screening**	**Randomisation**	**Follow up**					**EoS**[9]
Visit	**1**	**2a**	**2b**	**2c**	**3**	**4**	**5**[10]	**6**[10] **EoS**
	≤4.5 h	**D 1**	**1 h after start of admin**	**2 h after start of admin**	**D 2** 24 h	**D 8** 7 d/ discharge date	**30 d**	**90 d**
Time window for visits	none	none	±15 min	±30 min	±2 h	±2 d	-2 d or +7 d	±7 d
Informed consent[1]	X							
IRT	X							
Demographics	X							
Baseline conditions/ Medical history	X							
Non-contrast CT	X[2]				X[3]	(X)[4]	(X)[4]	
Physical exam	X[5]				X			
Vital signs	X	X	X	X	X	X	X[12]	X[12]
In-/Exclusion criteria	X							
Trial drug admin		X						
Lab test	X[6]				(X)[7]	X		(X)[7]
Pregnancy test	X[8]							(X)[7]
Concomitant therapy	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X
12-Lead ECG	X					X		
NIHSS[11]	X			X	X	X	X	X
Glasgow outcome score								X
Modified Rankin Scale	X (pre-stroke)						X	X
Barthel Index							X	X

Admin, administration; CT, computed tomography; D, day; ECG, electrocardiograms; EoS, end of study; h, hour(s); IMP, investigational medicinal product; NIHSS, National Institute of Health Stroke Scale

1. Informed consent needs to be signed before start of any procedure related to the study. In case subject cannot read the ICF by himself/herself, an impartial witness will be needed to attest the informed consent process. When it is signed, all AEs and concomitant treatment occurring after informed consent have to be recorded. Procedures which are performed as part of routine clinical care prior to obtaining the informed consent could be used for screening if they are within the allowed time window (after stroke onset and before drug administration, to be considered by investigator).
2. Non-contrast CT is mandatory before alteplase or tenecteplase administration to exclude intracranial haemorrhage (absolute contraindication) or mimic stroke (e.g. cerebral tumour) and to determine whether CT hypo-density of ischaemia is present. MRI is also acceptable.

   Investigator could make decision whether or not an imaging performed outside the site could be used for evaluation of inclusion/exclusion criteria. This evaluation should also include time elapsed from imaging and possible changes in clinical symptoms since imaging.
3. Non-contrast CT: between 22 and 36 h after starting the trial medication. MRI is also acceptable.
4. Optional in case of clinical deterioration (as determined by investigator)
5. Measurement of body weight and height is only required at screening visit. If it is hard to have an actual body weight/height due to certain reasons, an estimated body weight/height by investigator is acceptable.
6. For lab results used for eligibility evaluation, only the result of random glucose (POCT) is mandatory before drug administration. Hematology lab results including coagulation items and platelet count are necessary before drug administration only when

anticoagulants are used or there is suspicion of coagulopathy (as determined by investigator). Drug administration should not be delayed by other lab results.

7. Optional in case of clinical deterioration or clinical demand (as determined by investigator)
8. For women who are considered of childbearing potential (WOCBP), a urine pregnancy test is required at screening or at earliest possible; the test result should not delay the drug administration.
9. Trial completion: At the end of Visit 6 (D 90) for patients who will have completed the trial on treatment and completed V6 as planned
10. Remote visits will be acceptable based on especial conditions:
    - It's hard to perform the follow up on-site visit due to subject bad condition or other situations that on-site visit is restricted/not doable, determined by investigator.
    - Under pandemic of Covid-19 that the follow-up visits to the sites should be replaced with remote visits, shall be align with local /site related governance.
11. The distal motor function will be assessed when NIHSS is scored
12. The vital sign could be an optional item in case remote visit is performed.

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ABBREVIATIONS

AE	Adverse event
AESI	Adverse event of special interest
AHA	American Health Association
AIS	Acute ischaemic stroke
ALCOA	Attributable, legible, contemporaneous, original, accurate
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMI	Acute myocardial infarction
aPTT	Activated partial thromboplastin time
ASA	American Stroke Association
AST	Aspartate aminotransferase
BI	Boehringer Ingelheim
BP	Blood pressure
CA	Competent authority
CI	Confidence interval
CK	Creatine kinase
CRA	Clinical research associate
CRO	Contract research organisation
CT	Computed tomography
CTP	Clinical trial protocol
DBL	Database lock
DMC	Data Monitoring Committee
EAC	Endpoint Adjudication Committee
ECASS	European Cooperative Acute Stroke Study
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EMA	European Medical Agency
EoS	End of study
ES	Enrolled set

ESMINT	European Society for Minimally Invasive Neurological Therapy
ESO	European Stroke Organisation
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
GD	Gestational days
GMP	Good manufacturing practice
h	Hour(s)
HA	Health authority
Hb	Haemoglobin
HCT	Haematocrit
IB	Investigator's brochure
ICE	Intercurrent Events
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
INR	International normalised Ratio
IRB	Institutional Review Board
IRT	Interactive response technology
ISF	Investigator site file
iv	Intravenous
LDH	Lactate dehydrogenase
LOCF	Last observation carried forward
LPLT	Last patient last treatment
MedDRA	Medical Dictionary for Drug Regulatory Activities
min	Minute(s)
m	Month(s)
MRI	Magnetic resonance imaging
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
OPU	Operative unit

PAI-1	Plasminogen activator inhibitor 1
PK	Pharmacokinetic
POCT	Point-of-care testing
PPS	Per-protocol set
PROBE	Prospective, randomised, open label, blinded endpoint
PT	Preferred term
PT	Prothrombin time
RA	Regulatory authority
RBC	Red blood cell
rt-PA	Recombinant tissue plasminogen activator
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
sICH	Symptomatic intracranial haemorrhage
SITS-MOST	Safety Implementation of Thrombolysis in Stroke: Monitoring Study
SS	Safety set
SOC	System organ class
SOP	Standard operating procedure
STEMI	ST Elevation Myocardial Infarction
$t_{1/2}$	Half-life
t-PA	Tissue plasminogen activator
TSAP	Trial statistical analysis plan
vs	Versus
W	Week
WOCBP	Woman of childbearing potential

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

Stroke is one of the leading causes of death and disability worldwide. In 2016, stroke was the second leading cause of death globally (5.5 million deaths) and there were 13.7 million new stroke cases reported [R20-0900]. For more than a decade, stroke has been the leading cause of death in the Chinese population, responsible for an average of 1.96 million deaths/year [R19-3903] of which acute ischaemic stroke (AIS) accounts for 80% of the cases. As the highest single-disease disability rate, AIS brings enormous economic and social burden to the country [P19-03580] which further increases with the aging Chinese population.

Intravenous (iv) thrombolysis is of proven benefit for treatment of patients with AIS within 4.5 hours (h) of symptom onset, and alteplase is currently the standard of care for this indication. Alteplase has a short half-life ($t_{1/2}$) which requires a 60-minute (min) infusion.

Tenecteplase is a genetically engineered variant of alteplase. Tenecteplase is currently approved to treat acute myocardial infarction (AMI) in more than 90 countries around the world. Tenecteplase for AMI indication was approved in the United States in 2000 and in the European Union in 2001.

Tenecteplase is a promising thrombolytic agent for AIS. There is no completed registration study of tenecteplase in AIS worldwide. Several phase II and III studies initiated by investigators provided information on the efficacy and safety in patients with AIS. Additionally, the 2018 American Heart Association/American Stroke Association (AHA/ASA) Guidelines for the Early Management of Patients with AIS and 2018 China Guidelines for the Diagnosis and Treatment of AIS recommended that tenecteplase could be considered as an alternative to alteplase in selected AIS patients with minor neurological impairment and no major intracranial occlusion (Grade II recommendation, Grade B evidence) [P19-03892, P19-10855]. The 2019 European Stroke Organisation (ESO) - European Society for Minimally Invasive Neurological Therapy (ESMINT) guideline stated, “In large vessel occlusion-related ischaemic stroke patients eligible for iv thrombolysis before mechanical thrombectomy, 7 out of 11 experts suggest the use of tenecteplase (0.25 mg/kg) over alteplase (0.9 mg/kg), if the decision on iv thrombolysis is made after vessel occlusion status is known.” [P19-02504].

1.2 DRUG PROFILE

Mode of action

Tissue plasminogen activator (t-PA) is a serine protease which is pharmacologically classified as a thrombolytic enzyme. It converts plasminogen into plasmin, a circulating plasma protein that binds to and digests the solid-phase fibrin component of a thrombus, and thereby restores the vessel patency. The digestion of fibrin, or fibrinolysis, is regulated by the inhibitors α_2-antiplasmin and plasminogen activator inhibitor 1 (PAI-1).

Alteplase is a single-chain recombinant tissue plasminogen activator (rt-PA), with a property of fibrin-enhanced conversion of plasminogen to plasmin. Tenecteplase is a target-modified variant of alteplase with identical mechanism of action [P03-02906]. The modifications at three molecular sites allow tenecteplase to have increased fibrin specificity, reduced binding affinity to PAI-1, and prolonged plasma $t_{1/2}$.

Key pharmacokinetic (PK) characteristics

The PK data of tenecteplase is available in patients with AMI. In the phase I (TIMI 10A) study, PK parameters of tenecteplase administered as a single iv bolus were obtained over a wide dose-range of fixed doses of 5-50 mg in patients with AMI [P03-02906]. The clearance values decreased from 216 mL/min at the 5 mg dose group to 125 mL/min at the 50 mg dose group, indicating non-linear PK. In the phase II PK study (TIMI 10B), tenecteplase administered as a single bolus injection exhibits a biphasic disposition from the plasma [P03-02906]. Tenecteplase was cleared from the plasma with an initial $t_{1/2}$ of 20-24 min. The terminal phase $t_{1/2}$ of tenecteplase was 90-130 min. In 99 out of 104 patients treated with tenecteplase, mean plasma clearance had a range of 99-119 mL/min.

Limited PK data is available in patients with AIS [P05-02918], and the PK profile is not expected to be different from patients with AMI. A pilot dose-escalation safety study of tenecteplase in patients with AIS demonstrated safety and tolerability of tenecteplase with the dose of 0.1-0.4 mg/kg.

[redacted]

Clinically relevant differences in the PK of tenecteplase in comparison to alteplase due to race/ethnicity are not expected

Drug interactions

The interaction of a single iv bolus dose of tenecteplase with standard adjunctive therapies of acetyl salicylic acid and heparin was evaluated in dogs. Co-administration of acetyl salicylic acid and heparin did not potentiate the effect of tenecteplase on indices of blood coagulation, cause any additional toxicity, or affect the disposition of tenecteplase.

No formal interaction studies with alteplase have been performed.

Residual Effect Period

REP is defined as the number of days after the last trial medication administration for evaluating the causality of AEs. Although $t_{1/2}$ of tenecteplase and alteplase are quite short, the

REP is pharmacologically set to 7 days since symptoms of internal bleeds such as ICH may be delayed post lysis.

Data from non-clinical studies

Single-dose toxicology studies of tenecteplase were conducted in rats, rabbits and dogs with no unexpected toxicities identified. In general, tenecteplase was well tolerated with findings mainly restricted to the expected pharmacologic effects on the blood coagulation system and related sequelae. Daily iv doses of tenecteplase of 0.5, 1.5, or 5 mg/kg/day did not impact reproductive success when administered to rabbits during gestational days (GDs) 6-10. However, maternal mortality was observed in nearly all rabbits at all doses tested when administered during GDs 11-14 or 15-18.

In sub-chronic toxicity studies of alteplase in rats and marmosets, no unexpected undesirable effects were found. No indications of a mutagenic potential were found in mutagenic tests. In pregnant animals no teratogenic effects were observed after iv infusion of pharmacologically effective doses. In rabbits, embryotoxicity (embryolethality, growth retardation) was induced by >3 mg/kg/day. No effects on peri-postnatal development or on fertility parameters were observed in rats with doses up to 10 mg/kg/day.

Data from clinical studies

Alteplase

To date, alteplase is still the only licensed thrombolytic agent for AIS; a dose of 0.9 mg/kg is administered starting with an iv bolus of 10% of dose, immediately followed by iv infusion of the remaining of the dose over 60 min, maximum 90 mg, to thrombolysis eligible patients. The iv alteplase is proven clinical benefit for patients with AIS within 4.5 h of the onset of symptoms. In China, alteplase was approved for the treatment of AIS up to 3 h after onset, extended to within 4.5 h of the onset of symptoms on November 2019.

Tenecteplase

No prior Genentech/BI sponsored study of tenecteplase has been completed in AIS.

A meta-analysis of head-to-head trials between tenecteplase and alteplase in AIS was conducted [P19-06342]. A total of 5 trials enrolling 1585 patients (828 tenecteplase and 757 alteplase) included in the systematic analysis. For the primary endpoint, crude cumulative rates of disability-free (mRS score 0-1) at 3 months (m) were tenecteplase 57.9% vs alteplase 55.4%. The risk difference between the tenecteplase group and the alteplase group was 4% (95% CI -1%, 8%, 5 trials, n=1585). For the secondary efficacy endpoints, the rate of functional independence (mRS 0-2) at 3 months was 71.9% after tenecteplase and 70.5% after alteplase with a risk difference of 8% (95% CI -4%, 20%; 4 trials, n=1473). For safety endpoints, mortality and sICH rates did not differ between tenecteplase and alteplase.

For a more detailed description of the tenecteplase, please refer to the current investigator's brochure (IB) [c30996666], and for alteplase, besides the IB [U12-2040], the approved patient information leaflet by National Medical Product Administration.

1.3 RATIONALE FOR PERFORMING THE TRIAL

Ischaemic stroke is a major public health problem in the world. In China, stroke has become the leading fatal disease, with ischaemic stroke being the dominant type. Globally, the burden of stroke is still increasing. Stroke-care quality improvement should be a continuous effort.

Intravenous thrombolysis is of proven benefit for treatment of patients with AIS within 4.5 h of symptom onset, and alteplase is currently the standard of care for this indication. Alteplase has a short $t_{1/2}$ which requires a 60-min infusion.

