MASTER SYNOPSIS

Name of Company: **I.R.I.S.** **50 rue Carnot** **92284 Suresnes Cedex - FRANCE**		*(For National Authority Use only)*
Name of Finished Product: NA		
Name of Active Ingredient: S212958		
Individual Study Table Referring to Part of the Dossier	**Volume:**	**Page:**

Title of study:

Target involvement and exploratory biomarkers investigations in healthy volunteers and in patients with type 2 diabetes mellitus

Protocol No.: CL1-RTCMP-001

EudraCT No.: 2017-000045-42

The description of the study protocol given hereafter includes the modifications of the 1 substantial amendment to the protocol.

Structures involved in the study:

Clinical investigator: [redacted]

Biomarker Analytical centre: [redacted]

Publication (reference):

Not Applicable

Studied period:	**Phase of development of the study:**
Initiation date: 29 March 2017 Completion date: 15 September 2018	Phase I

Objective(s):

Primary objectives

The primary objectives were, in the study population [healthy volunteers (HVs) and type 2 diabetic patients], to:

- Assess the plasma and platelet levels of biopterins [tetrahydrobiopterin (BH4), dihydrobiopterin (BH2)].
- Assess the platelet level of cyclic guanosine monophosphate (cGMP).
- Identify genetic variants of genes such as guanylate triphosphate cyclohydrolase 1 (GCH-1) coding for the
- protein guanylate triphosphate cyclohydrolase (GTP-CH)*.
- Assess vascular endothelial function (VEF).
- Assess coronary flow reserve (CFR).

**Genomic analyses were not performed.*

Secondary objectives

The secondary objectives were to assess:

- The difference of plasma and platelet levels of biopterins between HVs according to their age group as well
- in comparison to patients with type 2 diabetes (T2D).
- The relationship between the plasma or platelet levels of biopterins and the age of HVs.
- The relationship between plasma or platelet levels of biopterins and CFR.
- The relationship between plasma or platelet levels of biopterins and VEF.
- The relationship between cGMP and plasma or platelet levels of biopterins and any other relevant
- parameters.
- The general safety.

Methodology:

This study was an interventional, prospective phase 1 monocentre study.
HVs and patients with T2D mellitus were selected within 3 weeks before inclusion [day (D) 0]. The investigations were performed on 2 days (D1 and D2): VEF assessment and blood collection on D1 and CFR on D2. Then, subjects were discharged at the end of the last investigation day (D2).
This study was performed in strict accordance with Good Clinical Practice.

Number of subjects:

Planned: 75 HVs in three age groups: 25 aged 18-30 years, 25 aged 50-59 years and 25 aged 60-70 years.
25 patients with T2D aged 50-70 years.
Included: 78 HVs in three age groups: 26 aged 18-30 years, 26 aged 50-59 years and 26 aged 60-70 years.
10 patients with T2D aged 50-70 years.

Diagnosis and main criteria for inclusion:

Healthy volunteers
Male and female HVs, aged [18-30], [50-59] and [60-70] years, except with skin types 5 and 6 with body weight ≥ 50 kg and BMI between [18.0-28.0] kg/m2 inclusive, non- or ex-smokers, with non-clinically relevant findings in the medical history and physical examination, especially with regards to cardiovascular system, lung, liver and renal function, and normal blood and urine laboratory tests.
Patients with T2D
Male and female patients, aged [50-70] years, except with skin types 5 and 6, with body weight ≥ 50 kg and BMI ≤ 35 kg/m2, non or ex-smokers, T2D patients according to American Diabetes Association criteria, currently treated with standards of medical care in T2D at stable doses for at least 3 months, and with antihypertensive drugs allowed except beta blockers and calcium antagonists.

Test drug:

NA

Reference product or placebo:

Acetylcholine (Ach) for endothelial function assessment – subcutaneous administration by iontophoresis, resulting in a total delivery of 125 nanomoles.
Adenosine for magnetic resonance imaging (MRI) assessment – intravenous infusion of at least 3 min at a constant rate of 140 $\mu g*kg^{-1}*min^{-1}$ [could be adjusted to a maximum of 210 $\mu g*kg^{-1}*min^{-1}$ if the adenosine effect was not detected by increase in heart rate (HR) of 10 bpm and/or decrease in blood pressure (BP) > 10 mmHg].

