2. SYNOPSIS

<table>
<tr><td colspan="2">Name of Sponsor:
I.R.I.S., 50 rue Carnot - 92284 Suresnes Cedex – France
Laboratorios Servier, S.L. - Avenida de los Madroños, 33 – 28043 Madrid - Spain</td><td>(For National Authority Use only)</td></tr>
<tr><td colspan="2">Test drug
Name of Finished Product:
Not applicable
Name of Active Ingredient:
Not applicable</td><td></td></tr>
<tr><td>Individual Study Table Referring to Part of the Dossier</td><td>Volume:</td><td>Page:</td></tr>
<tr><td colspan="3">Title of study: Assessment of Active Thrombin-Activatable Fibrinolysis Inhibitor (TAFIa) plasma kinetics in Patients at Acute Stage of Ischemic Stroke: Prospective, Multicentre, Open, Non-randomised, Biomarker Study.
Protocol No.:CL2-RTCCAR-001
France: IDRCB 2017-A01562-51
Spain: EudraCT No.: 2017-002760-41</td></tr>
<tr><td colspan="3">Main Investigator
International Coordinator: [redacted]</td></tr>
<tr><td colspan="3">Study countries:
2 centres in France, 3 centres in Spain.
2 countries included 37 patients: 2 patients in France and 35 in Spain.</td></tr>
<tr><td colspan="3">Publication (reference): Not applicable.</td></tr>
<tr><td colspan="2">Studied period:
Initiation date: 15-November-2017
Completion date: 05-April-2018</td><td>Phase of development of the study:
Phase II</td></tr>
<tr><td colspan="3">Objectives:
The primary exploratory objective was to assess the systemic plasma kinetics of the active Thrombin-Activatable Fibrinolysis Inhibitor (TAFIa) during the acute stage of ischemic stroke in patients eligible for recombinant tissue Plasminogen Activator (rtPA) thrombolysis alone or rtPA thrombolysis followed by endovascular thrombectomy (EVT).
Other exploratory objectives were:
- To assess systemic TAFIa levels according to cerebral arteries occlusion site in anterior circulation (proximal or distal artery).
- To assess systemic TAFIa levels in ischemic stroke patients treated by rtPA thrombolysis ± EVT.
- To assess TAFIa levels according to the stroke clinical severity [National Institute of Health Stroke Score (NIHSS)] at baseline and 24h.
- To assess tissue plasminogen activator (tPA) plasma kinetics during acute stage of ischemic stroke in patients eligible for rtPA-thrombolysis alone or followed by EVT.
- To assess several haemostasis parameters according to systemic TAFIa levels.
- To assess in situ peri-thrombus TAFIa level in EVT eligible patients via blood sampling collected upstream to proximal part of the thrombus through intra-arterial catheter used for EVT.
- To assess TAFIa levels according to the characteristics of the clot retrieved during EVT (clot composition, clot weight).</td></tr>
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Methodology:
Prospective, international, multicentre, open, non-randomised phase II biomarker study in stroke patients treated according to standard of care without additional treatment administration.

Adult patients at acute stage of ischemic stroke were included if eligible for rtPA thrombolysis alone (**group A**) or rtPA thrombolysis + EVT (**group B**) according to current clinical guidelines and had imaging documented cerebral artery occlusion in anterior circulation.

Blood samples were taken in all patients through an antecubital venous catheter (different from catheter for rtPA administration) during the first 24-hours after inclusion for TAFIa and tPA plasma levels, as well as haemostasis parameters measurements. In patients from group B, arterial blood was sampled *in situ* upstream to the proximal part of the thrombus through the EVT device. The characteristics of the clot retrieved during EVT were collected in centres used to perform this analysis locally.
In addition, some clinical data were collected. NIHSS score was assessed at baseline and 24 h.
This study was performed in strict accordance with Good Clinical Practice.

Number of patients:
Planned: 40 patients in total, 2 groups (20 treated with rtPA only, 20 treated with rtPA and EVT).
Included: 37 patients in total, 2 groups (N = 20 treated with rtPA only, N = 17 treated with rtPA and EVT.

Diagnosis and main criteria for inclusion:
Main inclusion criteria

- Adult patients (≥ 18 years old) within 4.5 hours after ischemic stroke symptoms onset (or last time known normal).
- Imaging evidence of cerebral artery occlusion in anterior circulation: proximal (internal carotid artery (ICA), middle cerebral artery M1 (MCA-M1) or distal (MCA-M2, MCA-M3) arteries.
- Eligible for pharmacological thrombolysis alone or followed by EVT according to current clinical guidelines.
- Complete informed consent signed (by the patient or an authorised representative according to local regulation) prior to participation in the trial or within 12 hours if an abbreviated informed consent was signed prior to the participation in the trial (by the patient or an authorised representative according to local regulation) or orally agreed by the patient and witnessed by a person according to local regulation.