Tenecteplase is a genetically engineered variant of alteplase. Owing to its longer $t_{1/2}$, it can be administered as a single iv bolus, which is more convenient compared to bolus immediately followed by infusion. Additionally, tenecteplase demonstrates 10-14 times greater fibrin-specificity than alteplase, which means that more fibrinogen will be available in human body and this potentially leads to lower risk of bleeding. The use of tenecteplase has been approved for the treatment of ST-elevation myocardial infarction (STEMI) in more than ninety countries. It has shown clinical equivalence and fewer systemic bleeding complications compared with alteplase in STEMI patients [P99-02520].

Tenecteplase is a promising thrombolytic agent for AIS due to its modified pharmacodynamics properties. In recent years, several phase II and phase III trials have investigated the efficacy and safety of tenecteplase for the treatment of AIS [P12-03304, P18-01526]. In 2019, a meta-analysis of 5 randomised trials was published, including 1585 patients in total (828 tenecteplase, 757 alteplase) [P19-06342]. It showed that the 3-m mRS 0-1 was 57.9% in the tenecteplase group, and 55.4% in the alteplase group, with a relative rate difference of 4% (95% CI -1%, 8%). In terms of safety endpoints, this meta-analysis concluded that tenecteplase and alteplase demonstrated a risk difference of 0% in sICH (95% CI -1%, 2%) and 3-m mortality (95% CI -3%, 2%).

The findings from the accumulated clinical trial data provide support for recent guidelines and recommendations. Both 2018 AHA/ASA Guidelines for the Early Management of Patients with AIS and 2018 China Guidelines for the Diagnosis and Treatment of AIS recommended that tenecteplase could be considered as an alternative to alteplase in selected patients with AIS with minor neurological impairment and no major intracranial occlusion (Grade II recommendation, Grade B evidence) [P19-03892, P19-10855]. The 2019 EU ESO ESMINT guideline states, "In large vessel occlusion-related ischaemic stroke patients eligible for iv thrombolysis before mechanical thrombectomy, 7 out of 11 experts suggest the use of tenecteplase (0.25 mg/kg) over alteplase (0.9 mg/kg), if the decision on iv thrombolysis is made after vessel occlusion status is known." [P19-02504].

Although there are accumulating emerging evidence supporting the use of tenecteplase in AIS patients, these data are based on trials conducted in the western population. Based on the current available data and guidelines, this phase III study aims to demonstrate that tenecteplase 0.25 mg/kg is non-inferior to alteplase 0.9 mg/kg for thrombolysis in AIS patients in the

Chinese population. The efficacy and safety results of this study will be used to register the use of tenecteplase to provide more treatment options for AIS patients in China.

1.4 BENEFIT-RISK ASSESSMENT

1.4.1 Benefits

Stroke-care quality improvement should be a continuous effort. This study aims to improve stroke-care quality by providing an alternative treatment that is at least as efficacious as the existing treatment that is potentially safer, and easy to administer.

Like alteplase, iv thrombolysis with tenecteplase aims at saving the ischaemic brain tissue by restoring blood circulation, thus reducing disability rate and potentially saving lives. A meta-analysis of five studies have shown that tenecteplase demonstrated non-inferior efficacy compared with alteplase in treating AIS [P19-06342]. Additionally, tenecteplase offers the following potential advantages over alteplase:

- Longer $t_{1/2}$ as demonstrated by a 4-fold slower plasma clearance compared to alteplase [P00-02456]. This allows tenecteplase to be administered as a single iv bolus instead of the traditional bolus followed by 90-min iv infusion of alteplase for the treatment of AIS
- Higher resistance to naturally occurring PAI-1, allowing for higher initial plasma concentration and enabling faster restoration of vessel patency [P03-02906]
- Evidence of faster vascular recanalization as demonstrated by several studies [P12-03304, P15-11262], which is correlated to excellent functional outcome

1.4.2 Risks

Table 1.4.2: 1 Summarises of the tenecteplase related risks.

Table 1.4.2: 1 Potential clinically important risks of tenecteplase, their rationale, and mitigation strategies

Possible or known risks of clinical relevance	Summary of data, rationale for the risk	Mitigation strategy
Bleeding	During clinical trials for AIS, the most common adverse reaction associated with tenecteplase is bleeding. The most severe bleeding is sICH.	A number of safety measures are defined to minimise the risk of sICH during this trial: • Patients with AIS will be excluded in the following situations because of increased haemorrhagic risk: o Known genetic predisposition to bleeding or significant bleeding disorder at present or within the past 6 m o Platelet count of below 100,000/mm3 at screening o Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery) o Recent traumatic external heart massage, obstetrical delivery, within the past 10 days, recent puncture of a non-compressive blood-vessel (e.g. subclavian or jugular vein puncture) o Known history of suspected intracranial haemorrhage or suspected subarachnoid haemorrhage from aneurysm o Neoplasm with increased haemorrhagic risk o Documented ulcerative gastrointestinal disease during the last 3 m, oesophageal varices, arterial-aneurysm, arterial/venous malformations o Any known disorder associated with a significant increased risk of bleeding • Clear guidance on the restricted concomitant medications such as heparin and anticoagulants • Tenecteplase 0.25 mg/kg selected in this trial has been supported by a number of completed studies, showing no increase in the risk of sICH compared to alteplase • Extensive safety monitoring processes will be put in place, including the setup of the Endpoint Adjudication Committee (EAC) and an independent DMC.
Hypersensitivity, including urticarial/anaphylactic reactions/ angioedema	Rare (<1%) after administration of tenecteplase (e.g., anaphylaxis, angioedema, laryngeal edema, rash, and urticaria)	Monitor patients treated with tenecteplase during and for several hours after infusion. If symptoms of hypersensitivity occur, appropriate therapy should be initiated.

Thrombolytic therapy is associated with a risk of bleeding, which applies for both tenecteplase and alteplase as well. The major risk remains sICH. Although the presence of oedema or mass effect on baseline CT scan is associated with higher risk of sICH, patients with these findings are more likely to have an excellent outcome if they received thrombolytic therapy [P97-10211].

Overall risks of alteplase are similar to those of tenecteplase. According to the local approved label dated at 15 Nov 2019, the most frequent adverse reaction associated with alteplase is bleeding. Hypersensitivity/anaphylactoid reactions (e.g. allergic reactions including rash, urticaria, bronchospasm, angio-oedema, hypotension, shock or any other symptom associated with hypersensitivity) are rarely reported. Serious anaphylaxis is very rarely observed.

In addition to the above mitigation strategies, extensive safety monitoring processes will be put in place, including the setup of an independent DMC. The DMC assesses the progress of the clinical trial and evaluates the data regularly in order to provide recommendation whether the trial should continue as planned, continue with modification, stop, or discussed with the sponsor. In addition, the evaluation and adjudication of all potential sICH events in a blind fashion will be a critical objective to be performed by DMC. Detailed information of DMC is available at Section 8.7.

Given the narrow time windows of treatment of AIS, timely emergency department (ED) evaluation and diagnosis is of utmost significance. As such, investigational sites are selected based on their ability to receive, identify, evaluate, treat, and/or refer patients with suspected stroke, as well as obtain access to stroke expertise when necessary for diagnostic or treatment purposes. All centers should be able to offer 24/7 (24 h/day, 7 days/week) comprehensive stroke care to acute stroke patients. The hospitals must be stroke ready centers with sufficient capability of efficiently evaluate, diagnose, and treat acute stroke. Besides key principles, guideline and procedures for site selection of clinical trials, the centers should fulfil below elements:

- Ability to administer iv tenecteplase and alteplase
- Ability to perform emergency brain imaging (e.g. CT) at all times
- Ability to conduct emergency laboratory testing at all times
- Availability of a system for the maintenance of stroke patient notes

In addition to the above, to minimise the risk and protect the safety of the subjects, all trial sites will be monitored closely and investigators will receive trainings, clear and timely updated supporting materials, and be notified of the availability of safety reports related to the product.

1.4.3 Discussion

The 2019 AHA/ASA AIS guideline point out that tenecteplase is a reasonable choice over alteplase in patients who are eligible for iv thrombolysis and mechanical thrombectomy, and 2018 China AIS guideline recognise tenecteplase as an alternative to alteplase. Additionally, a

meta-analysis of five studies has shown that tenecteplase demonstrated non-inferior efficacy compared to alteplase in treating AIS [P19-06342].

Some PK/PD advantages of tenecteplase over alteplase include higher fibrin specificity, reduced binding to the physiological PAI-1 and prolonged plasma $t_{1/2}$ enabling single bolus administration.

The major risk of all thrombolytic therapy remains sICH. In this trial, AIS patients will receive either the standard of care, alteplase, or the investigational product, tenecteplase, both of which have a similar risk profile for sICH. The greater fibrin specificity of tenecteplase mentioned above suggests that it potentially has a lower risk of sICH for patients who are assigned to this treatment.

Alteplase is the current standard of care for thrombolysis in AIS patients. This Phase III trial aims to determine whether tenecteplase is non-inferior to the current standard care alteplase in Chinese patients with AIS within 4.5 h after symptom onset. This study will provide evidence and formal support to consider tenecteplase as an alternative agent to alteplase in the treatment of AIS.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The primary objective is to assess whether tenecteplase is non-inferior to alteplase in favourable outcome, i.e., modified Rankin scale achieving 0 or 1 (mRS 0 or 1) in Chinese patients with AIS who are eligible for iv thrombolysis within 4.5 h of symptom onset.

The secondary objectives are to assess (1) whether it is safe to use tenecteplase in patients in this setting as well as (2) the efficacy in other key functional outcomes.

2.1.2 Primary endpoint

The primary efficacy endpoint is a mRS score of 0 or 1 at day 90.

2.1.3 Secondary endpoints

Secondary efficacy endpoints are defined as:

- Major neurological improvement at 24 h (NIHSS score of 0 or improvement of at least 4 points compared with baseline)
- mRS score of 0-2 at day 90
- Change from baseline of NIHSS score at day 90
- Distribution of mRS at day 90
- Barthel Index score ≥95 at day 90

Secondary safety endpoints are defined as:

- Symptomatic Intracerebral Haemorrhage (sICH[1]) per ECASS Ⅲ definition during on-treatment period.
- 90-day mortality
- mRS score of 5 or 6 at day 90

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN

This trial is a multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group phase III trial to compare the efficacy and safety of tenecteplase 0.25 mg/kg vs alteplase 0.9 mg/kg in Chinese patients with AIS within 4.5 h after symptom onset.

A total of approximately 1478 eligible AIS patients will be randomised into either the treatment group of tenecteplase or alteplase at 1:1 ratio stratified by NIHSS score at baseline (<6; 6-15; >15), and age (≤80, >80). Screening (Visit 1) will start when patients are admitted. Randomisation (Visit 2a) and administration of investigational medicinal products (IMPs) should be no later than 4.5 h after symptom onset. Visits on day 1 consist of two further visits (Visit 2b and Visit 2c) that will be performed 1 h and 2 h after the start of IMPs. Visit 3-5 for continuous follow-up will be performed on day 2, 8 and 30 after the start of IMPs. The primary efficacy endpoint will be assessed at Visit 6 (day 90). End of study (EoS) is defined as that the randomised patient completes 90-day follow-up visit after the treatment.

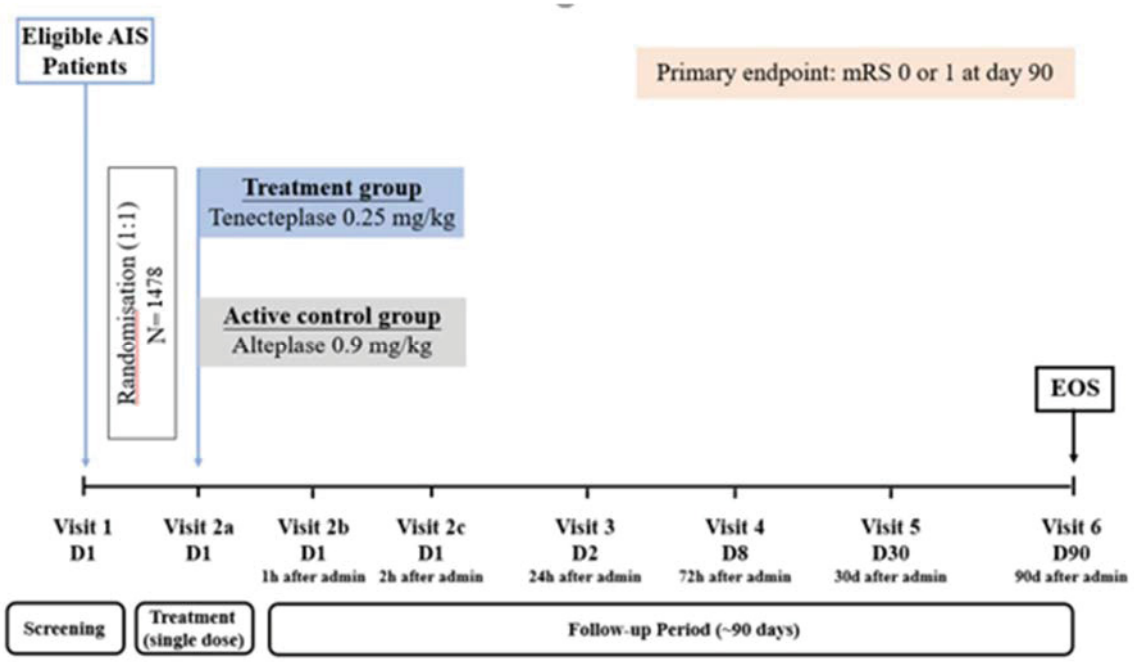

Figure 3.1:1 Study design

3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUP

Study design

This trial is designed to be a PROBE, active-controlled parallel group trial. EAC will be set for independent judgement of all sICH events described per protocol that occurred during the trial; independent DMC will be set for safety monitor of critical safety events, e.g., sICH, death, etc. For details, see Section 8.7.

Active-controlled and non-inferiority

Alteplase is the current standard of care for thrombolysis in eligible AIS patients within 4.5 h, and therefore it is chosen as the active control in this trial. Considering that there is a proven effective treatment, use of placebo in this case is ethically not acceptable. The objective of the trial is to prove that tenecteplase is non-inferior to the current standard of care in its efficacy. With its obvious advantage in convenience, tenecteplase, with similar or even better safety profile than alteplase [P12-03304, P18-01526], is expected to add value to AIS patients.