Duration of treatment:

Study period: Hospitalisation of 2 nights and 3 days (from INCL in the morning until D2), with single administration of each pharmacological agent (Ach on D1 and adenosine on D2). Participants were discharged from the clinical unit on D2 after magnetic MRI.

Follow-up period: The end of study was on D2 after the MRI.

Criteria for evaluation:

Efficacy measurements:

NA

Clinical endpoints and biomarker measurements:

- VEF (laser imaging with Ach iontophoresis, D1).
- CFR (MRI + adenosine infusion, D2).
- Blood sampling (performed on D1) for measurements of:
  - Plasma level of BH2, BH4.
  - Platelet levels of BH2, BH4 and cGMP.
  - Relevant genomic measurements including GCH-1 (deoxyribonucleic acid / ribonucleic acid) variants*.

**Genomic analyses were not performed.*

Safety measurements:

- The recording of BP at ASSE, INCL, and D1.
- The reporting of adverse events (AEs).

Statistical methods:

Analysis Sets:

The Included Set (IS) consisted of all enrolled participants included in the study.

The Biomarkers Set (BMKS) consisted of all included participants having performed a blood sampling for the biomarker analysis.

Efficacy analysis:

NA

Biomarkers and clinical endpoints (markers of target involvement):

Biomarkers (BH4, BH2, cGMP):

These analyses were performed in the BMKS and included:

- Descriptive statistics of biomarkers/markers of target involvement by group of interest (according to the healthy or diabetic status and age) were provided.
- The differences in levels of biopterins were assessed with Hodges-Lehmann estimates and tested with a Kruskal-Wallis (K-W) test:
  - Between HVs and patients with diabetes.
  - Between HV age groups (if the test was significant a multiple comparison was performed using the Dunn method).
- The relationship between biomarkers and clinical endpoints was explored by scatterplots, correlation coefficients and descriptive statistics.

Additional analyses were performed:

- The differences in cGMP levels, VEF and CFR were assessed with Hodges-Lehmann estimates and tested with a Kruskal-Wallis test:
  - Between HVs and patients with diabetes.
  - Between HV age groups (if the test was significant a multiple comparison was performed using the Dunn method).
- The distribution of VEF values by analysis date was analysed, and was found to be non-random, VEF was recalculated as maximum cutaneous blood flow (CBF) – basal CBF (i.e. ΔCBF) and thus cutaneous vascular conductance (CVC) was recalculated as ΔCBF/BP. The distribution of ΔCBF was also analysed by analysis date and found to be random.
- The relationship between biomarkers and the recalculated ΔCBF was explored by scatterplots, correlation coefficients and descriptive statistics
- The relationships between BH2 and BH4, between cGMP and biopterins, and between biomarkers and clinical endpoints were analysed in all participants.

Study participants: disposition, baseline characteristics and safety analysis: Descriptive statistics were provided in the IS.

SUMMARY – CONCLUSIONS

Disposition of participants and analysis sets

Disposition of included participants (N=88)

	HV 18-30 (N = 26)	HV 50-59 (N = 26)	HV 60-70 (N = 26)	T2D (N = 10)	All (N = 88)
Included	26	26	26	10	88
Withdrawn due to	1	1	1	-	3
- non-medical reason	1	1	1	-	3
Completed	25	25	25	10	85
Included Set (IS)	26	26	26	10	88
Biomarkers Analysis Set (BMKS)	25	25	25	10	85

A total of 88 participants were included in the study: 78 healthy participants (26 in the HV 18-30 group, 26 in the HV 50-59 group, and 26 in the HV 60-70 group), and 10 participants with T2D (T2D group). The study protocol indicated the inclusion of 20 participants with T2D, but the recruitment in this category was challenging and to maintain the time constraints of the study and considering the nature of the study this requirement was considered unnecessary.

Eighty-five participants completed the study and 3 (1 in the HV 18-30 group, 1 in the HV 50-59 group, and 1 in the HV 60-70 group) were withdrawn for non-medical reasons (endothelial function assessment not possible due to technical fault with the device) on D1.