Main non-inclusion criteria

- Any known serious disease (including active malignancy, active infection) likely to interfere with the conduct of the study, according to investigator's judgment.
- Known pregnant or breastfeeding woman.
- Patients unlikely to cooperate in the study.
- Participation in another interventional study at the same time or within the past 3 months (participation in non-interventional registries or epidemiological studies was allowed).

Test drug:
Not applicable.

Comparator (Reference product and/or placebo):
Not applicable.

Duration of observation:
After inclusion, blood samples were taken within the first day at prespecified time-points and clinical outcome was assessed at P001 (up to 28h).

Criteria for evaluation:
Efficacy measurements:
No efficacy assessments were performed.

The neurological deficit scale, NIHSS was used to grade patients at study entry and to track their study progression 24 hours later.

Safety measurements:
All adverse events and other situations relevant to the safety of the participants were followed up and fully documented.

Other measurements:

- Biomarker TAFIa:
  - Kinetic measurements in groups A and B: intravenous (IV) blood samples over 24h after rtPA administration, collected at 7 time-points in group A (patients treated with rtPA thrombolysis only) and at 8 time-points in group B (patients treated with rtPA thrombolysis and EVT).
  - Arterial blood sample in group B only:
    - One in-situ arterial blood sample collected through EVT catheter.
- Biomarker tPA:
  - tPA kinetic measurements in groups A and B: same time-points as TAFIa IV blood samples.
- Haemostasis parameters:
  - D-Dimers, Fibrinogen, Fibrin Degradation Products, plasminogen, plasmin-anti-plasmin complexes assessment in groups A and B: IV Blood samples collected over 24 h after rtPA administration.
- Main clinical assessment and collected data:
  - Groups A and B:
    - NIHSS score assessment at baseline and 24h.
    - Collection of stroke characteristics, imaging and clinical data. If performed in the hospital common practice, imaging thrombus characteristics and infarct volume at inclusion and 24 ± 4h, aetiology of the stroke, early ischemic change in MCA territory scored, platelet count at baseline.
  - Group B only if performed in the hospital common practice, collection of:
    - Thrombolysis in Cerebral Infarction (TICI) score at baseline (before thrombolysis), after EVT in case of follow-up imaging and at 24 ± 4 hours.
    - Characteristics of the clot retrieved during EVT.
- Safety measurements:
  - All adverse events and other situations relevant to the safety of the participants were followed up and fully and precisely documented in order to ensure that the sponsor had the necessary information to continuously assess the risk of the clinical trial.

Statistical methods:
Analysis Set:
Included Set (IS):
All enrolled patients included in the study.
Biomarker Set (BMKS):
All enrolled patients included in the study with a rtPA full dose administered.

Efficacy analysis:
Not applicable.
Biomarkers (Plasma TAFia levels, Plasma tPA levels) were described at each time-point. Individual evolution median graphs over time were provided, overall and by subgroup of interest and measurement assay [by High-Performance Liquid Chromatography (HPLC) and by Enzyme-linked Immunosorbent Assay (ELISA)] in the BMKS.

Study patients: disposition baseline characteristics and treatments analysis and Safety analysis:
Descriptive statistics were provided.

TAFIa kinetic analysis:
The TAFIa baseline profile was analysed using a population based approach.
A disease modelling analysis on plasma activity according to time was performed, with a K-PD model.

An interim blind review was performed on TAFIa kinetics after the inclusion of approximately 17 patients in the rtPA group and 9 patients in the rtPA+EVT group. The study continued until 37 patients were recruited.

SUMMARY - CONCLUSIONS

DISPOSITION OF PATIENTS AND ANALYSIS SETS

	rtPA group (N = 20)	**rtPA+EVT group (N = 17)**	**All (N = 37)**
Included	20	17	37
Withdrawn due to			
- adverse event	1	-	-
Completed	19	17	36
Included Set (IS)	20	17	37
Biomarker Set (BMKS)	20	16	36

BASELINE CHARACTERISTICS

A total of 37 patients (17 women and 20 men) were included in the study: 20 patients were enrolled in the rtPA group and 17 patients in the rtPA+EVT group. 36 patients completed the study.

Both groups were similar in terms of demographic characteristics. The mean age was 75.8 ± 15.2 years, with a range from 40 to 96 years.

The NIHSS total score at baseline was lower in the rtPA group with median at 9.0 (moderate) (interquartile range 6-16.5) than in the rtPA+EVT group with median at 18.0 (severe) (interquartile range 14-20).

Five patients in the rtPA group (25%) had at least one previous stroke versus one patient (5.9%) in the rtPA+EVT group.