Open label

Although the double-blind design is considered optimal for phase III randomised trials, the double-blind and double-dummy drug administration are not considered in this trial as it will significantly increase the complexity of the procedure in the very urgent medical situation. Additionally, double-dummy designs are limited by ethical issues for AIS patients. Though the treatment information is available to patients and investigators, the reviewer who is responsible for assessing the primary efficacy endpoint must be blinded to treatment. In addition, the BI analysis and data cleaning team will not have access to the treatment information during the trial conduct phase in order to reduce the risk of bias affecting the cleaning and planning for analysis of the data.

Study population

The trial will include patients with AIS, who are ≥18 years of age and are eligible for standard iv thrombolysis therapy within 4.5 h of stroke onset. The trial population is selected based on the current alteplase label. In addition, there are recent evidences supporting the use of iv thrombolysis in patients aged over 80 years [P10-14128, P10-12678, P20-06029]: a pooled analysis of randomised trial and registry data evaluated the benefit-risk profile of alteplase for AIS among elderly patients (>80 years) [P20-06029]. Among patients aged >80 years, alteplase vs placebo was associated with a higher proportion of good stroke outcome (mRS 0-1:19.1% vs 13.1%, p=0.0109) and similar 90-day mortality (29.5% vs 30.2%, p=0.8382). The odds of a good stroke outcome following alteplase allocation were independent of age (p=0.7383). In conclusion, alteplase for AIS has a positive benefit-risk profile among patients aged >80 years. Therefore, the patients with age ≥18 years without upper age limit will be enrolled in this trial. This is aligned with current international guidelines [P19-03892, P19-10385].

Interim analysis

No interim analysis is planned in this trial. Futility interim is usually considered when there is high uncertainty on the treatment difference between two treatment arms. As all the available data indicate that tenecteplase is not worse than alteplase [P19-06342], the potential value of the futility interim is quite limited.

3.3 SELECTION OF TRIAL POPULATION

A total of approximately 1478 patients are planned to be randomised at approximately 55 sites in China.

Screening of patients for this trial is competitive, i.e. screening for the trial will stop at all sites at the same time once a sufficient number of patients has been screened. Investigators will be notified about screening completion and will then not be allowed to screen additional patients for this trial.

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

If a patient is enrolled in error (does not meet all inclusion criteria or meets one or more exclusion criteria on the day of enrolment), the sponsor should be contacted immediately.

3.3.1 Main diagnosis for trial entry

Patients who are clinically suspected of acute ischaemic stroke within 4.5 h after symptoms onset will be assessed for thrombolytic treatment eligibility after CT scan of the brain.

Please refer to Section 8.3.1 for the documentation requirements pertaining to the in- and exclusion criteria.

3.3.2 Inclusion criteria

1. Age ≥18 years old
2. Diagnosis of ischaemic stroke with a measurable neurological deficit on NIHSS (0< NIHSS ≤25); if NIHSS <4, patients have to be with at least a measurable deficit on motor power (upper or lower limbs ≥1)
3. Stroke symptoms should have been present for at least 30 min without significant improvement prior to randomisation
4. Thrombolytic therapy can be initiated within 4.5 h of AIS onset
5. Patients with premorbid mRS 0 or 1
6. Signed and dated written informed consent in accordance with good clinical practice (GCP) and local legislation prior to trial admission

3.3.3 Exclusion criteria

1. Evidence of intracranial haemorrhage on the CT scan or symptoms suggestive of subarachnoid haemorrhage, even if the CT scan is normal
2. Patients who must or are expected to continue the intake of restricted medications or any drug considered likely to interfere with the safe conduct of the trial
3. Acute bleeding diathesis, including but not limited to
    1) Known genetic predisposition to bleeding or significant bleeding disorder at present or within the past 6 m
    2) Administration of heparin within the previous 48 h and activated partial thromboplastin time (aPTT) exceeding the upper limit of normal for laboratory measurement
    3) Current use of vitamin K based oral anticoagulants (e.g. warfarin) and a prolonged prothrombin time (INR >1.7 or PT >15 s) or current use of novel oral anticoagulants (i.e. dabigitran, rivaroxiban, or apixiban) with prolongation of aPTT and/or PT above the upper limit of the local laboratory reference range
    4) Platelet count of below 100,000/mm^3 at screening
    5) Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)
    6) Recent traumatic external heart massage, obstetrical delivery, or recent puncture of a non-compressive blood-vessel (e.g. subclavian or jugular vein puncture), within the past 10 days
    7) Known history of suspected intracranial haemorrhage or suspected subarachnoid haemorrhage from aneurysm
    8) Neoplasm with increased haemorrhagic risk
    9) Documented ulcerative gastrointestinal disease during the last 3 m, oesophageal varices, arterial aneurysm, or arterial/venous malformations
    10) Any known disorder associated with a significant increased risk of bleeding
4. Bacterial endocarditis or pericarditis at screening
5. Acute pancreatitis at screening
6. Significant trauma or major surgery (according to the investigator's assessment) in the past 3 m
7. Imaging demonstrates multi-lobar infarction (hypodensity >1/3 cerebral hemisphere)
8. Severe uncontrolled arterial hypertension, e.g. systolic blood pressure (BP) >185 mmHg or diastolic BP >110 mmHg
9. Blood glucose <50 mg/dL (2.8 mmol/L) at screening
10. Seizure at stroke onset
11. Known hypersensitivity to active substance alteplase or tenecteplase, gentamicin (a trace residue from the manufacturing process) or to any of the excipients.

12. Currently enrolled in another investigational device or drug study, or <30 days since ending another investigational device or drug study(s), or receiving other investigational treatment(s)
13. Women who are pregnant
14. Patient has a confirmed active infection with SARS-CoV-2 in medical history within the prior 3 m, based on which investigator considers likely to interfere with the safe conduct or the trial evaluation, procedures, or completion
15. Any other conditions that, in the opinion of the investigator or BI physician, if consulted, would pose a risk to subject safety or interfere with the trial evaluation, procedures or completion

3.3.4 Withdrawal of patients from treatment or assessments

Subjects may discontinue trial treatment or withdraw from trial participation as a whole ("withdrawal of consent") with very different implications; please see Sections 3.3.4.1 and 3.3.4.2.

For this trial, there's only one IMP administration, and no benefit could be expected to the subject withdrawing from the trial for possible reasons. Every effort should be made to keep the subjects in the trial, at least to collect important trial data.

Measures to control the withdrawal rate include careful patient selection, appropriate explanation of the trial requirements and procedures prior to trial enrolment, as well as the explanation of the consequences of withdrawal.

The decision to discontinue trial treatment or withdraw consent to trial participation and the reason must be documented in the patient files and eCRF. If applicable, consider the requirements for AE collection reporting (please see Sections 5.2.7.2.1 and 5.2.7.2).

3.3.4.1 Discontinuation of trial treatment

If a subject is randomised but not administrated study drug due to any reason, it will be identified as discontinuation of trial treatment. The discontinuation of trial treatment will be documented, and the reason should be noted in source.

3.3.4.2 Withdrawal of consent to trial participation

Subjects may withdraw their consent to trial participation at any time without the need to justify the decision. In case of a subject elect to withdraw from consent after IMP administration, his/her vital/functional status at 90-day will still be collected and used for trial analysis if possible, which will be specified in ICF.

For all subjects the date and the reason for withdrawal should be recorded in the eCRF. These data will be included in the trial database and reported.

In case vital status is not yet available at Visit 6 (90-day), the follow up should be conducted as early as possible but within last patient last visit, and every effort should be made to obtain the patient vital status.

3.3.4.3 Discontinuation of the trial by the sponsor

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

- Failure to meet expected enrolment goals overall or at a particular trial site
- New efficacy or safety information invalidating the earlier positive benefit-risk-assessment, please see Section 3.3.4.1
- Deviations from GCP, the trial protocol, or the contract impairing the appropriate conduct of the trial

Further follow up of patients affected will occur as described in Section 3.3.4.2.

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

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENT

4.1.1 Identity of the investigational medicinal products

Table 4.1.1:1 Test product 1: tenecteplase

Substance:	tenecteplase (TNK-tPA) Metalyse®
Pharmaceutical formulation:	Powder and solvent for solution for injection
Source:	Boehringer Ingelheim Pharma GmbH & Co. KG
Unit strength:	1 vial contains 40 mg tenecteplase; 1 pre-filled syringe contains 8 mL solvent (water for injection)
Posology:	The reconstituted solution contains 5 mg tenecteplase/mL Single dose bolus
Mode of administration:	iv bolus over 5-10 seconds (s)

Table 4.1.1: 2 Test product 2: alteplase

Substance:	alteplase (rt-PA) Actilyse®
Pharmaceutical formulation:	Powder and solvent for solution for injection and infusion
Source:	Boehringer Ingelheim Pharma GmbH & Co. KG
Unit strength:	1 vial contains 50 mg alteplase 1 vial contains 50 mL solvent (water for injection)
Posology	The reconstituted solution contains 1 mg alteplase/mL Single dose bolus plus 1 h infusion
Mode of administration:	10% of the total dose administered as an initial iv bolus and remaining 90% of the total dose administered as an iv infusion over 1 h

For detail instructions for tenecteplase using information, please refer to ISF.

The package insert of commercial product Actilyse® can be taken as the reconstitution and administration instruction for alteplase. Details please refer to the package insert of Actilyse® archived in ISF.

4.1.2 Selection of doses in the trial and dose modifications

The dose of both alteplase and tenecteplase is body weight adjusted; therefore, investigators will try their best to assess the actual body weight of the patient. If it is not possible to have an actual body weight due to any reasons, an estimated body weight by the study nurse is acceptable.

Alteplase will be administered as a total single dose of 0.9 mg/kg. The upper dose limit is set to 90 mg. Ten percent of the total dose will be administered as a bolus. The remaining 90% of the dose will be given by continuous iv infusion over 60 min. The alteplase dose is consistent with the locally approved label for AIS treatment.

Tenecteplase will be administered as a total single dose of 0.25 mg/kg. The upper total dose limit is set to 25 mg. The total dose will be administered as a bolus over 5-10 seconds.

The tenecteplase dose is based on the previous studies [P19-02504, P12-03304, P18-01526] which have demonstrated the safety and efficacy of tenecteplase 0.25 mg/kg.

The meta-analysis of five randomised trials published in 2019 [P19-06342] included 1585 patients in total (828 in tenecteplase group, 757 in alteplase group). The proportion of patients in tenecteplase group at the concentration of 0.1 mg/kg, 0.25 mg/kg, and 0.4 mg/kg were 6.8%, 24.6%, and 68.6%, respectively. Compared with alteplase 0.9 mg/kg, tenecteplase 0.25 mg/kg and 0.4 mg/kg groups showed equivalent efficacy in primary outcome (90-day mRS 0-1), and the point estimation value of tenecteplase 0.25 mg/kg was even at the further right side of the forest plot, which suggested better efficacy (although statistically non-significant). See Figure 4.1.2: 1 below.

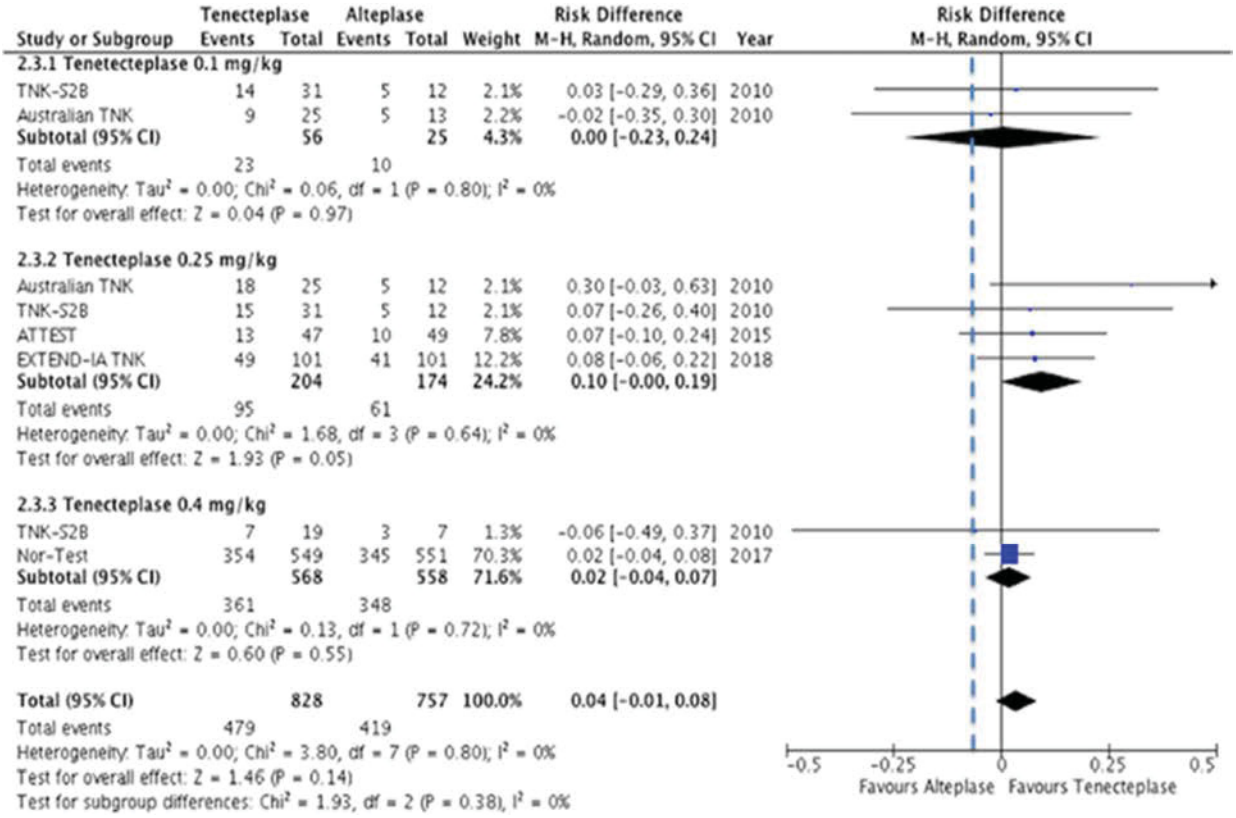

Figure 4.1.2: 1 Comparison of primary outcome between tenecteplase at different doses and alteplase forest plot without disability (mRS 0-1)

Compared with alteplase 0.9 mg/kg, the point estimate of tenecteplase 0.25 mg/kg showed a trend of lower incidence of sICH and death. The risk difference of sICH is 0% for tenecteplase 0.25 mg/kg compared to alteplase 0.9 mg/kg (95% CI -3%, 2%). The risk difference of 90-day mortality is -5% for tenecteplase 0.25 mg/kg compared to alteplase 0.9 mg/kg (95% CI -12%, 2%).