Baseline Characteristics

Main demographic characteristics in the Included Set (N = 88)

		HV 18-30 (N = 26)	HV 50-59 (N = 26)	HV 60-70 (N = 26)	T2D (N = 10)
Age (years)	mean ± SD	26.0 ± 3.4	55.5 ± 3.2	64.0 ± 3.0	61.0 ± 4.1
	Min ; Max	19 ; 30	50 ; 59	60 ; 70	56 ; 69
Weight (kg)	mean ± SD	70.3 ± 9.9	77.9 ± 10.3	77.1 ± 9.5	91.7 ± 14.7
	Min ; Max	50 ; 90	55 ; 97	56 ; 94	69 ; 112
BMI (kg/m^2)	mean ± SD	22.79 ± 2.08	25.03 ± 2.26	25.75 ± 1.86	28.34 ± 3.44
	Min ; Max	18.3 ; 26.9	20.1 ; 28.0	22.0 ; 28.0	21.7 ; 31.7
Gender	Female (%)	14 (53.85)	7 (26.92)	7 (26.92)	1 (10.00)
	Male (%)	12 (46.15)	19 (73.08)	19 (73.08)	9 (90.00)

The included participants corresponded well to the inclusion criteria in terms of demographic characteristics and medical criteria for their respective groups. There was some heterogeneity between the groups in the men:women ratio with ~1:1 to 1:3 in the HV groups and 1:9 in the T2D group. All participants were white Caucasian.

The T2D group had an age range of 56-69 years and thus was fairly close that of oldest HV group (60-70 years). The mean body weight and BMI of the T2D group were higher than those of the HV 50-70 group at 91.7 ± 14.7 kg versus 77.5 ± 9.8 kg and 28.3 ± 3.4 kg/m² versus about 25.4 ± 2.1 kg/m², respectively. The participants in the T2D group had a mean (± SD) duration of T2D of 7.2 ± 4.3 years, ranging from 1 to 14 years; 6 of the participants (60.0%) had a family history of T2D.

Clinical endpoints and biomarkers: results

Biopterin levels, vascular endothelial function and coronary flow reserve in the Biomarkers Analysis Set (N=85) – Median values

	HV 18-30 (N=25)	HV 50-59 (N = 25)	HV 60-70 (N = 25)	HV 50-70* (N = 50)	T2D (N = 10)
VEF (%)	977.0	392.0	297.0	326.50	257.0
ΔCBF (VEF recalc)	73.6	67.6	56.4	62.3	46.7
CFR	4.210	4.910	4.060	4.240	5.000
BH2 in plasma (ng/mL)	1.490	1.840	1.920	1.880	2.200
BH2 by platelet level ($pmol/10^9$ platelets)	3.860	4.970	6.770	5.370	7.850
BH4 in plasma (ng/mL)	0.810	0.620	0.670	0.655	0.960
BH4 by platelet level ($pmol/10^9$ platelets)	2.440	3.090	3.000	3.090	4.965
BH4/BH2 in plasma	0.470	0.350	0.380	0.365	0.480
BH4/BH2 by platelet levels	0.665	0.630	0.540	0.550	0.625
cGMP ($fmol/10^9$ platelets)	1602	2217	2662	2317	2114

**This group is composed of the participants in the HV 50-59 group and the participants in the HV 60-70 group.*
VEF vascular endothelial function; CVC cutaneous vascular conductance; CBF cutaneous blood flow; CFR Coronary flow reserve

- **In healthy participants**

In healthy participants, there was very high intragroup variability in VEF (%), and moderate-high intragroup variability in CVC and CFR.

VEF (%) decreased with age, and there was evidence of a relationship between VEF (%) and age group ($p < 0.0001$, K-W).

There was no relevant difference in CFR between the age groups ($p = 0.3833$) and no evidence of a relationship between CFR and VEF (%).

Due to the non-random distribution of VEF (%) by analysis date, VEF was recalculated as ΔCBF and, as a consequence. Using this new definition of VEF, which showed no effect of the analysis date, the intragroup variability was much lower than for VEF (%). Decreases in ΔCBF were observed with age (but not tested

statistically), and no correlation was observed between ΔCBF and CFR.
There was high intragroup variability in plasma BH2 and BH4 levels in all groups, with particularly high variability in the HV 50-59 group. There was a trend towards an increase in BH2 levels in plasma and by platelet level with age. This relationship appears significant on the K-W test: p=0.0108 for plasma levels and p=0.0363 for levels corrected by platelet concentration. (This correlation was also evident on Spearman estimation 038 [0.20; 0.53]; p = 0.0006). On the other hand, there was no evidence of a relationship between age group and BH4 plasma levels (p = 0.8104) or BH4/BH2 ratio (p = 0.2846) or by platelet levels. For BH2 and BH4 by platelet levels, a correlation was seen for BH2 (p = 0.0363), but not for the other parameters.
There was high intragroup variability in cGMP (by platelet level) in all groups. There no evidence of a relationship between cGMP and age group.
No marked correlations were observed between biopterin levels and cGMP, due to the high variability observed in the results in all groups.
There were no marked correlations between biopterin levels (or ratios) and clinical endpoints except for the following:

- a positive correlation between BH4/BH2 ratio (on platelet corrected values) and CFR, with a Spearman correlation coefficient of 0.43 [0.23; 0.59] and p = 0.0006.
- a positive correlation between BH2 (in plasma) and age: Spearman = 0.38 [0.20; 0.53] (p = 0.0006).
- a positive correlation between BH2 (by platelet level) and age: Spearman = 0.37 [0.17; 0.54] (p = 0.0030).
- a negative correlation between BH2 (in plasma) and ΔCBF: Spearman = -0.33 [-0.50; -0.13] (p = 0.0077).
- a negative correlation between BH2 (by platelet level) and ΔCBF: Spearman = -0.42 [-0.59; -0.21] (p = 0.0013).
- a positive correlation between BH4/BH2 ratio (on platelet values) and ΔCBF: Spearman = 0.32 [0.10; 0.51] (p = 0.0157).

- **In participants aged 50 to 70 years (healthy versus diabetic)**

Intragroup variability was high for VEF (%) and CVC and moderate for CFR.
VEF (%) and CVC tended to be lower in the T2D group than in the HV 50-70 group, and there was evidence of a relationship between health status (diabetic or non-diabetic) and VEF (%) (p = 0.0475).
There was no evidence of an effect of T2D on CFR (p = 0.8049).
There was a positive correlation between ΔCBP and CFR in this more elderly population (HV and T2D combined), with a Spearman correlation coefficient of 0.29 [0.06; 0.49] and p = 0.0342.
There were trends towards higher BH2 and BH4 levels and BH4/BH2 ratios in plasma and by platelet level in the T2D group than in the HV 50-70 group.
There was evidence of a relationship between BH2 levels in plasma and diabetic/non-diabetic status in participants aged 50 to 70 (p-value = 0.0758), but no evidence of a relationship between BH4 plasma levels (p = 0.2666) or BH4/BH2 ratio ((p = 0.6269). For BH2 and BH4 by platelet levels, a correlation was seen for BH4 (p = 0.0762), but not for the other parameters.
There were no marked correlations between biopterin levels (or ratios) and clinical endpoints except for the following:

- a positive correlation between BH4/BH2 ratio (on platelet corrected values) and CFR, with a Spearman correlation coefficient of 0.41 [0.20; 0.59] and p = 0.0023.
- a positive correlation between BH2 (by platelet level) and age: Spearman = 0.29 [0.06; 0.48] (p = 0.0352).

- **In all participants**

As expected, there was a highly positive correlation between BH2 levels and BH4 levels (platelet level), with a Spearman correlation coefficient of 0.60 [0.45; 0.71] (p < 0.0001).
No marked correlations were observed between biopterin levels and cGMP, due to the high variability observed in the results in all groups.
There was a negative correlation between BH2 and BH4 levels by platelet level versus VEF (%), with Spearman estimates of -0.31 [-0.49; -0.11] (p = 0.0108) and -0.25 [-0.43; 0.04] (p = 0.0443), respectively. No correlation was observed between BH4/BH2 ratio and VEF (%).
There was a trend towards a negative correlation between BH2 and BH4/BH2 ratio versus CFR, with Spearman estimates of -0.26 [-0.44; -0.06] (p = 0.0296) and 0.38 [0.19; 0.54] (p = 0.0014), respectively. No correlation was observed between BH4 levels.
There was a positive correlation between BH2 and BH4 (by platelet level) versus age, with Spearman estimates of 0.39 [0.20; 0.54] (p = 0.0008) and 0.29 [0.10; 0.46] (p = 0.0136), respectively.
There was a trend towards a negative correlation between cGMP levels and VEF (%), with a Spearman correlation coefficient of -0.22 and p = 0.0773. No correlation was observed between cGMP levels and CFR.
There was a trend towards a positive correlation between cGMP levels and age, with a Spearman correlation coefficient of 0.25 and p = 0.0333.

Overall, the results concerning clinical endpoints and biomarkers should be interpreted with caution, due to the high variability observed in the data.