Nearly two thirds of patients in the included set (IS) had a history of hypertension (62.2%), almost one third had dyslipidaemia (29.7%), and type II diabetes was reported by 18.9% of patients.
Overall, there were 10 patients with a history of atrial fibrillation: 7 patients (35%) in the rtPA group and 3 patients (17.6%) in the rtPA+EVT group.

At the time of inclusion, 11 rtPA patients (55.0%) were treated by antithrombotic agents versus 5 rtPA+EVT patients (29.4%).

A majority of patients in the rtPA group had a distal occlusion (14 patients (70%) had their MCA M2 or M3 affected), and 5 patients (25%) had a proximal occlusion (MCA-M1). In the rtPA +EVT group, all patients except one (94%) had their ICA or MCA M1 affected. A cardioembolism aetiology was identified in 12 patients in the rtPA group (60.0%) and for 7 patients in the rtPA+EVT group (41.2%), according to the TOAST classification. The median ASPECT score was 10 in both groups [interquartile range (10; 10) in rtPA group and (9; 10) in rtPA+EVT group].

In conclusion, as expected occlusion of the cerebral artery in patients treated by rtPA alone was more often distal and stroke was less severe according to NIHSS than in patients treated by rtPA+EVT, whose occlusion was more often proximal, and resulting stroke was more severe.

SUMMARY – CONCLUSIONS (Cont'd)

BASELINE CHARACTERISTICS (Cont'd)

The main stroke baseline characteristics are summarized in the following table:

			rtPA (N = 20)	**rtPA+EVT (N = 17)**	**ALL (N = 37)**
Age (years)		n	20	17	37
		Mean ± SD	77.8 ± 17.0	73.5 ± 13.0	75.8 ± 15.2
		Median	83.5	73.0	80.0
		Min ; Max	40 ; 96	46 ; 94	40 ; 96
History					
	Hypertension	n (%)	11 (55%)	12 (70.6%)	23 (62.2%)
	Dyslipidaemia	n (%)	4 (20.0%)	7 (41.2%)	11 (29.7%)
	Type 2 diabetes mellitus	n (%)	4 (20.0%)	3 (17.6%)	7 (18.9%)
	Atrial fibrillation	n (%)	7 (35.0%)	3 (17.6%)	10 (27.0%)
	Antithrombotic agents	n (%)	11 (55%)	5 (29.4%)	16 (43.2%)
NIHSS at Baseline		n	20	17	37
		Mean ± SD	11.3 ± 6.0	16.9 ± 4.5	13.9 ± 6.0
		Median	9.0	18.0	15.0
		Q1 ; Q3	6.0 ; 16.5	14.0 ; 20.0	8.0 ; 19.0
		Min ; Max	3 ; 23	8 ; 23	3 ; 23
		n	20	17	37
	[1;5]	n (%)	2 (10.0)	-	2 (5.4)
	[6;13]	n (%)	11 (55.0)	4 (23.5)	15 (40.5)
	>= 14	n (%)	7 (35.0)	13 (76.5)	20 (54.1)
Location of cerebral artery occlusion		n	20	17	37
	Internal Carotid Artery	n (%)	-	3 (17.7)	3 (8.1)
	MCA M1	n (%)	5 (25.0)	13 (76.5)	18 (48.7)
	MCA M2	n (%)	9 (45.0)	3 (17.7)	12 (32.4)
	MCA M3	n (%)	5 (25.0)	-	5 (13.5)
	Other	n (%)	2 (10.0)	-	2 (5.4)

Percentages are based on n/N

EXTENT OF EXPOSURE

Not applicable.

SUMMARY – CONCLUSIONS (Cont'd)

RESULTS

The primary exploratory endpoint was the plasma activity of TAFIa over the first 24 hours following inclusion (hospitalisation). A rise in TAFIa activity was seen in both groups of patients at acute stage of ischemic stroke after rtPA administration, followed by a rapid decrease to baseline values 3 hours thereafter in patients treated or not by thrombectomy. At T1h after rtPA administration, the median peak of TAFIa activity reached in each arm was 0.8 U/L in the rtPA group and 2.53 U/L in the rtPA+EVT group.

Similarly, the measurement of TAFIa/TAFIai concentration using the ELISA method also showed a peak at about T1h. However a slower decrease of TAFIa/TAFIai concentration (up to 7 hours) was observed.

In the rtPA group, median TAFIa/TAFIai concentration showed an increase from baseline (53 ng/mL) until T1.5h (93 ng/mL), followed by a plateau until T3h (86 ng/mL) before decreasing to baseline values from T7h (49 ng/mL at T7h and 57 ng/mL at T24h).
In the rt-PA+EVT group, median TAFIa/TAFIai concentration reached a peak at T1h (204 ng/mL), then decreased at $T_{END-EVT}$ (164 ng/mL). A plateau was observed until T3h (157 ng/mL) followed by a slow decrease to baseline value observed at T24h (37ng/mL).
The median peak TAFIa/TAFIai concentration reached at T1h tended to be higher in the rtPA+EVT group (204ng/mL) than in the rtPA group (90 ng/mL), followed by a slower return to baseline values.