The EXTEND-IA TNK Part 2 study published in 2020 showed a dose of 0.40 mg/kg, compared with 0.25 mg/kg of tenecteplase did not significantly improve cerebral reperfusion prior to endovascular thrombectomy in patients with large vessel occlusion ischaemic stroke, and provided further evidence to suggest that 0.25 mg/kg of tenecteplase may be an appropriate dose for ischaemic stroke [P20-01928].

4.1.3 Method of assigning patients to treatment groups

An Interactive Response Technology (IRT) will be used to screen eligible patients, perform drug assignment and manage initial/re-supply ordering of drug supplies. The investigator will receive all necessary instructions to access the IRT from the Sponsor. Detailed IRT functions and procedures will be documented in the user requirement specifications mutually agreed to by the sponsor and the IRT vendor.

After the patient's eligibility has been confirmed, the treatment will be assigned via IRT at Visit 2a. Patients will be randomised to receive tenecteplase or alteplase in a ratio of 1:1 within each stratum, which is stratified by NIHSS score at baseline (<6; 6-15; >15) and age (≤80, >80). The assignment will occur in an open label fashion via IRT.

Medication numbers will be assigned by IRT at randomization and will be entered in the eCRF.

4.1.4 Drug assignment and administration of doses for each patient

Following the screening period, patients who qualify according to entry criteria will be randomised to one of the two treatment groups.

The patients will receive either iv alteplase (at the standard licensed dose of 0.9 mg/kg up to a maximum of 90 mg, 10% as bolus and the remainder over 1 h) or iv tenecteplase (0.25 mg/kg, maximum 25 mg, administered as a bolus over 5-10 seconds). Alteplase and tenecteplase will be given as a single dose. The trial medications will be administered as iv bolus (tenecteplase) or iv bolus immediately followed by iv infusion (alteplase) by authorised study personnel.

4.1.5 Blinding and procedures for unblinding

The double-blind design and double-dummy designs are not applicable in the trial. Though the treatment information is available to patients and investigators, the reviewer who is responsible for assessing the primary efficacy endpoint, i.e. an mRS score of 0 or 1 must be blinded to treatment. Please refer to Section 3.2. In addition, the BI analysis and data cleaning team will not have access to the treatment information during the trial conduct phase in order to reduce the risk of bias affecting the cleaning and planning for analysis of the data.

4.1.6 Packaging, labelling, and re-supply

The IMPs will be provided by BI or a designated contract research organization (CRO). They will be packaged and labelled in accordance with the principles of good manufacturing practice (GMP). Re-supply to the sites will be managed via an IRT system, which will also monitor expiry dates of supplies available at the sites.

For details of packaging and the description of the label, refer to the ISF.

4.1.7 Storage conditions

Drug supplies will be kept in their original packaging and in a secure limited access storage area according to the recommended storage conditions on the medication label. A temperature log must be maintained for documentation.

If the storage conditions are found to be outside the specified range, the Clinical Research Associate (CRA) or the Clinical Trial Manager, as provided in the list of contacts, must be contacted immediately.

If the storage conditions are found to be outside the specified range, the process outlined in the ISF should be followed.

4.1.8 Drug accountability

The investigator or designee will receive the investigational drugs delivered by the sponsor when the following requirements are fulfilled:

- Approval of the clinical trial protocol (CTP) by the Institutional Review Board (IRB)/ Independent Ethics Committee (IEC)
- Availability of a signed and dated trial contract between the sponsor and the investigational site
- Approval/notification of the regulatory authority (RA), e.g. competent authority (CA)
- Availability of the curriculum vitae of the Principal Investigator
- Availability of a signed and dated CTP
- Availability of the proof of a medical license for the Principal Investigator

Investigational drugs are not allowed to be used outside the context of this protocol. They must not be forwarded to other investigators or clinics.

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

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

For overall drug accountability process of medication, please refer to Medication Handling Instruction which will be provided in ISF.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

There are no special emergency procedures to be followed. However, potential side effects of tenecteplase and alteplase were to be treated symptomatically.

4.2.1.1 Recommendation of managing bleeding

The most common complication encountered during tenecteplase and alteplase therapy is bleeding. The type of bleeding associated with thrombolytic therapy can be divided into two broad categories:

- Internal bleeding, involving intracranial and retroperitoneal sites, or the gastrointestinal, genitourinary, or respiratory tracts.
- Superficial or surface bleeding, observed mainly at vascular puncture and access sites (e.g., venous cutdowns, arterial punctures) or sites of recent surgical intervention.

Standard management of AIS should be implemented concomitantly with tenecteplase/ alteplase treatment. Arterial and venous punctures should be minimized. Noncompressible arterial puncture must be avoided, and internal jugular and subclavian venous punctures should be avoided to minimize bleeding from the noncompressible sites.

Should serious bleeding occur, especially cerebral haemorrhage, concomitant heparin administration should be terminated immediately. Administration of protamine should be considered if heparin has been administered within 4 hours before the onset of bleeding. In the few patients who fail to respond to these conservative measures, judicious use of transfusion products may be indicated. Transfusion of cryoprecipitate, fresh frozen plasma, and platelets should be considered with clinical and laboratory reassessment after each administration. A target fibrinogen level of 1g/l is desirable with cryoprecipitate infusion. Antifibrinolytic agents are available as a last alternative. Should an arterial puncture be necessary during the first few hours following tenecteplase or alteplase therapy, the use of an upper extremity vessel that is accessible to manual compression is preferable. Pressure should be applied for at least 30 min, a pressure dressing applied, and the puncture site checked frequently for evidence of bleeding.

Each patient being considered for therapy with tenecteplase and alteplase should be carefully evaluated and anticipated benefits weighed against potential risks associated with therapy.

4.2.1.2 Recommendation of managing hypersensitivity

Hypersensitivity, including urticarial/anaphylactic reactions, have rarely (<1%) been reported after administration of tenecteplase and alteplase (e.g., anaphylaxis, angioedema, laryngeal oedema, rash, and urticaria).

Immune-mediated hypersensitivity reactions associated with the administration of Actilyse/Metalyse can be caused by the active substance alteplase or tenecteplase, gentamicin (a trace residue from the manufacturing process), any of the excipients, or the stopper of the glass vial with Actilyse/Metalyse powder which contains natural rubber (a derivative of latex). No sustained antibody formation to the recombinant human tissue-type plasminogen activator molecule has been observed after treatment. There is no systematic experience with re-administration of Actilyse/Metalyse.

There is also a risk of hypersensitivity reactions mediated through a non-immunological mechanism.

Angio-oedema represents the most common hypersensitivity reaction reported with Actilyse/Metalyse. This risk may be enhanced in the indication acute ischaemic stroke and/or by concomitant treatment with ACE inhibitors. Patients treated for any authorised indication should be monitored for angio-oedema during and for up to 24h after infusion.

If a severe hypersensitivity reaction (e.g. angio-oedema) occurs, the infusion should be discontinued, and appropriate treatment promptly initiated. This may include intubation.

4.2.1.3 Recommendation of managing other adverse events

During and following a subject's participation in a trial, the investigator should ensure that adequate medical care is provided to a subject for any AEs, including clinically significant laboratory values, related to the trial.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

Additional thrombolytic medication (e.g. prolinase or streptokinase) is forbidden for either study group of tenecteplase or the active control group of alteplase during the whole study period

Heparin

Intravenous heparin at any dose is forbidden before and during the trial drug administration and during the initial 24 h after completion of drug administration. Thereafter iv heparin must not be started before the second CT is evaluated and reveals no signs of intracerebral bleeding.

In exceptional circumstances, low dose subcutaneous heparin may be given concomitantly during the first 24 h in order to prevent deep venous thrombosis provided that the aPTT will not be prolonged for >2 fold over the baseline value. The aPTT must be determined regularly during the administration of heparin SC. The SC heparin dose must not exceed 10,000 IU of sodium heparin during 24 h or equivalent doses of low molecular weight heparin. Simultaneous administration of heparin and other antithrombotic drugs is strongly discouraged.

Other medication

Administration of oral anticoagulants (including warfarin, dabigatran, rivaroxaban, apixaban and edoxaban) and antiplatelet agents (including aspirin, clopidogrel, ticagrelor and cilostazol), is prohibited during the first 24 h after completion of the trial drug administration.

4.2.2.2 Restrictions on diet and lifestyle

Not applicable.

4.3 TREATMENT COMPLIANCE

The investigator will maintain accurate records of receipt of all trial medication, including the date, exact time point of receipt. In addition, accurate records will be kept regarding the total dosage administered to each individual patient in the trial.

Actual weighing of the patient is preferred when it can be performed without causing delays. The treatment effect is time-dependent; therefore, treatment must be started as early as possible within 4.5 h after onset of stroke symptoms.

5. ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

Primary and secondary efficacy endpoints are performed by authorised medical staff at each site who are masked to treatment allocation. Site staff should try best to ensure the face-to-face follow-up visit for evaluation. Among further endpoints, evaluation of all the functional scores is blinded except NIHSS score at 2h which is usually not feasible. In the case that on-site visit is by no means possible, remote evaluation via video or telephone could be accepted (Section 10.1). All assessments should be done by trained observers.

The primary study endpoint is the favourable outcome as measured by a mRS score of 0 or 1 at day 90. [redacted]

Modified Rankin Scale (mRS)

The mRS is a tool used to evaluate disability after a stroke and will be collected at day 30 and day 90 (see Appendix 10.2.1).

Barthel Index Score

The Barthel Index score will be used to evaluate the recovery of neurological functions at day 30 and day 90. The index describes 10 tasks, which include feeding, bathing, grooming, dressing, bowels, bladder, toilet use, transfers (bed to chair and back), mobility (on level surfaces), and stairs use. Each task is scored according to the amount of time or assistance required by the patient. Total score is from 0 to 100, with lower scores representing greater nursing dependency (see Appendix 10.2.2).

Glasgow Outcome Scale

The Glasgow Outcome Scale will be used to measure an outcome at day 90. It applies to patients with brain damage allowing the objective assessment of their recovery in five categories. This allows a prediction of the long-term course of rehabilitation to return to work and everyday life (see Appendix 10.2.3).

National Institutes of Health Stroke Scale (NIHSS)

The clinical outcome will be assessed by NIHSS scoring at baseline, 2 h, 24 h, day 8, day 30 and day 90. Details of NISHSS are described in Appendix 10.2.4.

The NIHSS will be recorded, if it had been collected at the time of qualifying (index) stroke. In patients, where NIHSS has not been done at the time of index stroke, it will be performed at trial entry (at Visit 1). It is a tool used by healthcare providers to objectively quantify the impairment caused by a stroke. The NIHSS is composed of 11 items, and for each item a score

of 0 typically indicates normal function in that specific ability, while a higher score is indicative of some level of impairment. The individual scores from each item are summed in order to calculate a patient's total NIHSS score. The maximum possible score is 42, with the minimum score being a 0 [R13-4008, R13-4023, P13-10973].

Only if the patient has physical barrier or other specific situation that make it impossible to respond, e.g., amputation or joint fusion at the shoulder/hip, or blind etc., the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. Refer to the items scored with 9 in Appendix 10.2.4.

Additionally, distal motor function will also be assessed.

5.2 ASSESSMENT OF SAFETY

For assessment of safety, incidence and severity of AEs including mortality at day 90 will be evaluated. An ECASS III-defined sICH were assessed as one of secondary safety endpoints. [redacted]

The definitions of sICH used in the study list below:

Definition	Clinical criteria	Radiographic criteria	Causality of Neurological Deterioration	Time Frame
ECASS[1] II	Clinical deterioration or adverse events indicating clinical worsening (e.g., drowsiness, increase of hemiparesis) or causing an increase in NIHSS score of ≥4 points	Any haemorrhage on CT	Regardless of causal relationship	CT done at 22-36 h and 7 days after stroke onset
ECASS III	Clinical deterioration defined by an increase of ≥4 points in NIHSS score or that lead to death	Any haemorrhage	Haemorrhage as the predominant cause of the neurological deterioration	CT/MRI required at 22-36 h after stroke onset
SITS-MOST[2]	Neurological deterioration indicated by an NIHSS score that was ≥4 points higher than the baseline value or the lowest value between baseline and 24 h or haemorrhage leading to death	Local or remote parenchymal hematoma -2 (PH-2)	Regardless of causal relationship	CT/MRI required at 22-36 h after stroke onset

[1] ECASS: European Cooperative Acute Stroke Study

[2] SITS-MOST: Safety Implementation of Thrombolysis in Stroke: Monitoring Study

5.2.1 Cerebral imaging

A non-contrast CT at screening is to be used simply to exclude patients presenting with ICH. Usually, the infarction will not show in CT at visit 1 but in the imaging at the following visits, which should be regarded as the disease under study. At Visit 3, a repeated cerebral imaging will be performed between 22 and 36 h after starting the trial medication. The cerebral imaging at Visit 4 and Visit 5 is optional in case of clinical deterioration determined by investigator.

The brain imaging should be interpreted by a physician with expertise in reading CT. MRI is also acceptable.

Investigator could make decision whether or not an imaging performed outside the site could be used for evaluation of inclusion/exclusion criteria. This evaluation should also include time elapsed from imaging, possible changes in clinical symptoms since imaging.