Safety results

- **Adverse events**

		HV 18-30 (N = 26)	HV 50-59 (N = 26)	HV 60-70 (N = 26)	T2D (N = 10)
Participants having reported at least one:					
AE	n (%)	3 (11.5%)	-	6 (23.1%)	-
Protocol-related AE	n (%)	-	-	-	-
Serious AE* (including death)	n (%)	-	-	-	-
Participants who died	n (%)	-	-	-	-

No AEs were reported in the HV 50-59 group and the T2D group. Four (4) AEs were reported in 3 participants (11.5%) in the HV 18-30 group and 7 AEs were reported in 6 participants in the HV 60-70 group. The most frequently affected system organ class was Cardiac disorders, with 1 AE in 1 participant (3.8%) in the HV 18-30 group and 3 AEs in 3 participants (11.5%) in the HV 60-70 group. No AE was reported in more than 1 participant.

All AEs were mild.

At the end of the study, 2 AEs in 1 participant in the HV 18-30 group (pleural effusion and pericardial effusion) were recovering/improving; 1 AE in 1 participant in the HV 18-30 group (atrial septal defect) and 4 AEs in 3 participants in the HV 60-70 group (cardiac hypertrophy, cardiac septal hypertrophy, aortic dilatation and aortic valve incompetence) had unknown outcome. According to the Investigator comments, these AEs were all incidental findings during the MRI examination.

- **Laboratory tests**

NA, as laboratory tests were only performed at ASSE and INCL.

- **Other tolerance criteria (vital signs)**

Decreases from baseline in mean (± SD) supine systolic BP were observed on D1 after the last blood sampling in the HV 50-59 group (baseline: 124.3 ± 10.1 mmHg; D1: 115.3 ± 8.4 mmHg; change from baseline: -8.6 ± 8.6 mmHg) and in the T2D group (baseline: 130.2 ± 8.0 mmHg; D1: 115.5 ± 8.0 mmHg; change from baseline: -14.7 ± 6.1 mmHg). No participant presented a supine BP abnormality and no participant experienced an AE concerning supine BP.

CONCLUSION

In this exploratory phase I study, without test product, healthy volunteers were enrolled according to 3 age groups (18-30, 50-59 and 60-70 years; n = 26 for each), along with a group of patients with type 2 diabetic (T2D; aged 56-69 years; n = 10). The study duration was 2 days for each participant.

The primary objectives were to measure to measure vascular endothelial function (VEF; using laser imaging) and coronary flow reserve (CFR; by magnetic resonance imaging), as well as circulating levels of biopterins (BH2, BH4) and cGMP, and to assess if correlations exist within these biomarkers and clinical endpoints or between any of them and age or diabetic status. A planned genetic investigation was not performed due to the small size of the final sample population.

Main results:

- High intragroup variability was observed in the clinical endpoints and biomarkers.
- Median VEF% and an alternative measure, which was found to be more robust in the present investigation, ΔCBF (i.e. the change between resting and acetylcholine stimulated in cutaneous blood flow) tended to decrease with age. This relationship was evidenced statistically by the Kruskal-Wallis test between age groups (VEF% and ΔCBF) and by the Spearman correlation coefficient (age as a continuous variable) for VEF%. There was no effect of age on CFR, and no correlation between VEF% or ΔCBF and CFR.
- Median VEF% and ΔCBF tended to be lower in the T2D group than in the combined HV 50-70 year group; statistically significant on Kruskal-Wallis test. There was no effect of diabetic status on CFR. There was a positive correlation between ΔCBP and CFR in this more elderly population (HV50-70 and T2D combined).
- There was a trend towards an increase in BH2 levels with age, with statistical evidence of a relationship on age group. There was no evidence of relationships between BH4 or BH4/BH2 ratio with age.
- There was a slight trend towards higher BH2 levels in the T2D group than in the HV 50-70 group, but without statistical significance. No relationship between BH4 or BH4/BH2 ratio levels and diabetic status were evidenced.
- There was no evidence of a relationship between cGMP levels and age group, nor between cGMP levels and diabetic status. There was no correlations between cGMP levels and biopterin parameters

- There were no consistent correlations between biopterin levels and clinical endpoints, although some
- trends could invite further investigation.

This investigational protocol was safe and well tolerated; adverse events were infrequent and mild.

Date of the report: 13 March 2020

Signature of the report (study responsible):

Clinical and Translational Research Senior Project Leader [redacted]