The peri-thrombus in situ TAFIa activity was assessed in rtPA+EVT group, but no clear difference was observed when compared with TAFIa activity measured in systemic plasma samples.

In general, there was a large inter-individual variability in both TAFIa activity and TAFIa/TAFIai concentration. In addition to this about 40% of samples were unexploitable due to haemolysis that jeopardized TAFI measurements.

There was no clear relationship between the site of arterial occlusion and TAFIa activity.

The kinetic of tPA observed during the study showed peak of tPA concentration at T1h with a rapid drop thereafter in both groups.

No correlation was found between the NIHSS score and TAFIa activity or TAFIa/TAFIai concentration at any time-point.

Haemostasis parameters were not described as there were no impacts on statistical analysis strategy.

In conclusion, this study does provide some new information about the pharmacokinetics of TAFIa activity and TAFIa/TAFIai concentration, particularly in patients undergoing EVT. In addition, the study demonstrates the different kinetics between TAFIa activity and TAFIa/TAFIai concentration and the link between both.

SAFETY RESULTS

No investigational product was used in this study, therefore no emergent adverse event could be imputed.
The table below summarises the main adverse events in the Included Set.

Overall summary for adverse events in the Included Set

		rtPA (N = 20)	**rtPA+EVT (N = 17)**
Patients having reported at least one:			
EAE	n (%)	-	-
Serious AE (including death)	n (%)	3 (15)	4 (23.5)
Study protocol-related serious EAE	n (%)	-	-
EAE leading to study withdrawal	n (%)	-	-
Serious EAE leading to Study withdrawal	n (%)	-	-
Patients who died	n (%)	1 (5)	1 (5.9)

SUMMARY – CONCLUSIONS (Cont'd)

SAFETY RESULTS (Cont'd)

Overall, 14 out of 37 patients reported a total of 31 adverse events. 8 patients were affected in the rtPA group and 6 in the rtPA+EVT group.

The most frequently affected SOCs (in more than 10% of the patients) were gastrointestinal disorders and metabolism and nutrition disorders.

The most frequent affected PT was hyperglycaemia: 3 patients in the rtPA group (15%).

There were 6 serious adverse events reported in the rtPA group: 1 haemorrhagic transformation stroke, 1 coma, 1 generalised tonic clonic seizure, 1 syncope, 1 bradycardia and 1 hypertension.

There were 6 serious adverse events reported in the rtPA+EVT group: 1 haemorrhagic transformation stroke, 1 myoclonic epilepsy, 1 atrial fibrillation, 1cardiac failure, 1 hypertension, 1 aspiration.

No study related emergent adverse event was observed.

Two patients died:

- One in the rtPA group died during the study period, from haemorrhagic transformation stroke, coma and general tonic clonic seizure (all considered as fatal serious adverse events).
- One in the rtPA+EVT group died after the end of the study, from a haemorrhagic transformation stroke as fatal serious adverse event.

CONCLUSION

The CL2-RTCCAR-001 was a prospective, multicentre, open, non-randomised, biomarker study without investigational medicinal product. The primary exploratory objective was to assess the systemic plasma kinetics of the activated Thrombin-Activatable Fibrinolysis Inhibitor (TAFIa) during the acute stage of ischemic stroke in patients eligible for recombinant tissue plasminogen activator (rtPA) thrombolysis alone or rtPA thrombolysis with endovascular thrombectomy (EVT). The attribution of patients to one of the two treatment groups was made by the investigator according to his/her clinical assessment and guidelines.

A rise in TAFIa activity was seen in both groups after rtPA administration in patients at acute stage of ischemic stroke, followed by a rapid decrease thereafter in patients treated or not by thrombectomy. An increase in TAFIa/ai was similarly observed at the end of rtPA infusion but the return to baseline value was slower due to overestimation of TAFI activity as both TAFIa and TAFIai are measured with ELISA test.

A large inter-patient variability was observed. Patients from both groups could present a high or low rise in TAFIa activity. TAFIa activity measured in arterial blood sampled peri-thrombus was comparable with the one measured in systemic venous sample.

It was not possible to define the relationship between NIHSS at baseline and TAFIa activity, or between NIHSS at 24h and TAFIa due to the high variability observed and to the low plasma sample availability (haemolysed tubes).

The reported adverse events were consistent with the underlying pathology. No adverse event was related to the study protocol.

Date of the report: 11 February 2019

Version of the report: Final version