5.2.2 Physical examination

A physical examination as per standard care will be performed at the Visit 1 and Visit 3. It includes at a minimum general appearance, neck, lungs, cardiovascular system, abdomen, extremities, and skin. Measurement of height and body weight will be performed at the screening visit specified in the Flow Chart.

The results must be included in the source documents available at the site. The abnormal results will be recorded in eCRF.

5.2.3 Vital signs

Vital signs include blood pressure, pulse rate, respiratory rate and temperature. It should be measured on each visit and the result shall be noted in source.

5.2.4 Safety laboratory parameters

Safety laboratory parameters to be assessed are listed in Table 5.2.4: 1. For the sampling time points please see the Flow Chart. All analyses will be performed by local laboratory, the respective reference ranges will be provided in the ISF.
Patients have to be fasted for the blood sampling for the safety laboratory except for screening (Visit 1).

Table 5.2.4: 1 Safety laboratory tests

Category	Laboratory test
Point-of-care testing (POCT)	Glucose [1] (non-fasting is acceptable for Visit 1)
Haematology	Red blood cell (RBC) count Haemoglobin (Hb) Haematocrit (HCT) Mean corpuscular volume White blood cell count including differential Platelet count[1]
Biochemistry	Aspartate aminotransferase (AST) Alanine transaminase (ALT) Gamma-glutamyl transferase (GGT) Alkaline phosphatase (ALP) Creatine kinase (CK) Lactate dehydrogenase (LDH) Total protein Total bilirubin Creatinine Glucose[1] (non-fasting is acceptable for Visit 1. Fasting is required at the Visit 3-6) Uric acid
Electrolytes	Sodium Potassium Calcium Inorganic phosphorus
Coagulation[1]	International normalised ratio (INR) Activated partial thromboplastin time (APTT) Prothrombin time (PT)
Urinalysis[2]	pH, glucose, erythrocytes, leukocytes, protein, nitrite (semi quantitative measurements; (–, +, ++, +++), pregnancy test

1. During screen (Visit 1) the lab values related to eligibility for the trial, only the result of random glucose (POCT) is mandatory before drug administration. Hematology lab results including coagulation items and platelet count are necessary before drug administration only when anticoagulants are used or there is suspicion of coagulopathy (as determined by investigator). Drug administration should not be delayed by other lab results.
2. If the routine urine test during screening is not available, investigator should judge patient's baseline condition and decide whether the skipping of urine test will impact the eligibility and should fully document in medical source. Urine test should be conducted at the earliest possible afterwards for safety consideration.

Clinically relevant abnormal findings will be reported either as baseline condition (if identified at the screening visit) or otherwise as AEs and will be followed up and/or treated as medically appropriate.

5.2.5 Electrocardiogram

The 12-lead electrocardiograms (ECGs) should be performed by a qualified personnel and results will be recorded as scheduled in the Flow Chart. The investigator or a designee will evaluate whether the ECG is normal or abnormal and assess clinical relevance. ECGs may be repeated for quality reasons and the repeated recording will be used.

Clinically relevant abnormal findings will be reported either as baseline condition (if identified at the screening visit) or otherwise as AEs and will be followed up and/or treated as medically appropriate.

5.2.6 Other safety parameters

For further safety parameters, the frequency and severity of AE will be recorded and calculated throughout the whole trial.

5.2.7 Assessment of adverse events

5.2.7.1 Definitions of adverse events

5.2.7.1.1 Adverse event

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

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

The following should also be recorded as an AE in the eCRF and BI SAE form (if applicable):

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

If such abnormalities already exist prior to trial inclusion, they will be considered as baseline conditions and should be collected on Medical history/pages related to baseline condition in the eCRF only.

5.2.7.1.2 Serious adverse event

An SAE is defined as any AE, which fulfils at least one of the following criteria:

- Results in death
- Is life-threatening, which refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if more severe
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity

- Is a congenital anomaly/birth defect
- Is deemed serious for any other reason if it is an important medical event when based on appropriate medical judgement which may jeopardise the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse

5.2.7.1.3 AEs considered "Always Serious"

In accordance with the EMA initiative on Important Medical Events, BI has set up a list of AEs, which by their nature, can always be considered to be "serious" even though they may not have met the criteria of an SAE as defined above.

The latest list of "Always Serious AEs" can be found in the electronic data capture (EDC) system. A copy of the latest list of "Always Serious AEs" will be provided upon request. These events should always be reported as SAEs as described in Section 5.2.7.1.2.

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the drug and must be reported as described in Section 5.2.7.2, subsections "AE Collection" and "AE reporting to sponsor and timelines".

5.2.7.1.4 Adverse events of special interest (AESI)

No AESIs have been defined for this trial.

5.2.7.1.5 Intensity (severity) of adverse events

The intensity (severity) of the AE should be judged based on the following:

Mild:	Awareness of sign(s) or symptom(s) that is/are easily tolerated
Moderate:	Sufficient discomfort to cause interference with usual activity
Severe:	Incapacitating or causing inability to work or to perform usual activities

The intensity (severity) of AEs should be classified and recorded in the eCRF.

Causal relationship of adverse events

Medical judgement is to be used to determine whether there is a reasonable possibility of a causal relationship between the AE and the given trial treatment, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history.

Claims that may suggest that there is a reasonable possibility of a causal relationship:

- The event is consistent with the known pharmacology of the drug

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

Claims that may suggest that there is no reasonable possibility of a causal relationship:

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

5.2.7.2 Adverse event collection and reporting

5.2.7.2.1 Adverse event collection

The investigator shall maintain and keep detailed records of all AEs in the patient files.

The following must be collected and documented on the appropriate eCRF by the investigator:

- From signing the informed consent onwards until the last follow-up visit (Visit 6): all AEs (non-serious and serious)
- After the individual patient's end of the study, the investigator does not need to actively monitor the patient for new AEs but should report any occurrence of cancer of new histology and any trial treatment related SAEs of which the investigator may become aware of by any means of communication, e.g. phone call. Those AEs should be reported on the BI SAE form (see Section 5.2.7.2.2), but not on the eCRF

Vital Status Data Collection

Patients who are administrated study drugs but discontinue study prematurely, who agree to be contacted further but do not agree to on-site visits, should be followed up as described in

Section 3.3.4.2. From then on until the individual patient's end of the study the investigator must report all deaths/fatal and AEs regardless of relationship and trial treatment related SAEs.

5.2.7.2.2 AE reporting to the sponsor and timelines

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

With receipt of any further information to these events, a follow-up SAE form has to be provided. For follow-up information, the same rules and timeline apply as for initial information. All (S)AEs, including those persisting after individual patient's end of trial must be followed up until they have resolved, have been assessed as "chronic" or "stable", or no further information can be obtained.

5.2.7.2.3 Pregnancy

In rare cases, pregnancy might occur in a trial. Once a patient has been enrolled in the trial and has taken trial medication, the investigator must report any drug exposure during pregnancy in a trial participant immediately (within 24 h) by means of Part A of the Pregnancy Monitoring Form to the sponsor's unique entry point.

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

The ISF will contain the Pregnancy Monitoring Form for Clinical Studies (Part A and B).

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

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

Not applicable.

5.4 ASSESSMENT OF BIOMARKER

Not applicable.

5.5 BIOBANKING

Not applicable.

5.6 OTHER ASSESSMENTS

Not applicable.

5.7 APPROPRIATENESS OF MEASUREMENTS

All measurements conducted in the trial are using standard methods.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

The trial will consist of six visits. The schedule for trial visits is summarised in the Flow Chart including time windows for trial visits. All visit dates are calculated from the date of drug administration. In the event that visits are missed or out of sequence, subsequent visits will be planned according to the date of visit 1.

No protocol waivers will be given (e.g. Sponsor will not grant permission to include a known ineligible patient). In the case of medical emergencies, prior approval from the Sponsor for protocol deviations (e.g. visit schedule) will not be required, but BI should be notified as soon as possible. The relevance of any such protocol deviation will be assessed prior to analysing the data.

Remote visits will be acceptable under specific situations. The decision to replace a site visit with a remote visit will be done by the investigator based on a thorough risk assessment or after discussion with the sponsor.

The procedures to be conducted at each visit are provided in the Flow Chart and further described below.

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

All subjects should adhere to the visit schedule as specified in the Flow Chart. If any visit has to be rescheduled, subsequent visits should follow the original visit date schedule.

6.2.1 Screening period

Informed consent of all trial patients will be obtained in compliance with ICH and GCP guidelines and the principles stipulated in the Declaration of Helsinki prior to any trial related procedure.

After informed consent procedure, a patient may be enrolled into the trial when entering the clinic with acute stroke symptoms and being liable to be treated within 4.5 h after stroke onset. All inclusion and exclusion criteria have to be checked prior to administration of the test dose of trial medication. The cerebral imaging scan and its evaluation has to be done with due diligence. Investigator and/or co-investigator must agree with the radiologist that there are no imaging exclusion criteria prior to administration of the test dose of trial medication.

6.2.2 Treatment period

After the consent procedure has been completed, eligibility has been confirmed, and all randomisation procedures have been completed, the drug can be administered (Visit 2a). Please refer to the Flow Chart for details.

Alteplase will be administered as a total single dose of 0.9 mg/kg. The upper dose limit is 90 mg. Ten percent of the total dose will be administered as bolus. The remaining 90% of the dose will be given through continuous iv infusion over 60 min. The alteplase dose is consistent with the local approved label for AIS treatment.

Tenecteplase will be administered as a total single dose of 0.25 mg/kg. The upper dose limit is 25 mg/patient. The total dose will be administered as a bolus over 5-10 seconds.

6.2.3 Follow-up period and trial completion

The vital signs will be measured, as well as AEs and concomitant medication will be documented for all patients at all predefined treatment time points (Visit 2b and Visit 2c) as of the administration day and Visit 3, 4, 5 and 6. For detailed assessment of the follow-up period and trial completion, please refer to the Flow Chart.

Subjects will complete the trial (end of study, i.e. EoS) on treatment and the follow up visit around day 90 as per protocol (Visit 6). Data should have been entered in eCRF when subject has terminated the study. In case vital status is not yet available at Visit 6, the follow up should be conducted as early as possible but within last patient last visit, and every effort should be made to obtain the patient vital status.

Based on especial conditions, remote visits will be acceptable for Visit 5 and Visit 6. It should be determined by investigator, and also shall keep align with the regulations from local governance/ site EC. E.g., when subject has a very bad condition, or under the pandemic of Covid-19 on-site visits are strictly limited for subject's benefit.

If a subject is randomised but not administrated any study drug, or a subject discontinues from the trial after treatment, either situation will be documented and the date and reason for discontinuation will be recorded in the eCRFs. For any reason if a subject cannot perform a follow up visit to site, remote follow up visits including EoS evaluation (Visit 6, day 90) will be performed as complete as possible, and the data collected from these subjects will be included in the trial database and will be reported.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

This trial is a multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group phase III trial. The primary objective is to establish the non-inferiority of in efficacy between tenecteplase and alteplase in patients with AIS based on a statistical comparison of the following criteria:

- Proportion of patients achieving modified Rankin Scale 0 or 1 (mRS0-1) at day 90

Based on the meta-analysis results of historical randomised trials of intravenous alteplase for treatment of AIS [P14-11754], the unadjusted risk ratio for the effect of rt-PA versus placebo for the endpoint of mRS 0-1 was 1.24 (95% CI 1.14 to 1.36). Taking lower bound 1.14 as M1 and f=0.5 (preserve at least 50% of the effect of alteplase), M2 will be 1.0677. Therefore, the non-inferiority margin (1/M2) for RR will be set as 0.937.

Based upon these design considerations, the trial will be analysed using log binomial regression model with subsequent transformation of estimated parameters to risk ratio including the respective CI, which will include terms for NIHSS at baseline, time to drug administration and age as covariates. The primary treatment comparison will be as randomised, including the effects of performing thrombectomy. Details of the model are presented in Section 7.2.3.

The secondary objectives of this trial are to assess the safety and efficacy in other key functional outcomes in patients with AIS.

7.1 NULL AND ALTERNATIVE HYPOTHESES

The test will be performed with respect to tenecteplase versus alteplase.

Based on the non-inferiority setup, the hypotheses for the non-inferiority test can be written as follows:

H_{01}: Risk ratio in mRS0-1 response rates at day 90 (tenecteplase/alteplase) is ≤ 0.937

versus the alternative hypothesis

H_{11}: Risk ratio in mRS0-1 response rates at day 90 (tenecteplase/alteplase) is > 0.937

Tests will be based on a two-sided 95% CI (using a two-sided alpha of 5%) for risk ratio in mRS0-1 response rates at day 90. To conclude non-inferiority, the null hypotheses need to be rejected, which means that:

- the 95% CI for risk ratio in mRS0-1 response rates at day 90 has to be contained in the predefined non-inferiority regions $(0.937, +\infty)$

Once the non-inferiority hypothesis test specified above concludes non-inferiority of tenecteplase over alteplase, the following superiority hypotheses will be tested in risk ratio in mRS0-1 response rates at day 90 in a hierarchical manner with overall type I error control. Failure to demonstrate the non-inferiority of tenecteplase over alteplase implies that no further formal testing in the hierarchical sequence below will be conducted.

The hypotheses for the superiority test can be written as follows:

H_{02}: Risk ratio in mRS0-1 response rates at day 90 (tenecteplase/alteplase) is ≤ 1

versus the alternative hypothesis

H_{12}: Risk ratio in mRS0-1 response rates at day 90 (tenecteplase/alteplase) is > 1

Tests will also be based on a two-sided 95% CI (using a two-sided alpha of 5%) for risk ratio in mRS0-1 response rates at day 90. No adjustment of the alpha of 5% for constructing the confidence interval is necessary under the hierarchical manner. To conclude superiority, the null hypotheses need to be rejected, which means that:

- the 95% CI for risk ratio in mRS0-1 response rates at day 90 has to be contained in the superiority regions $(1, +\infty)$

7.2 PLANNED ANALYSES

7.2.1 General considerations

There will be 4 main patient populations in this trial for analyses: the enrolled set (ES), the safety set (SS), the full analysis set (FAS), and the per-protocol set (PPS).

Enrolled Set

This patient set includes all patients who signed informed consent. It will be used for analyses of patient disposition.

Safety Set

This patient set includes all patients who were randomised and received any dose of treatment. It will be the main analysis set for presentation of safety. Patients will be analysed according to the actual treatment.

Full Analysis Set

This patient set includes all randomised patients who received any dose of treatment. Treatment assignment will be as randomised. This is the primary analysis set for presentation of efficacy according to the intention-to-treat principle.

Per-Protocol Set

This patient set includes all patients in the FAS who adhered to the CTP without any important protocol deviations (IPDs) relevant for efficacy which lead to exclusion from the PPS. A protocol deviation (PD) will be considered important if it can be expected to have a distorting influence on the assessment of the primary endpoint, which will be assessed on a case-by-case basis. [redacted]

Standard statistical parameters (number of non-missing values, mean, standard deviation (SD), median, quartiles, minimum and maximum) or frequency tables (including patient frequencies and percentages) will be calculated where appropriate.

The final trial analysis is planned to be performed at the end of the study once all randomised patients have completed the study (including any applicable follow-up period). The final analysis will include all trial data. Details of the analysis to be performed will be described in the trial statistical analysis plan (TSAP).

A Clinical Trial Report will be prepared at the end of the study.

7.2.2 Handling of intercurrent events

The expected intercurrent events (ICE) of interest in this trial are listed in Table 7.2.2:1 together with handling strategy.

Table 7.2.2:1 Intercurrent events (ICE) and handling strategy

ICE	Handling strategy	Comments
Perform thrombectomy	Treatment policy (Include all available data)	This event reflects the clinical practice and will be included in efficacy evaluation
Death	Will not be considered as ICE for primary objective	Death event is included in mRS variable (death is equivalent to score 6)

The primary estimand in evaluating the primary objective is **Treatment-Regimen Estimand** which is specified as below:

1) Target population: The treatment effect will be estimated for trial patients with AIS as specified by the inclusion and exclusion criteria.

2) Treatment regimen under evaluation will be the randomised treatment taken (tenecteplase and alteplase).

3) Endpoints: Efficacy will be evaluated using endpoint “mRS score of 0 or 1 at day 90”.

4) Intercurrent events: For patients who performed thrombectomy, observed variable values will be used for the analysis.

5) Population-level summary: The proportion of patients with "mRS score of 0 or 1 at day 90" will be compared between two treatment groups in terms of risk ratio.

7.2.3 Primary endpoint analyses

Comparisons between treatment groups regarding the binary endpoint variable mRS 0-1 at day 90 will be performed using log binomial regression model with subsequent transformation of the estimated parameters to risk ratio including the respective CI. The difference in log (proportion of mRS0-1 at day90) between tenecteplase and alteplase will be estimated with 95% CI adjusting for continuous covariates (NIHSS at baseline, age and time to drug administration). The estimate and CI will be exponentiated to return to the ratio scale.

The analysis will be related to the defined primary estimand and performed on FAS. Patients will be assigned to treatment groups as randomised. Multiple imputation method mentioned in Section 7.3 will be used to handle the missing data of primary endpoint.

[redacted]

7.2.4 Secondary endpoint analyses

Secondary endpoints will be presented in an exploratory manner. Frequencies and percentages will be presented for categorical endpoints; descriptive statistics will be used for continuous data. If applicable, 95% CI will also be displayed. Details will be described in TSAP.

[redacted]

7.2.6 Safety analyses

AEs will be coded using the Medical Dictionary for Drug Regulatory Activities (MedDRA). Standard BI summary tables and listings will be produced.

All treated patients will be included in the safety analysis. In general, safety analyses will be descriptive in nature and will be based on BI standards. No hypothesis testing is planned. Statistical analysis and reporting of AEs will concentrate on treatment-emergent AEs. To this end, all AEs occurring between start of treatment and end of the residual effect period (REP) will be considered 'treatment-emergent'; the REP is defined as 7 days after the end of treatment, will be assigned to the on-treatment period for evaluation. Adverse events that start before first drug intake and deteriorate under treatment will also be considered as 'treatment-emergent'. The frequency of all AEs collected until the end of the study will also be summarized.

Frequency, severity, and causal relationship of AEs will be tabulated by system organ class (SOC) and preferred term (PT) after coding according to the current version of the MedDRA at database lock (DBL).

Laboratory data will be analysed qualitatively and quantitatively. Treatment groups will be compared descriptively with regard to frequency and percentage of patients with abnormal values or clinically relevant abnormal values.

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

7.2.7 Interim Analyses

No interim analysis is planned.

7.3 HANDLING OF MISSING DATA

Every effort will be made to collect complete data at all visits. However, for functional outcome parameters of surviving patients missing values might occur.

For primary endpoint, multiple imputation approach will be applied for the primary analysis. Three steps will be done for multiple imputation process:

Step 1: Multiple imputation for missing data: Fully Conditional Specification (FCS) method will be used for MI process. Logistic regression method will be used to impute missing values by using the ordering of the class level in each variable.

Step 2: Analysis of completed datasets: The imputed complete dataset will be used to analyze the primary endpoint and the corresponding statistical analysis specified above will be performed.

Step 3: Combine the results. For risk ratio, the distribution is not expected to be approximately normal, before using Rubin's rules to combine the analysis results from step 2 and generate valid statistical inferences by accounting for the variability introduced by MI process, normal transformation of the statistics estimated from step 2 will be done first.

7.4 RANDOMISATION

BI will arrange for the randomisation and the packaging and labelling of trial medication. The randomisation list will be generated using a validated system, which involves a pseudo-random number generator so that the resulting treatment will be both reproducible and non-predictable. Access to the codes will be controlled and documented.

Stratification for NIHSS score (<6; 6-15; >15) at baseline and age (≤80; >80) will be done in order to assure the balance of NIHSS score and age between the two treatment groups; these strata will be included into the analyses of efficacy endpoints. Within each stratum of NIHSS at baseline and age, patients will be randomised in a 1:1 ratio (tenecteplase vs alteplase). The randomisation will be done in blocks to achieve balanced allocation. The block size of the randomisation will be documented in the CTR.

The process of randomisation is done via an IRT. Practical aspects of the treatment allocation process are detailed in Section 4.1.3.

7.5 DETERMINATION OF SAMPLE SIZE

In NORTEST study [P17-08885], which had a similar enrolment criteria with this trial, the proportion of patients with mRS 0 or 1 at 90 days was 63% in alteplase group. In the China

single arm 3-4.5 h alteplase study [P20-04860], the proportion of patients with mRS 0 or 1 at 90 days was 63%. Thus, it is assumed that 63% of patients in alteplase group will achieve mRS 0 or 1 at day 90. Recent ACT study results showed that the proportion of patients with mRS 0 or 1 at 90-120 days in Canadian was 36.9% in tenecteplase group and 34.8% in alteplase group [P22-05053]. The unadjusted absolute relative risk was 1.06. Based on the absolute relative risk 1.06, proportion of patients with mRS 0 or 1 at day 90 in tenecteplase group will be 66.8%. For conservative consideration, it is assumed as 65.8%.

The power estimates for detecting the non-inferiority were performed using R (Version 4.01).

With an expected response rate of 65.8% in tenecteplase group, 63% in alteplase group for the primary endpoint, a type I error of 0.025(1-sided) and a non-inferiority margin of 0.937, for a total sample size of 1478 patients, there is an 80% power to demonstrate non-inferiority. Approximately 739 patients will be randomised in either group.

8. INFORMED CONSENT, TRIAL RECORDS, DATA PROTECTION, PUBLICATION POLICY, AND ADMINISTRATIVE STRUCTURE

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Guideline for GCP, relevant BI standard operating procedures (SOPs), and other relevant regulations. Investigators and site staff must adhere to these principles. Deviation from the protocol, the principles of ICH GCP or applicable regulations as will be treated as "protocol deviation".Standard medical care (prophylactic, diagnostic and therapeutic procedures) remains the responsibility of the treating physician of the patient.

The investigator will inform the sponsor immediately of any urgent safety measures taken to protect the trial patients against any immediate hazard, as well as of any serious breaches of the protocol or of ICH GCP.

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

The certificate of insurance cover is made available to the investigator and the patients and is stored in the ISF.

8.1 TRIAL APPROVAL, PATIENT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective IRB/IEC and CA according to national and international regulations. The same applies for the implementation of changes introduced by amendments.

Prior to patient participation in the trial, written informed consent must be obtained from each patient (or the patient's legally accepted representative) according to ICH GCP and to the regulatory and legal requirements of the participating country. Each signature must be personally dated by each signatory and the informed consent and any additional patient-information form retained by the investigator as part of the trial records. A signed copy of the informed consent and any additional patient information must be given to each patient or the patient's legally accepted representative."

The patient must be given sufficient time to consider participation in the trial. The investigator or delegate obtains written consent of the patient's own free will with the informed consent form after confirming that the patient understands the contents. The investigator or [redacted] delegate must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent.

Re-consenting may become necessary when new relevant information becomes available and should be conducted according to the sponsor's instructions.

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

8.2 DATA QUALITY ASSURANCE

A risk-based approach is used for trial quality management. It is initiated by the assessment of critical data and processes for trial subject protection and reliability of the results as well as identification and assessment of associated risks. An Integrated Quality and Risk Management Plan documents the rationale and strategies for risk management during trial conduct including monitoring approaches, vendor management and other processes focusing on areas of greatest risk.

Continuous risk review and assessment may lead to adjustments in trial conduct, trial design or monitoring approaches.

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

8.3 RECORDS

The eCRFs for individual patients will be provided by the sponsor. For drug accountability, refer to Section 4.1.8.

8.3.1 Source documents

In accordance with regulatory requirements, the investigator should prepare and maintain adequate and accurate source documents and trial records that include all observations and other data pertinent to the investigation on each trial patient. Source data as well as reported data should follow the "Attributable, Legible, Contemporaneous, Original, Accurate (ALCOA) principles" and be **a**ttributable, **l**egible, **c**ontemporaneous, **o**riginal and **a**ccurate. Changes to the data should be traceable (audit trail).

Data reported on the eCRF must be consistent with the source data or the discrepancies must be explained.

The current medical history of the patient may not be sufficient to confirm eligibility for the trial and the investigator may need to request previous medical histories and evidence of any diagnostic tests. In this case, the investigator must make at least one documented attempt to retrieve previous medical records. If this fails, a verbal history from the patient would be acceptable if applicable. It will be determined by the investigator whether the patient is eligible to participate the trial according to all the available information which should be documented in source.

If the patient is not compliant with the protocol, any corrective action such as re-training must be documented in the patient file.

For the eCRF, data must be derived from source documents, for example:

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

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

8.3.2 Direct access to source data and documents

The investigator/institution will allow site trial-related monitoring, audits, IRB/IEC review and regulatory inspections. Direct access must be provided to the eCRF and all source documents/data, including progress notes, copies of laboratory and medical test results, which must be available at all times for review by the CRAs, auditor and regulatory inspector (e.g. FDA). They may review all eCRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in section 8.3.1. The sponsor will also monitor compliance with the protocol and GCP.

8.3.3 Storage period of records

Trial site(s):

The trial site(s) must retain the source and essential documents (including ISF) according to contract or the local requirements valid at the time of the end of the trial (whichever is longer).

Sponsor:

The sponsor must retain the essential documents according to the sponsor's SOPs.

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

BI is responsible to fulfil their legal and regulatory reporting obligation in accordance with regulatory requirements.

8.5 STATEMENT OF CONFIDENTIALITY AND PATIENT PRIVACY

Data protection and data security measures are implemented for the collection, storage and processing of patient data in accordance with the principles 7 and 12 of the WHO GCP handbook.

Individual patient data obtained as a result of this trial is considered confidential and disclosure to third parties is prohibited with the following exceptions:

Personalised treatment data may be given to the patient's personal physician or to other appropriate medical personnel responsible for the patient's welfare. Data generated at the site as a result of the trial need to be available for inspection on request by the participating physicians, the sponsor's representatives, by the IRB/IEC and the RAs.

8.5.1 Collection, storage and future use of biological samples and corresponding data

Not applicable.

8.6 TRIAL MILESTONES

The start of the trial is defined as the date when the first patient in the whole trial signs informed consent.

The end of the trial is defined as the date of the last visit of the last patient in the whole trial ("Last Patient Completed"). The "Last Patient Last Treatment" (LPLT) date is defined as the date on which the last patient in the whole trial is administered the last dose of trial treatment (as scheduled per protocol or prematurely). Individual investigators will be notified of suspected unexpected serious adverse reactions occurring with the trial medication until 30 days after LPLT at their site.

Early termination of the trial is defined as the premature termination of the trial due to any reason before the end of the trial as specified in this protocol.

Temporary halt of the trial is defined as any unplanned interruption of the trial by the sponsor with the intention to resume it.

Suspension of the trial is defined as an interruption of the trial based on a Health Authority (HA) request.

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by BI.

In order to ensure the patient's safety during the trial, fully external EAC and DMC will be set up respectively, independent of the trial and project teams. The EAC will be responsible to provide consistent independent judgement of all sICH events described per protocol that occurred during the trial. Events will be blinded, adjudicated on an ongoing basis and EAC meetings will be scheduled approximately after every 15 - 18 events reported since first-patient-in or at a frequency determined by the EAC.

The DMC will review all available un-blinded safety data (group tenecteplase and group alteplase not disclosing the actual treatment) based on EAC review outcome report at a similar frequency and provide review recommendation. A DMC statistical analysis plan (SAP) which describes the analyses required for assessment by the DMC will be produced. Further details will be provided in a DMC charter.

The two committees, EAC and DMC, each consist of members independent of BI.

- The EAC members will be specialists with proven expertise in pertaining therapeutic areas.
- The DMC members include physicians experienced in the treatment of the disease under investigation and a statistician.

The DMC will assess the progress of the trial, focusing on the safety outcomes for the benefit-risk evaluation at pre-specified time points, and to recommend to the sponsor whether to continue, modify, or stop the trial. The DMC will also receive urgent significant safety concerns for immediate evaluation. DMC meetings will be held at a frequency determined by the DMC or with regular intervals. The DMC will recommend continuation, modification or termination of the trial as detailed in the DMC charter. DMC recommendations as well as the final BI decision (if needed) will be reported to the appropriate RAs/HAs, IRBs/ECs, and to investigators as requested by local law. The tasks and responsibilities of the DMC are specified in DMC charter.

Relevant documentation on the participating (Principal) Investigators (e.g. their curricula vitae) will be filed in the ISF. The investigators will have access to the BI web portal Clinergize to access documents provided by the sponsor.

BI has appointed a Clinical Trial Leader, responsible for coordinating all required activities, in order to

- Manage the trial in accordance with applicable regulations and internal SOPs,
- Direct the trial team in the preparation, conduct, and reporting of the trial,
- Ensure appropriate training and information of Clinical Trial Managers, CRAs, and investigators of participating countries.

The organisation of the trial in the participating countries will be performed by the respective local or regional BI-organisation (Operating Unit, OPU) in accordance with applicable regulations and BI SOPs, or by a CRO with which the responsibilities and tasks will have been agreed and a written contract filed before initiation of the trial.

Tasks and functions assigned in order to organise, manage, and evaluate the trial are defined according to BI SOPs. A list of responsible persons and relevant local information can be found in the ISF.

Local laboratories and an IRT vendor will be used in this trial. Details will be provided in the IRT Manual and Local Laboratory Manual, available in the ISF.

9. REFERENCES

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10. APPENDICES

10.1 COVID-19 RELATED CONTENTS

Potential risks for trial participants due to the COVID-19 pandemic have been evaluated together with the principal investigator.

Both IMPs, tenecteplase and alteplase, are classified as tissue plasminogen activators (t-PA). The t-PA is a serine protease which is pharmacologically classified as a thrombolytic enzyme. It converts plasminogen into plasmin, a circulating plasma protein that binds to and digests the solid-phase fibrin component of a thrombus, and thereby restores the vessel patency. Based on the mode of action, the trial treatment is not expected to impose an increased susceptibility to infections or immune suppression. According to previous clinical trials no impaired lung function is observed or reported.

Patients have confirmed active infection with SARS-CoV-2 within the prior 3 months, as recorded in medical histories, will be excluded during screening visit.

In case of a confirmed infection after study drug treatment, appropriate measures for monitoring, treatment and quarantine will be implemented. Remote follow-up visits will be performed to replace any visits to site.

Due to local travel restrictions or lock down policy caused by COVID-19 pandemic, the trial conduct may need to be adjusted and remote visits could be performed as follows:

- For Visit 5 and Visit 6, if a patient is not able to come to the site for the visit(s), a remote visit (by video call or telephone, video call is highly recommended) should be performed within time window instead. The following assessment can be done remotely: Adverse Events, concomitant therapy, mRS, Barthel Index, Glasgow outcome score. The remote assessment of NIHSS may not be completely reliable on certain criteria, e.g., the assessment of motor function: some/no effort against gravity. The remote assessment of NIHSS is not judged by the investigator to be reliable unless, for example, the patient did not have motor function score during the previous NIHSS assessments, and the patient did not report an associated AE. The remote NIHSS should be clearly documented in case the investigator judges the result could be reliable.
- Safety lab tests during the following visit of Visit 6

  If by investigator's determination, any local safety lab analyses are necessary, the blood sample should be collected with every effort to reduce the risk virus infection. The results of the lab tests must be transferred to the investigator who ensures medical review and proper documentation in the eCRF.

10.2 SCALES OF EFFICACY ASSESSMENT

10.2.1 Modified Rankin Scale, MRS

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead
Total (0-6): _____	

10.2.2 Barthel index

Item	Score	Categories
Bowels	0	Incontinent or needs enemas
	5	Occasional incontinence (<once/week)
	10	Continent
Bladder	0	Incontinent/unable to manage catheter
	5	Occasional accident (<once/day)
	10	Continent
Grooming	0	Needs help with shaving, washing, hair or teeth
	5	Independent
Toilet use	0	Dependent
	5	Needs some help
	10	Independent on, off, dressing and cleaning
Feeding	0	Dependent
	5	Needs some help (e.g. with cutting, spreading)
	10	Independent if food provided within reach
Transfer (e.g. bed to chair)	0	Unable and no sitting balance
	5	Needs major help
	10	Needs minor help
	15	Independent
Mobility	0	Unable
	5	Wheelchair independent indoors
	10	Walks with help or supervision
	15	Independent (but may use aid)
Dressing	0	Dependent
	5	Needs some help
	10	Independent including fasteners
Stairs	0	Unable
	5	Needs some help or supervision
	10	Independent up and down
Bathing	0	Dependent
	5	Independent in bath or shower
Total	**100**	

10.2.3 Glasgow Outcome Scale

Grade	Description	Definition
1	Good recovery	Patient can lead an independent life, with or without minimal neurological deficit
2	Moderately disabled	Patient has neurological or intellectual impairment but is independent
3	Severely disabled	Patient conscious but totally dependent on others to get through daily activities
4	Vegetative survival	Patient is unresponsive, but alive; a "vegetable" in lay language
5	Dead	

10.2.4 National Institutes of Health Stroke Scale, NIHSS

1a. Level of consciousness	**0**	Alert	
	1	Not alert, but arousable with minimal stimulation	
	2	Not alert, requires repeated stimulation to attend	
	3	Coma	
1b. Ask patient the month and their age	**0**	Answers both correctly	
	1	Answers one correctly	
	2	Both incorrect	
1c. Ask patient to open/close eyes and form/release fist	**0**	Obeys both correctly	
	1	Obeys one correctly	
	2	Both incorrect	
2.Best gaze (only horizontal eye movements)	**0**	Normal	
	1	Partial gaze palsy	
	2	Forced gaze deviation	
3. Visual field testing	**0**	No visual field loss	
	1	Partial hemianopsia	
	2	Complete hemianopsia	
	3	Bilateral hemianopsia (blind, incl. Cortical blindness)	
4. Facial paresis (Ask patient to show teeth or raise eyebrows and close eyes tightly)	**0**	Normal symmetrical movement	
	1	Minor paralysis (flattened nasolabial fold, asymmetry on	
	2	Partial paralysis (total or near total paralysis of lower face)	
	3	Complete paralysis of one or both sides (absence of facial movement in the upper and lower face)	
5a. Motor Function-right arm	**0**	Normal (extends arm 90º or 45º for 10 sec without drift)	
	1	Drift	
	2	Some effort against gravity	
	3	No effort against gravity	
	4	No movement	
	9	Untestable (joint fused or limb amputated)	
5b. Motor Function-left arm	**0**	Normal (extends arm 90º or 45º for 10 sec without drift)	
	1	Drift	
	2	Some effort against gravity	
	3	No effort against gravity	
	4	No movement	
	9	Untestable (joint fused or limb amputated)	
6a. Motor Function -right leg	**0**	Normal (holds leg in 30º position for 5 sec without drift)	
	1	Drift	
	2	Some effort against gravity	
	3	No effort against gravity	
	4	No movement	
	9	Untestable (joint fused or limb amputated)	

10.2.4 National Institutes of Health Stroke Scale, NIHSS (continued)

6b. Motor Function -left leg	**0**	Normal (holds leg in 30º position for 5 sec without drift)	
	1	Drift	
	2	Some effort against gravity	
	3	No effort against gravity	
	4	No movement	
	9	Untestable (joint fused or limb amputated)	
7. Limb ataxia	**0**	No ataxia	
	1	Present in one limb	
	2	Present in two limbs	
8. Sensory (use pinprick to test arms, legs trunk and face, compare side to side)	**0**	Normal	
	1	Mild to moderate decrease in sensation	
	2	Severe to total sensory loss	
9. Best language (describe picture, name items)	**0**	No aphasia	
	1	Mild to moderate aphasia	
	2	Severe aphasia	
	3	Mute	
10. Dysarthria (read several words)	**0**	Normal articulation	
	1	Mild to moderate slurring of words	
	2	Near unintelligible or unable to speak	
	9	Intubated or other physical barrier	
11. Extinction and inattention (use visual double stimulation or sensory double stimulation)	**0**	Normal	
	1	Inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities	
	2	Severe hemi-inattention or hemi-inattention to more than one modality	

11. DESCRIPTION OF GLOBAL AMENDMENTS

11.1 GLOBAL AMENDMENT 1

Date of amendment		17 May 2021
BI trial number		1123-0040
BI Investigational Medicinal Product		Metalyse®, tenecteplase
Title of protocol		A phase III multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group trial to assess efficacy and safety of tenecteplase versus alteplase in Chinese patients with acute ischaemic stroke within 4.5 hours after stroke onset
Global Amendment due to urgent safety reasons		
Global Amendment		X
Section to be changed		**Lay Title**
Description of change		● Replaced "improve long-term physical activity" with "improve recovering of physical activity".
Rationale for change		● Updated the wording to describe the drug effect and objective with better accuracy.
Section to be changed		**FLOW CHART**
Description of change		● Updated the 2nd footnote. Added "This evaluation should also include time elapsed from imaging and possible changes in clinical symptoms since imaging."
Rationale for change		● Clarified that the investigator should fully evaluate whether to use the imaging performed in the transfer hospital, including time elapsed from imaging and possible changes in clinical symptoms since imaging.
Description of change		● Removed the description of pregnancy test
Rationale for change		● Pregnancy test (blood) takes a long time for the testing result, not suitable for eligibility evaluation in this trial.

Description of change		● Updated the footnote 6
Rationale for change		● Clarified only the result of random glucose (POCT) is mandatory before drug administration. Hematology lab results including coagulation items and platelet count are necessary before drug administration only when anticoagulants are used or there is suspicion of coagulopathy. Therefore, drug administration should not be delayed by other lab results.
Description of change		● Updated the footnote 8
Rationale for change		● Clarified a urine pregnancy test is required for WOCBP at screening or at earliest possible; the test result should not delay the drug administration.
Description of change		● Added the footnote 11 to NIHSS "The distal motor function will be assessed when NIHSS is scored."
Rationale for change		● Data on distal motor function improvement will be collected.
Section to be changed		**ABBREVIATIONS**
Description of change		● Added the abbreviation of POCT: Point - of- care testing
Rationale for change		● The random blood glucose test during screening was updated, to be performed via POCT.
Section to be changed		**2.1.3 Secondary endpoints**
Description of change		● Added footnote to sICH: "Both investigator and DMC will evaluate and report the sICH events. The conclusion on endpoints will rely on the adjudication outcome by DMC."
Rationale for change		● Clarified that the DMC's adjudication of sICH events should play the primary role in the endpoint conclusion.
Section to be changed		[redacted]
Description of change		

Rationale for change		[redacted]
Section to be changed		**3.3.3 Exclusion criteria**
Description of change		● Added "Current use of vitamin K based oral anticoagulants (e.g. warfarin) and a prolonged prothrombin time (INR >1.7 or PT >15 s) or current use of novel oral anticoagulants (i.e. dabigitran, rivaroxiban, or apixiban) with prolongation of aPTT and/or PT above the upper limit of the local laboratory reference range" under "Acute bleeding diathesis"
Rationale for change		● To exclude patient currently using oral anticoagulants to lower the risk of bleeding might occur after IMP administration.
Section to be changed		**5.1 ASSESSMENT OF EFFICACY**
Description of change		● Added the assessment of distal motor function together with NIHSS
Rationale for change		● Data on distal motor function improvement will be collected.
Section to be changed		**5.2.1 Cerebral imaging**
Description of change		● Added "This evaluation should also include time elapsed from imaging and possible changes in clinical symptoms since imaging."
Rationale for change		● Clarified that the investigator should fully evaluate whether to use the imaging performed in the transfer hospital, including time elapsed from imaging and possible changes in clinical symptoms since imaging.
Section to be changed		**Table 5.2.4:1 Safety laboratory tests**
Description of change		● Added the category of Point-of- care testing for glucose test for eligibility
Rationale for change		● The POCT of random glucose will benefit a rapid analysis outcome, which is proper and preferred for the eligibility evaluation for this trial
Description of change		● Updated the note 1
Rationale for change		● Clarified in screen only the result of random glucose (POCT) is mandatory before drug administration.

		Hematology lab results including coagulation items and platelet count are necessary before drug administration only when anticoagulants are used or there is suspicion of coagulopathy (as determined by investigator). Drug administration should not be delayed by other lab results
Description of change		● Updated the note 2
Rationale for change		● Editorial change to improve the wording.
Section to be changed		**5.2.5 Electrocardiogram**
Description of change		● Revised “administered” to “performed”.
Rationale for change		● Updated wording for better expression.
Section to be changed		**7.1 NULL AND ALTERNATIVE HYPOTHESES**
Description of change		● Changed “H02” to “H11”and “H11” to “H02”
Rationale for change		● Corrected the typo error.

11.2 GLOBAL AMENDMENT 2

Date of amendment		29 Sep 2022
BI trial number		1123-0040
BI Investigational Medicinal Product		Metalyse®, tenecteplase
Title of protocol		A phase III multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group trial to assess efficacy and safety of tenecteplase versus alteplase in Chinese patients with acute ischaemic stroke within 4.5 hours after stroke onset
Global Amendment due to urgent safety reasons		
Global Amendment		X
Section to be changed		**Clinical Trial Protocol Synopsis**
Description of change		Updated the site amount
Rationale for change		● Adjust according to the actual situation
Description of change		Updated total number of patients randomised, number of patients on each treatment and non-inferiority margin for risk difference for pre-defined regions
Rationale for change		● See rationale in Sections 7 and 7.5
Description of change		Updated the criteria for Covid-19 infected subjects
Rationale for change		● As the virus evolves, the impact to patient safety and study evaluation/completion will change. It'll be up to the investigator.
Section to be changed		**FLOW CHART**
Description of change		Added footnote 2 "MRI is also acceptable"
Rationale for change		● In very rare cases, MRI at screening will happen, which is also acceptable.
Description of change		Added footnote 12 to vital sign during V5 and V6
Rationale for change		● The vital sign could be an optional item in case remote visit is performed.

Section to be changed		**ABBREVIATIONS**
Description of change		Added the abbreviation of EAC: Endpoint Adjudication Committee
Rationale for change		● Responsibility of independent adjudication of all potential sICH events is picked out from DMC and to be conducted by Endpoint Adjudication Committee (EAC)
Section to be changed		**1.4.2 Risks**
Description of change		Added "Endpoint Adjudication Committee (EAC)" for extensive safety monitoring processes
Rationale for change		● Clarified the responsibility will be conducted by EAC
Section to be changed		**2. TRIAL OBJECTIVES AND ENDPOINTS**
Description of change		The footnote was changed/added for sICH in Sections 2.1.3
Rationale for change		● To keep the consistence with EAC responsibility
Section to be changed		**3.1 OVERALL TRIAL DESIGN**
Description of change		Updated total number of eligible AIS patients in context and Figure 3.1.1
Rationale for change		● See rationale in Section 7 and Section 7.5
Section to be changed		**3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUP**
Description of change		Added EAC for sICH events adjudication
Rationale for change		● Responsibility of independent adjudication of all potential sICH events was picked out from DMC and to be conducted by EAC
Description of change		Replaced the wording 'some local site staffs may be aware of the treatment' to 'the treatment information is available to patients and investigators'
Rationale for change		● Small adaptation for accurate expression
Description of change		The following new text was added: In addition, the BI analysis and data cleaning team will not have access to the treatment information during the trial conduct phase in order to reduce the risk of bias affecting the cleaning and planning for analysis of the data.

Rationale for change		● Explained that the BI BDS team will conduct a blind review to ensure that the PROBE design is well implemented.
Section to be changed		**3.3 SELECTION OF TRIAL POPULATION**
Description of change		Updated total number of patients randomised
Rationale for change		● See rationale in Section 7 and Section 7.5
Section to be changed		**3.3.3 Exclusion criteria**
Description of change		Updated the criteria for Covid-19 infected subjects
Rationale for change		● As the virus evolves, the impact to patient safety and study evaluation/completion will change. It'll be up to the investigator.
Section to be changed		**3.3.4.2 Withdrawal of consent to trial participation**
Description of change		The following new text was added: In case vital status is not yet available at Visit 6 (90-day), the follow up should be conducted as early as possible but within last patient last visit, and every effort should be made to obtain the patient vital status.
Rationale for change		● To mitigate the risk of endpoint missing for V6
Section to be changed		**4.1.5 Blinding and procedures for unblinding**
Description of change		Replaced the wording 'some local site staffs may be aware of the treatment' to 'the treatment information is available to patients and investigators'
Rationale for change		● Small adaptation for accurate expression
Description of change		The following new text was added: In addition, the BI analysis and data cleaning team will not have access to the treatment information during the trial conduct phase in order to reduce the risk of bias affecting the cleaning and planning for analysis of the data.
Rationale for change		● Explained that the BI BDS team will conduct a blind review to ensure that the PROBE design is well implemented.
Section to be changed		**4.2.2.1 Restrictions regarding concomitant treatment**
Description of change		Added the specific drug names for oral anticoagulants and antiplatelet agents
Rationale for change		● Clarified the restricted medication in detail

Section to be changed		**5.1 ASSESSMENT OF EFFICACY**
Description of change		[redacted]
Rationale for change		• Clarified the efficacy endpoints for which blind review should be performed.
Description of change		Added the instruction of NIHSS evaluation for specific and rare situations
Rationale for change		• Clarified the NIHSS assessment for untestable items
Section to be changed		**5.2.1 Cerebral imaging**
Description of change		The following new sentence was added "Usually, the infarction will not show in CT at visit 1 but in the imaging at the following visits, which should be regarded as the disease under study"…." MRI is acceptable"
Rationale for change		• To emphasize the limitation of early imaging and the particularity of lysis and avoid inappropriate or erroneous AE/SAE reporting and keep consistence.
Section to be changed		**5.2.3 Vital signs**
Description of change		Deleted the wording "armpit" and sentence "(the locations should be specified in the medical notes and the same location should be used at each time point when body temperature is measured)" for temperature.
Rationale for change		• To keep in accordance with clinical practice
Section to be changed		**Table 5.2.4: 1 Safety laboratory tests**
Description of change		Replaced Partial thromboplastin time (PTT) with Activated partial thromboplastin time (APTT) for coagulation test
Rationale for change		• Laboratory test was updated according to clinical practice and to keep consistence of exclusion criteria
Section to be changed		**5.2.7.2.3 Pregnancy**
Description of change		Deleted the requirement for pregnancy report from a pregnant partner of a male trial participant.
Rationale for change		• The previous requirement is not applicable
Section to be changed		**6.2.1 Screening period**

Description of change		Removed the description of pregnancy test at screening is mandatory for WOCBP and the identification of WOCBP
Rationale for change		● Pregnancy test should not delay the drug administration. For women who are considered of childbearing potential (WOCBP), a urine pregnancy test is required at screening or at earliest possible.
Section to be changed		**6.2.3 Follow-up period and trial completion**
Description of change		Added "In case vital status is not yet available at Visit 6, the follow up should be conducted as early as possible but within last patient last visit, and every effort should be made to obtain the patient vital status"
Rationale for change		● To emphasis the important of vital status and mitigate the risk of endpoint missing for V6
Section to be changed		**7 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE**
Description of change		Based on the meta-analysis results of historical randomised trials of intravenous alteplase for treatment of AIS [P14-11754], the unadjusted risk ratio for the effect of rt-PA versus placebo for the endpoint of mRS 0-1 was 1.24 (95% CI 1.14 to 1.36). Taking lower bound 1.14 for RR and assumed the response rate for alteplase group was 63%, the corresponding M1 for risk difference will be 7.7%; Taking f=0.35 (preserve 35% of M1), M2 will be 5.0%. Therefore, the non-inferiority margin (-M2) for risk difference will be -5.0%.
Rationale for change		● In accordance with CDE guideline of non-inferiority trials design, update of the non-inferiority margin
Section to be changed		**7.1 NULL AND ALTERNATIVE HYPOTHESES**
Description of change		Changed the non-inferiority margin (from -9.5% to -5.0%)
Rationale for change		● See rationale to Sections 7
Section to be changed		**7.2.2 Primary endpoint analyses**
Description of change		Added the fold words in sentence 'Secondary analysis of the primary endpoint will include **but not limited to**:'
Rationale for change		● To improve the accuracy

Description of change		[redacted]
Rationale for change		• To add more credibility for analysis of primary endpoint
Description of change		[redacted]
Rationale for change		• [redacted]
Description of change		[redacted]
Rationale for change		• Clarification
Section to be changed		**7.2.5 Safety analyses**
Description of change		The following text was removed: To this end, all AEs occurring between start of treatment and end of the study will be considered 'treatment-emergent'. Adverse events that start before first drug intake and deteriorate under treatment will also be considered as 'treatment-emergent.
Rationale for change		• Correction
Description of change		The following new text was added: The frequency of all AEs collected until the end of the study will also be summarized.
Rationale for change		• Clarification
Section to be changed		**7.5 DETERMINATION OF SAMPLE SIZE**
Description of change		Updated the whole section with updated margin, response rate assumptions and power which lead to a new sample size
Rationale for change		• In accordance with CDE guideline of non-inferiority trials design, updated of the non-inferiority margin; Based on recent ACT study results, updated of the response rate assumption for this study. The sample size determination therefore was updated accordingly.

Section to be changed		**8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL**
Description of change		Added Endpoint Adjudication Committee (EAC) for the independent adjudication of all potential sICH events after randomisation
Rationale for change		● To clarify the responsibility of adjudication is independently performed by EAC to keep a complete blind adjudication on safety data (sICH events)
Section to be changed		**9 REFERENCES**
Description of change		Added published references P14-11754, P22-05053, and P20-04860 in Section 9.1, and deleted unpublished reference c21624583 in Section 9.2
Rationale for change		● To keep consistent with statistical changes in Section 7.
Section to be changed		**10.1 COVID-19 RELATED CONTENTS**
Description of change		Added the instruction on remote assessment of NIHSS
Rationale for change		● To ensure high-quality data, helping investigators to get reliable conclusions from remote NIHSS assessment.
Section to be changed		**Throughout the protocol**
Description of change		Small adaptations and corrections throughout the document
Rationale for change		● Minor corrections and/or clarifications that have no effect on the way the study is to be conducted was done to keep consistency and/ or improve the accuracy.

11.3 GLOBAL AMENDMENT 3

Date of amendment		23 Feb 2023
BI trial number		1123-0040
BI Investigational Medicinal Product		Metalyse®, tenecteplase
Title of protocol		A phase III multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group trial to assess efficacy and safety of tenecteplase versus alteplase in Chinese patients with acute ischaemic stroke within 4.5 hours after stroke onset
Global Amendment due to urgent safety reasons		
Global Amendment		X
Section to be changed		**Clinical Trial Protocol Synopsis**
Description of change		Updated total number of patients randomised, number of patients on each treatment, statistical methods part including non-inferiority margin for risk ratio for pre-defined regions, analysis model and superiority margin for risk ratio for predefined regions.
Rationale for change		● See rationale to Sections 7 and 7.5
Section to be changed		**2.1.3 Secondary endpoints**
Description of change		Added the bold text in the endpoint definition: • Symptomatic Intracerebral Haemorrhage (sICH) per ECASS III definition **during on-treatment period**
Rationale for change		● Clarify the timepoints for endpoint definition
Section to be changed		[redacted]
Description of change		
Rationale for change		

Section to be changed		**3.1 OVERALL TRIAL DESIGN**
Description of change		Updated total number of eligible AIS patients in context and Figure 3.1.1
Rationale for change		● See rationale to Section 7.5
Section to be changed		**3.3 SELECTION OF TRIAL POPULATION**
Description of change		Updated the number of patients planned to be randomised.
Rationale for change		● See rationale to Section 7.5
Section to be changed		**5.2 ASSESSMENT OF SAFETY**
Description of change		Changed 'An ECASS III-defined sICH were assess' to: 'An ECASS III-defined sICH were assessed'
Rationale for change		● Correction
Section to be changed		**7 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE**
Description of change		Updated the non-inferiority margin determination paragraph: Based on the meta-analysis results of historical randomised trials of intravenous alteplase for treatment of AIS [P14-11754], the unadjusted risk ratio for the effect of rt-PA versus placebo for the endpoint of mRS 0-1 was 1.24 (95% CI 1.14 to 1.36). Taking lower bound 1.14 as M1 and f=0.5 (preserve at least 50% of the effect of alteplase), M2 will be 1.0677. Therefore, the non-inferiority margin (1/M2) for RR will be set as 0.937.
Rationale for change		● In accordance with CDE guideline of non-inferiority trials design, the non-inferiority margin was reset based on risk ratio and consider f≥0.5. ● It was believed that risk ratio met 'constancy', then risk ratio was chosen as the statistics for primary endpoint analysis.
Description of change		The following bold text was revised, "Based upon these design considerations, the trial will be analysed using **log binomial regression model with subsequent transformation of estimated parameters to risk ratio including the respective CI**, which will include terms for NIHSS at baseline, time to drug administration and age as covariates".

Rationale for change		● Considering that the statistics revised from risk difference to risk ratio, the primary analysis model was revised accordingly.
Description of change		Added the sentence "The primary treatment comparison will be as randomised, including the effects of performing thrombectomy."
Rationale for change		● Clarify details related to the primary estimand.
Section to be changed		**7.1 NULL AND ALTERNATIVE HYPOTHESES**
Description of change		Updated non-inferiority margin for risk ratio for pre-defined regions and superiority margin for risk ratio for predefined regions.
Rationale for change		● See rationale to Section 7
Section to be changed		**7.2.1 General considerations**
Description of change		Changed 'all randomised patients received any dose of treatment' to 'all randomised patients who received any dose of treatment'
Rationale for change		● Correction
Section to be changed		**7.2.2 Handling of intercurrent events**
Description of change		Added definition for primary estimand and specified the handling strategy for intercurrent events.
Rationale for change		● Re-consider the trial design from the perspective of estimand with reference to ICH E9 (R1) and present the attributes for the primary estimand.
Section to be changed		**7.2.3 Primary endpoint analyses**
Description of change		Primary endpoint analysis model was revised to log binomial regression model.
Rationale for change		● Considering the statistics for primary endpoint change from risk difference to risk ratio, the analysis model was revised accordingly.
Description of change		Added the sentence "The analysis will be related to the defined primary estimand and performed on FAS. Patients will be assigned to treatment groups as randomised. Multiple imputation method mentioned in Section 7.3 will be used to handle the missing data of primary endpoint".
Rationale for change		● Clarify the imputation approach used for the primary analysis.

Description of change		[redacted]
Rationale for change		• [redacted]
Section to be changed		**7.2.6 Safety analyses**
Description of change		Added the bold text in sentence "Laboratory data will be analysed qualitatively **and quantitatively**. "
Rationale for change		• Further clarify the analysis for lab data.
Section to be changed		**7.3 HANDLING OF MISSING DATA**
[redacted]		[redacted]
[redacted]		[redacted]
Description of change		Reworded the details for imputation using LOCF
Rationale for change		• Further clarify the details for imputation using LOCF regarding the missing data for surviving patients
Section to be changed		**7.5 DETERMINATION OF SAMPLE SIZE**
Description of change		The following bold part was revised "With an expected response rate of 65.8% in tenecteplase group, 63% in alteplase group for the primary endpoint, a type I error of 0.025(1-sided) and a non-inferiority margin of **0.937**, for a total sample size of **1478** patients, there is an **80%** power to demonstrate non-inferiority. Approximately **739** patients will be randomised in either group".
Rationale for change		• Updated the sample size based on new non-inferiority margin and power.

APPROVAL / SIGNATURE PAGE

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Document Name: clinical-trial-protocol-version-04

Title: A phase III multi-centre, prospective, randomised, open label, blinded endpoint (PROBE), active-controlled parallel group trial to assess efficacy and safety of tenecteplase versus alteplase in Chinese patients with acute ischaemic stroke within 4.5 hours after stroke onset

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Approval-Biostatistics	[redacted]	23 Feb 2023 10:16 CET
Approval-Clinical Trial Leader		23 Feb 2023 10:30 CET
Approval		24 Feb 2023 11:52 CET
Approval-Team Member Medical Affairs		01 Mar 2023 14:31 CET
Verification-Paper Signature Completion		02 Mar 2023 02:42 CET

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed