CLINICAL TRIAL PROTOCOL

Protocol N° BPS0115 / OP099516.DOM
EudraCT n° : 2016-003629-41

Version n°6.0 - Date: - 27-MAR-2018

An Ascending Dose Tolerability Study and Pharmacokinetic Assessment in Healthy Male and Female Volunteers after Single & Multiple Oral Administration of DF2755A

Version	Modification	Author	Date	Application
1.0	Final Version	Eurofins Optimed	*05-OCT-2016*	NA
2.0	Inconsistency between text and flow-chart rectified and escalation dose procedure rectified	Eurofins Optimed	*23-NOV-2016*	
3.0	Maximum dose justified and duration of contraception extended	Eurofins Optimed	*30-NOV-2016*	
4.0	Change timing leukocytes subset for logistical reasons of laboratory and implementation according to the last ANSM requests: implementation stopping rules; Addition female in all cohort; updating concerning the modalities of immediate declaration for vigilance data; Implementation time window between all subjects of each cohort; start the MAD after all of SAD cohorts; change the highest dose of SAD from 900 mg to 700 mg and the highest dose of MAD from 400 bid to 300 bid; explanation about randomization for first two subjects; implementation about contraceptive method; Duration of subjects follow up; adding of pharmacokinetic profile in the dog; change of manufacturer	Eurofins Optimed/Dompé Farmaceutici spa	*07-APR-2017*	
5.0	A preliminary quantitative analysis of metabolites identified will be planned during the SAD; In order to proceed from SAD to MAD, a substantial	Eurofins Optimed/Dompé Farmaceutici spa	*24-MAY-2017*	

Version	Modification	Author	Date	Application
	amendment to the protocol with the results of SAD part of the study must be submitted to ANSM.			
6.0	**In order to better qualify the PD markers and add collection of blood for PD markers (Leucocyte subsets) during the SAD, starting from the second ascending dose.** **In order to extend the range of age and reduce the minimum number of enrolled women in SAD and MAD.** **In order to modify criteria 6.**	**Eurofins Optimed/Dompé Farmaceutici spa**	***27MAR2018***	

NAMES AND ADRESSES OF PARTICIPANTS

Sponsor:	**DOMPE FARMACEUTICI S.P.A** Via Santa Lucia 6 – 20122 Milano – Italy Tel.: + 39 0258 38 31 – Fax: +39 02 5838 33 24
Represented by:	**Marcello ALLEGRETTI, ChemD** Via Campo di Pile, snc – 67100 L'Aquila – Italy Tel.: + 39 0862 338 212 – Fax: +39 0862 338 219
Coordinating investigator/Principal investigator:	**Yves DONAZZOLO, MD, MSc,** **EUROFINS OPTIMED** **Clinical Pharmacology Unit** 1, rue des Essarts - 38610 Gières - FRANCE Tel: + 33 438 372 747 - Fax: + 33 438 372 741
Investigators:	**Mathilde LATREILLE, MD** **EUROFINS OPTIMED** Tel: +33 438 374 750 - Fax: +33 438 372 741 **Valentina HODAJ, MD** **EUROFINS OPTIMED** Tel: +33 438 374 752 - Fax: +33 438 372 741
Sponsor's Project manager:	PPD
Sponsor's Medical expert:	PPD
EUROFINS-Optimed Project manager:	PPD
Monitor:	PPD

Biological assays:	PPD
Bioanalytical determination for Pharmacokinetics	PPD
Pharmacokinetics assays:	PPD
Pharmacovigilance	PPD
Drug distribution center:	PPD
Data Manager:	PPD
Statistician:	PPD

CLINICAL STUDY PROTOCOL AGREEMENT

Protocol N°: BPS0115 / OP099516.DOM

Title: An Ascending Dose Tolerability Study and Pharmacokinetic Assessment in Healthy Male and Female Volunteers after Single & Multiple Oral Administration of DF2577A

The sponsor and the investigator agree to conduct the study in compliance with the clinical study protocol, International Conference on Harmonization (ICH) guidelines for current Good Clinical Practice (cGCP) in accordance with the Declaration of Helsinki and applicable regulatory requirements.

Sponsor:

Date: 28 MAR 2018 (dd-mmm-yyyy) ON BEHALF OF Signature :

Marcello ALLEGRETTI, ChemD
Chief Scientific Officer
DOMPÉ FARMACEUTICI S.P.A
Via Campo di Pile, snc
67100 l'Aquila – Italy

Date: 28 MAR 2018 (dd-mmm-yyyy) Signature :

Pier Adelchi RUFFINI, MD
Medical Expert
DOMPÉ FARMACEUTICI S.P.A
Via Santa Lucia, 6
20122 Milano – Italy

Date: 28 MAR 2018 (dd-mmm-yyyy) Signature :

Mauro P. FERRARI, PharmD
Early Clinical Development Manager
DOMPÉ FARMACEUTICI S.P.A
Via Santa Lucia, 6
20122 Milano – Italy

Principal Investigator:

Date: 29 MAR 2018 (dd-mmm-yyyy) Signature :

Yves DONAZZOLO, MD, MSc
EUROFINS OPTIMED
1, rue des Essarts
38610 Gières – France

SYNOPSIS

Title:	An Ascending Dose Tolerability Study and Pharmacokinetic Assessment in Healthy Male and Female Volunteers after Single & Multiple Oral Administration of DF2755A
Study product	DF2755A
Protocol No.:	BPS0115 / OP099516.DOM
Sponsor:	**DOMPÉ FARMACEUTICI S.P.A** Via Campo di Pile s.p.a 67100 L'Aquila – Italy
Number of study centers	Single center study
Principal Investigator:	Yves DONAZZOLO, M.D., M.Sc. EUROFINS OPTIMED, GIERES – France
Study Design:	The study is a phase I, single center, double-blind, placebo controlled, randomized, ascending single and repeated doses study in healthy male and female volunteers. The design consists of a double blind comparison of the test compound versus placebo in which the dose is increased in successive treatment periods. The Multiple Ascending Dose (MAD) will be initiated only after the authorization, from ANSM of a substantial amendment that will be presented in order to notify the results of Single Ascending Dose (SAD) part of the study. Besides in order to decide to move forward from SAD to MAD, the pharmacokinetic data for all doses administered during SAD will be evaluated (i.e. plasma: quantitative determination of DF2755A bound and unbound; qualitative and preliminary quantitative determination of known metabolites; urine and faeces: qualitative and quantitative determination of parent compound and qualitative and preliminary quantitative investigation of known metabolites). Dose will be escalated in order to achieve enough safety information on an interval of doses possibly encompassing both the effective dose and the maximum tolerated dose (defined as the highest dose devoid of any clinical signs/symptoms). Part A: Single doses of 50 mg oad, 150 mg oad, 450 mg oad or 700 mg oad of DF2755A are planned to be tested in healthy male and female volunteers. Part B: Repeated doses of 100 mg bid, 200 mg bid or 300 mg bid of DF2755A are planned to be tested in healthy male and female volunteers.
Study Objectives:	**Primary Objective:** • To evaluate the tolerability and safety of ascending single and repeated doses of DF2755A in healthy adult male and female volunteers. **Secondary Objectives:** • To determine the pharmacokinetics parameters of DF2755A • To establish a dose concentration-response relationship over a wide range of doses in order to select a narrower range of dose and dosing regimen to be subsequently studied in patients after single and multiple administration • To evaluate the effect of ascending single and repeated doses on the pharmacodynamics parameters • To compare metabolites pathway in Human with the one observed in animals

Investigational Treatment:	Name of the compound: DF2755A Pharmaceutical form: Capsules of 50 mg, and 200 mg Dose per administration: Part A: 50 mg oad, 150 mg oad, 450 mg oad or 700 mg oad Part B: 100 mg bid, 200 mg bid or 300 mg bid Timing for administration: Part A: Single oral dose administration on D1 according to the randomization. The administration will take place at around 8:00 a m with 200 ml of tap water, in sitting position, in fasting conditions. Part B: Repeated oral administration from Day 1 to Day 14. The administration will take place at around 8:00 a m and at around 8:00 p.m with 200 ml of tap water, in sitting position, in fasting conditions. Name of the compound: Placebo Pharmaceutical form: Matching capsule Dose per administration: NA Timing for administration: Part A: Single oral dose administration on D1 according to the randomization. The administration will take place at around 8:00 a.m with 200 ml of tap water, in sitting position, in fasting conditions. Part B: Repeated oral administration from Day 1 to Day 14. The administration will take place at around 8:00 a m and at around 8:00 p.m with 200 ml of tap water, in sitting position, in fasting conditions.
Subjects:	Part A: Eight healthy male and female subjects (6 active and 2 placebo) will be included in the four dose level. At least two healthy female subjects will be included in each cohort. One of them should be allocated to placebo. In total, 32 subjects will be enrolled. Part B: Twelve healthy male and female subjects (9 active and 3 placebo) will be included in each dose level. Between three and six healthy female subjects will be included in the cohort. At least one of them should be allocated to placebo. In total, 36 subjects will be enrolled.
Main Evaluation Criteria:	**Primary evaluation criteria:** AE, vital signs, 12-lead ECG, laboratory exams. **Secondary evaluation criteria:** Measurement of pharmacokinetics parameters: C_{max}, t_{max}, λ_z, $t_{1/2}$, $AUC_{0\text{-last}}$, AUC_{inf}, V_z/F, CL/F, $Ae_{0\text{-last}}$, CL_R, total amount in faeces, parent:metabolite(s) ratio. Measurement of Cytokine Inflammation Markers (part B only) and Leukocyte markers (CD11b & CD18), part A except for first cohort and part B.
Study Duration:	Part A: Screening within 21 days prior to the first administration. Hospitalization approximately for 4 days (D-1 morning to D4 morning). Consideration should be given to the hospitalization after the last dose, in fact it could be reviewed according to PK parameters of the compound after each dose of SAD. Expected duration: approximately 4 weeks for each participating subject. Part B: Screening within 21 days prior to the first administration. Hospitalization for 17 days (D-1 morning to D17 morning) Consideration should be given to the hospitalization after the last dose, in fact it could be reviewed according to PK parameters of the compound after each dose of MAD. Expected duration: approximately 6 weeks for each participating subject.
Statistics	**Sample size:** No formal sample size calculation was made. The subjects treated with test product and the subjects treated with placebo are considered sufficient to detect drug effects and determine pharmacokinetic parameters before escalating to the next dose level.

Table 1: STUDY FLOW CHART – PART A : from cohort 1 to cohort 4

Visit/Period	Screening	Inclusion	Treatment period			End of study visit
Day	D-21 to D-2	D-1	D1	D2	D3	D4
Informed consent	X					
Inclusion/Exclusion criteria[1]	X	X				
Previous Medical / Surgical History	X					
Prior/concomitant medications	X	X	X	X	X	X
Physical/Medical examinations	X	X				X
Body weight, height	X	X				X
Haematology	X	X		X		X
Hemostasis	X	X		X		X
Biochemistry	X	X		X		X
Urinalysis	X	X		X		X
Serology	X					
Urine test for drug abuse	X	X				
Alcohol breath test	X	X				
Admission		X				
Discharge						X
Randomization			X			
Study Drug / Placebo Administration[2]			X			
Vital signs : Blood pressure / Heart rate / Oral body temperature	X	X	X	X		X
12-lead ECG recording[3]	X	X	X	X	X	X
Blood sample for Pharmacokinetics			X	X	X	X
Blood sample for Pharmacodynamic[5]			X	X	X[6]	X[6]
Urine collection		X	X	X	X	X
Faeces collection		X	X	X	X	X
AE collection[4]	←					→

[1]Confirmation of the Inclusion/exclusion criteria prior to randomization number assignment;
[2]Dose: Cohort 1, 2; 3 & 4; Single dose in the morning; All subjects will be in fasting conditions from the evening before (at least 10 h, overnight);
[3]Safety ECG;
[4]All AEs will be reported
[5] Starting only from the second ascending dose for leucocytes markers only;
[6] T48h or T72h: time will be determined after results of the first dose administered.
When ECG, vital signs and PK are required at the same time, this specific order will be followed: ECG first, then vital signs, then and blood sample for PK at the end.

Table 2: DETAILED FLOW CHART – PART A

Visit/Period	**Inclusion**	**Treatment period**																			
Day	**D-1**	**D1**														**D2**			**D3**		**D4**
Theoretical time (h)		**Pre dose**	**T0**	**T0,5**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T12**	**T16**	**T24**	**T36**	**T40**	**T48**	**T60**	**T72**
Inclusion/Exclusion criteria	X																					
Prior/concomitant medications	←																					→
Physical/Medical examinations	X																					X
Body weight; height	X																					X
Haematology	X																X					X
Hemostasis	X																X					X
Biochemistry	X																X					X
Urinalysis	X																X					X
Urine test for drug abuse	X																					
Alcohol breath test	X																					
Admission	X																					
Discharge																						X
Randomization			X																			
Study Drug Administration			X																			
Blood pressure / Heart rate / Oral Body Temperature	X	X			X		X		X			X			X		X					X
12-lead ECG recording	X	X			X	X	X	X		X		X			X		X			X		X
Blood sample for Pharmacokinetics		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood sample for Pharmacodynamics		X	Cmax then Cmax +3h												X		X			X[1]		X[1]
Urine collection	← X		X								X					X			X		X	→
Faeces collection	← X		X																X		X	→
AE collection																						

[1] T48h or T72h: time will be determined after results of the first dose administered

Table 3: STUDY FLOW CHART – PART B : from cohort 5 to cohort 7

Visit/Period	Screening	Inclusion	Treatment period																End of study visit
Day	D-21 to D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17
Informed consent	X																		
Inclusion/Exclusion criteria[1]	X	X																	
Previous Medical / Surgical History	X																		
Prior/concomitant medications	←																		→
Physical/Medical examinations	X	X	X				X		X			X				X			X
Body weight; height	X	X																	X
Haematology[1]	X	X					X		X			X				X			X
Hemostasis[1]	X	X					X		X			X				X			X
Biochemistry[1]	X	X					X		X			X				X			X
Urinalysis[1]	X	X					X		X			X				X			X
Serology	X																		
Urine test for drug abuse	X	X																	
Alcohol breath test	X	X																	
Admission		X																	
Discharge																			X
Randomization			X																
Study Drug / Placebo Administration[3]			X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Blood pressure / Heart rate / Oral body temperature	X	X	X				X		X			X				X			X
12-lead ECG recording	X	X	X	X	X	X	X	X	X	X		X	X			X	X		X
Blood sample for Pharmacokinetics			X	X			X	X				X	X			X	X	X	X
Cytokine plasma collection			X				X									X		X	X
Leukocytes subsets blood collection			X					X[5]								X	X		
Urine collection		X	X	X			X	X				X	X			X	X	X	X
Faeces collection		X	X	X			X	X				X	X			X	X	X	X
AE collection[4]	←																		→

[1]Confirmation of the Inclusion/exclusion criteria prior to randomization number assignment;

[4]All AEs will be reported
[5]Time window from D5 to D7: Tpredose et T_{cmax}

When ECG, vital signs and PK are required at the same time, this specific order will be followed: ECG first, then vital signs,and blood sample for PK at the end.

Table 4: DETAILED FLOW CHART – PART B

Day	D-1		D1														
Theoretical time (h)	-24	-12	predose	0	0,5	1	2	3	4	5	6	7	8	9	10	12	16
Inclusion/exclusion criteria	X																
Prior/concomitant medications	←→																
Physical/Medical examinations	X		X														
body weight; height	X																
Haematology	X																
Hemostasis	X																
Biochemistry	X																
Urinalysis	X																
Urine test for drug abuse	X																
Alcohol breath test	X																
Admission	X																
discharge																	
Randomization				X													
Study drug administration				X												X	
Vital signs	X		X														
12-lead ECG recording	X		X		T_{cmax}											X	
Blood sample for pharmacokinetics			X		X	X	X	X	X	X	X	X	X	X	X	X	X
Cytokine			X														
Leukocyte subsets			X		T_{Cmax}												
Urine collection	X			X													X
Faeces collection	X			X													
AE collection	←→																

Day	D2			D3			D4		D5				D6		D7			D8		D9	
Theoretical time (h)	0/24	T_{cmax}	12	0/24	T_{cmax}	12	0/24	12	0	T_{cmax}	12	T_{cmax}	0/24	12	0	T_{cmax}	12	0/24	12	0	12
Inclusion/exclusion criteria																					
Prior/concomitant medications																					
Physical/Medical examinations									X						X						
body weight; height																					
Haematology									X						X						
Hemostasis									X						X						
Biochemistry									X						X						
Urinalysis									X						X						
Urine test for drug abuse																					
Alcohol breath test																					
Admission																					
discharge																					
Randomization																					
Study drug administration	X		X	X		X	X	X	X		X		X	X	X		X	X	X	X	X
Vital signs									X						X						
12-lead ECG recording	X^{a}	X	X	X^{a}	X	X	X^{a}			X	X		X^{a}			X	X	X^{a}			
Blood sample for pharmacokinetics	X								X	X	X	X	X								
Cytokine									X												
Leukocyte subsets									X												
Urine collection									X												
Faeces collection									X												
AE collection																					

Day	D10				D11		D12/13		D14														D15			D16		D17
Theoretical time (h)	0	T_{cmax}	12	T_{cmax}	0/24	12	0	12	0	0,5	1	2	3	4	5	6	7	8	9	10	12	16	24	36	40	48	60	72
Inclusion/exclusion criteria																												
Prior/concomitant medications																												←→
Physical/Medical examinations	X								X																			X
body weight; height																												X
Haematology	X								X																			X
Hemostasis	X								X																			X
Biochemistry	X								X																			X
Urinalysis	X								X																			X
Urine test for drug abuse																												
Alcohol breath test																												
Admission																												
discharge																												X
Randomization																												
Study drug administration	X		X		X	X	X	X	X																			
Vital signs	X								X																			X
12-lead ECG recording		X	X		X^{a}					T_{Cmax}											X		X					X
Blood sample for pharmacokinetics	X	X	X	X	X				X	X	X	X	X	X	X	X	X	x	X	X	X	X	X	X	X	X	X	X
Cytokine									X																	X		X
Leukocyte subsets																							X					
Urine collection	X								X													X		X			X	
Faeces collection	X								X															X			X	
AE collection																												←→

[5]Time window from D5 to D7: Tpredose et T_{cmax}

[a] 12-lead ECG will be record before administration (predose morning)

TABLE OF CONTENTS

1. SCIENTIFIC JUSTIFICATION AND GENERAL DESCRIPTION OF THE RESEARCH .18

1.1. Introduction and background 18
1.2. Summary of available results of non-clinical studies and clinical studies pertinent to the biomedical research concerned 18
1.2.1. Non clinical studies 18
1.2.1.1. Non clinical primary pharmacology 18
1.2.1.2. Safety Pharmacology 20
1.2.1.3. Pharmacokinetics 20
1.2.1.4. Toxicology 26
1.3. Summary of the potential risk and benefits to human subjects 28
1.4. Description and justification for the dosage regimen 28
1.5. Ethical considerations 29
1.6. Description of the population to be studied 30
1.7. References 30

2. STUDY OBJECTIVES AND PURPOSE 30

2.1. Principal objective 30
2.2. Secondary objectives 30

3. STUDY DESIGN 31

3.1. Evaluation criteria 31
3.1.1. Primary endpoint 31
3.1.2. Secondary endpoints 31
3.2. Design 31
3.3. Description of the measures taken to minimize and avoid bias 32
3.3.1. Randomization 32
3.3.2. Blinding 32
3.3.3. Risk assessment, study conduct and method of dose escalation 32
3.3.3.1. Number of subjects receiving the study drug simultaneously 32
3.3.3.2. Dose Escalation 32
3.4. Expected duration of subject participation 33
3.4.1. Description and duration of trial periods 33
3.4.2. Duration of follow-up 34
3.5. Stopping rules 34
3.5.1. Stopping rule applicable at any time 34
3.5.2. Stopping rule for a subject 34
3.5.3. Stopping rules within a group 34
3.5.4. Stopping rule for dose escalation 34
3.6. Removal of Subjects from Therapy or Assessment 35
3.7. Blind and procedures for unblinding 35
3.7.1. Coding list 36
3.7.2. Breaking the blind 36

4. STUDY POPULATION 36

4.1. Subject inclusion criteria 36
4.2. Subject non-inclusion criteria 37
4.3. Subject Identification 38
4.3.1. Screening number 38
4.3.2. Inclusion number 38
4.3.3. Randomization number 38
4.4. Subject withdrawal criteria 38
4.4.1. Definitive or temporary stop of a person's participation in the research 38
4.4.1.1. List of withdrawal criteria 38
4.4.1.2. Reasons for withdrawal 38
4.4.2. Definitive or temporary stop of a part or the totality of the research 38

4.4.2.1. Decided by the Sponsor....38
4.4.2.2. Decided by the Investigator....39
4.5. Premature discontinuation of subject....39
4.5.1. Data to be collected, and time of recording of these data....39
4.5.2. Methods of replacement....39
4.5.3. Methods of follow-up of premature discontinuations....39
4.6. Exclusion period....39

5. STUDY PRODUCT/TREATMENT....40

5.1. Description of the treatment(s)....40
5.1.1. Pharmaceutical form....40
5.1.2. Unit form, manufacture, packaging and labelling....40
5.1.3. Route and mode of administration....42
5.2. Accountability procedures for the investigational product(s)....42
5.2.1. Responsibilities....42
5.2.2. Accountability....43
5.2.3. Return and /or destruction of IMP....43
5.3. Medication(s)/treatment(s) permitted and no permitted before and/or during the trial....43
5.4. Procedures of monitoring subject compliance....43

6. ASSESSMENT OF PHARMACOKINETIC....44

6.1. Pharmacokinetic endpoints....44
6.2. Specification of pharmacokinetic parameters....46
6.3. Methods and timing for assessing, recording, and analysing pharmacokinetic parameters....47
6.3.1. Collection, treatment and storage of blood samples....47
6.3.2. Collection, treatment and storage of urine samples....50
6.3.3. Collection, treatment and storage of faeces samples....50
6.4. Analytical methods....51

7. ASSESSMENT OF PHARMACODYNAMIC....51

7.1. Cytokine Inflammation Marker analysis....51
7.2. Leukocytes subset blood samples....52

8. ASSESSMENT OF SAFETY....53

8.1. Specification of safety parameters....54
8.1.1. Clinical parameters....54
8.1.1.1. Blood pressure and heart rate....54
8.1.1.2. Physical examination....54
8.1.1.3. Body weight and height....54
8.1.1.4. Electrocardiogram (ECG)....54
8.1.2. Biological parameters....54
8.1.2.1. Routine laboratory observations....54
8.1.2.2. Urinalysis....55
8.1.2.3. Drug of abuse screening tests....55
8.1.2.4. Serologies....55
8.1.3. Other parameters....55
8.1.3.1. Alcohol breath test....55
8.2. Study Procedure....55
8.2.1. Screening procedures....55
8.2.2. Description by type of visit....56
8.2.3. Sampled blood volume....60
8.3. Evaluation of Adverse Event and Safety Information....61
8.3.1. Recording of adverse events....63
8.3.2. Analysis of adverse events....63
8.4. Procedures in place for the recording and notification of serious adverse events, SUSAR and new events....64
Periodical Reporting to Regulatory Authorities and Investigators....67

8.5. Type and duration of the follow-up of subjects after adverse events 67
8.6. PREGNANCY IN THE CLINICAL TRIAL 67
8.7. OVERDOSE 68
9. DATA MANAGEMENT 68
9.1. Definition of source data 68
9.2. Source document requirements 68
9.3. Use and completion of the eCRF and additional request 68
9.3.1.1. Data collection 68
9.3.1.2. Responsibilities 68
9.3.1.3. Data Management 69
9.4. Study monitoring 69
9.5. Access to data by the subjects 69
10. STATISTICS 70
10.1. Description of the statistical methods 70
10.1.1. Descriptive statistics 70
10.1.1.1. Subject demographic characteristics, medical history and diagnoses 70
10.1.1.2. Previous medications 70
10.1.1.3. Baseline safety parameters 70
10.1.1.4. Study drug and concomitant therapy 70
10.1.1.5. Pharmacokinetics 70
10.1.2. Analysis of principal criteria 71
10.1.2.1. Adverse event (for each part) 71
10.1.2.2. Physical examination, ECG and vital signs (for each part) 71
10.1.2.3. Laboratory parameters (for each part) 72
10.1.1. Analysis of secondary criteria 72
10.1.1.1. Formal statistics for pharmacokinetic 72
10.1.1.2. Correlation between pharmacokinetic and pharmacodynamic parameters 73
10.1.2. Calendar for analyses 73
10.2. Sample size 73
10.3. Level of significance to be used 73
10.4. Interim Analysis 73
10.5. Procedure for accounting, for missing, unused, and spurious data 73
10.6. Procedures for reporting any deviation(s) from original statistical plan 74
10.7. Selection of subjects to be included in the analyses 74
11. QUALITY CONTROL AND ASSURANCE 74
11.1. Quality Assurance 74
11.2. Quality Control 74
11.3. Sponsor audits and inspections by regulatory agencies 74
12. ETHICS 74
12.1. Informed consent form 74
12.2. Ethics Committee and Competent Authorities 75
12.3. Protocol amendments 75
12.4. Protocol deviations 75
13. DATA HANDLING AND RECORD KEEPING 75
13.1. Archival 75
13.2. Confidentiality 75
13.3. Ownership of results 75
14. FINANCING AND INSURANCE 76
15. PUBLICATION 76
16. LIST OF APPENDICES 76

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

General terms

ANSM	:	Agence Nationale de sécurité du médicament et des produits de santé
cGCP	:	Current Good Clinical Practice
CPP	:	Comité de Protection des Personnes
CRF	:	Case Report Form
CRO	:	Contract Rresearch Organisation
CSP	:	Code de la Santé Publique
CSR	:	Clinical Study Report
CQA	:	Clinical Quality Assurance
EC	:	Ethics committee
EOS		End Of Study Visit
FDA	:	Food and Drug Administration
FIM	:	First in men
GCP	:	Good Clinical Practice
GLP	:	Good laboratory practice
ICH	:	International Conference on Harmonization
IMP	:	Investigational Medicinal Product
IRB/IEC	:	Institutional review board / ethical review board
MedDRA	:	Medical Dictionary for Regulatory Activities
NTEAE	:	Non-Treatment Emergent Adverse Event
PI	:	Principal Investigator
OTR	:	On Treatment Response
SAD	:	Single administration dose
SmPC	:	Summary of Product Characteristics

Safety

AE	:	Adverse Event
AR	:	Adverse Reaction
LD50	:	Median Lethal Dose
MTD	:	Maximum Tolerated Dose
SAE	:	Serious Adverse Event
SAR	:	Serious Adverse Reaction
SUSAR	:	Suspected Unexpected Serious Adverse Reaction
TEAE	:	Treatment Emergent Adverse Event

Medical

BP	:	Blood pressure
bpm	:	beats per minute
BW	:	Body Weight
ECG	:	Electrocardiogram

HR	:	Heart Rate
IV	:	Intravenous
SBP	:	Systolic Blood Pressure

Biologics

ALT	:	Alanine Leucine Transferase
AST	:	Alanine serine transferase
CHC	:	Chronic viral hepatitis C
CPK	:	Creatine phosphokinase
GGT	:	Gamma Glutamy1 Transferase
HBs	:	Hepatitis B surface antigen
HCV	:	Hepatitis C virus
HIV	:	Human Immunodeficiency Virus
HIV1	:	Human Immunodeficiency Virus type 1
HIV2	:	Human Immunodeficiency Virus type 2
MCH	:	Mean Corpuscular Hemoglobin
MCHC	:	Mean Corpuscular Hemoglobin Concentration
MCV	:	Mean Corpuscular Volume
PNM		Polimorphonuclear leukocytes
RBC	:	Red Blood Cells
WBC	:	White Blood Cells

Bioanalysis

ALQ		Above limit of quantification
LOQ	:	Limit of quantification
ULOQ		Upper limit of quantification

Pharmacokinetics

Ae	Amount excreted in urine
$Ae_{0\text{-last}}$	Cumulative urinary excretion up to the final urinary collection interval
AUC	Area under the plasma concentration-time curve
$AUC_{0\text{-last}}$	
AUC_{inf}	Area under the plasma concentration-time curve from time zero to infinity
$AUC_{0\text{-}12}$	Area under the plasma concentration-time curve from time zero to 12 hours post-dosing
$AUC_{0\text{-}24}$	Area under the plasma concentration-time curve from time zero to 24 hours post-dosing
$AUC_{0\text{-}72}$	Area under the plasma concentration-time curve from time zero to 72 hours post-dosing
AUC_{τ}	Area under the plasma concentration-time curve within a 12-hour dosing interval at steady state
CL/F	Apparent clearance
CL_R	Renal clearance
CL_{ss}/F	CL/F at steady state

C_{max}		Maximum plasma concentration
$C_{ss,av}$		Average plasma concentration at steady state
$C_{ss,max}$		Maximum plasma concentration at steady state
$C_{ss,min}$		Measured plasma concentration at the end of the dosing interval
PK		Pharmacokinetic(s)
PTF%		Peak trough fluctuation
$t_{½}$		Terminal half-life
t_{max}		Time of maximum plasma concentration
$t_{ss,max}$		Time to maximum plasma concentration at steady state
Vd	:	Apparent volume of distribution
Vdss	:	Apparent volume of distribution at steady state
λ_z		Terminal phase rate constant

Data Management & Statistics

CI	:	Confidence Interval
DCF	:	Data Clarification Form
DRF	:	Data Resolution Form
eCRF	:	Electronic Case Report Form
GM		Geometric mean
Min		Minimum
Max		Maximum
N		Number
SD	:	Standard Deviation
SEM	:	Standard Error of the Mean

1. SCIENTIFIC JUSTIFICATION AND GENERAL DESCRIPTION OF THE RESEARCH

1.1. Introduction and background

DF2755A is a novel small molecular weight selective CXCR1/2 (chemokine receptor 1 and 2) inhibitor, aimed at providing an oral therapy for Interstitial cystitis/bladder pain syndrome (IC/BPS) and other inflammatory or pain-related conditions. DF2755A is a potent and selective allosteric modulator of both CXCR1 and CXCR2 and inhibits CXCL-1 and CXCL-8 (IL-8)-related human neutrophil chemotaxis while showing selectivity over other chemokines.

1.2. Summary of available results of non-clinical studies and clinical studies pertinent to the biomedical research concerned

1.2.1. Non clinical studies

1.2.1.1. Non clinical primary pharmacology

DF2755A inhibits CXCR1 and CXCR2 signalling after IL-8 binding, in human polymorphonuclear leukocytes (PMNLs), by a non-competitive allosteric mechanism of action. Pre-incubation of PMNLs with DF2755A at 1 µM demonstrated that DF2755A inhibits IL-8-induced G-protein activation without modifying either the number or the affinity of the receptors. The allosteric binding mode was demonstrated by site-specific mutagenesis studies and showed that DF2755A induces an uncoupled conformation that cannot transduce the IL-8 signal. DF2755A did not affect the migration of PMNLs induced by C5a and fMLP or monocyte migration induced by CCL2. Similarly, no effect of DF2755A was observed on the production of PGE2 induced by LPS. These results suggest that inhibition of IL-8-induced signalling is related to CXCR1/2 inhibition rather than other chemokines or cyclo-oxygenase activity. A panel screen with DF2755A at 10 µM against a series of adrenergic, histaminic, muscarinic, neurokinin, opioid and serotoninergic receptors revealed no inhibitory properties, while specific studies in cell-based assays identified an agonistic effect against the cannabinoid receptor 2 (CB2) with an EC50 of 17.4 µM but no effects towards the CB1 receptor, the bradykinin receptors B1 or B2 or dopamine receptor D2 or D3.

DF2755A inhibited CXCL1- and IL-8-induced migration of human PMNLs in a concentration-dependent manner with IC50 values of 2.1 and 4.2 nM for CXCL1 and IL-8, respectively, Figure 1. In the absence of chemokine stimulation, DF2755A did not modulate spontaneous migration.

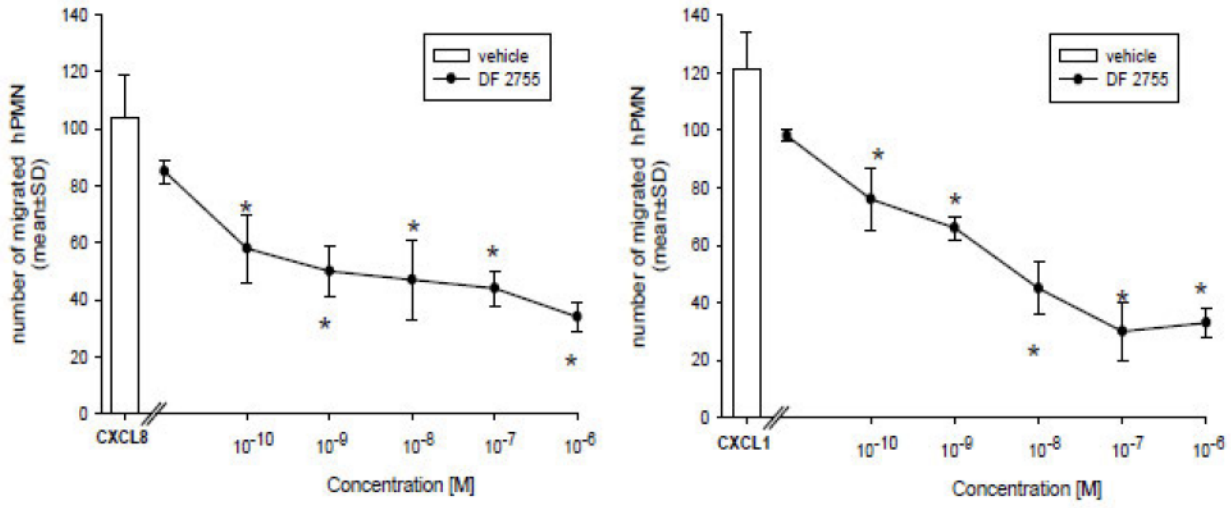

Figure 1. Human PMNLs were pre-incubated at 37°C for 15 min with vehicle or DF2755A. hPMNLs were then tested for their ability to migrate in response to IL-8 (1 nM) or CXCL1 (10 nM). Data are expressed as Mean ± SD of 5-6 independent experiments. Spontaneous hPMNL migration was 7 ± 11.

Statistical analysis was performed by Student's t test and Mann-Whitney U test. The significance thresholds were set at $p<0.05$. hPMNL migration was determined as described previously.

The effects of DF2755A were evaluated in a visceral pain model induced by repeated administration of cyclophosphamide in conscious rats. Treatment with cyclophosphamide at 75 mg/kg (i.p., three doses over 10 days), induced a strong decrease in withdrawal thresholds to noxious stimulus measured at the level of the lower abdomen and the hind paws.

DF2755A or vehicle were administered orally to rats at dose levels of 1, 3, 7, 10 and 30 mg/kg for two to nine days after the last administration of cyclophosphamide. Nociceptive thresholds were not affected by vehicle treatment while DF2755A produced a significant increase in mechanical thresholds, both in the abdomen and hind paw plantar surface; the effect was dose-dependent and appeared to plateau at the dose of 10 mg/kg, as no further increase in the effect was observed at 30 mg/kg, (Figure 2 – data for 7 and 30 mg/kg not shown). Statistically significant ($p<0.01$) effects were seen from 3 mg/kg, which was considered the minimally efficacious dose (MED).

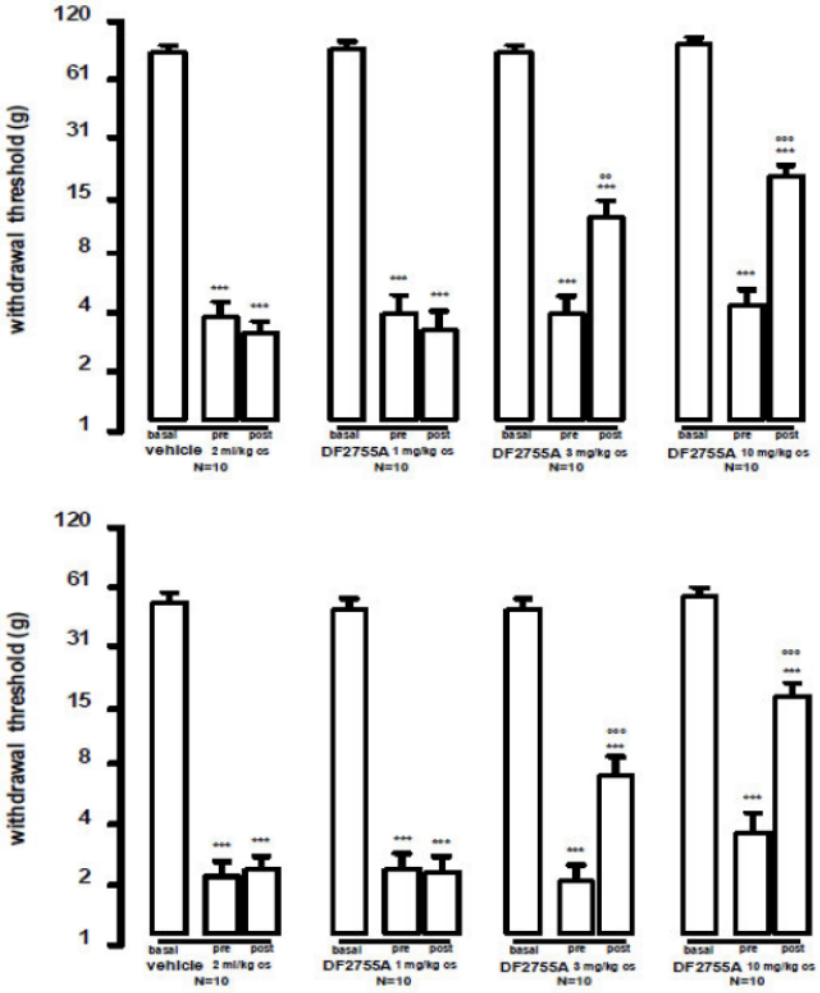

Figure 2. Effect of oral administration of vehicle, DF2755A at 1, 3 and 10 mg/kg on the abdominal (left) and hind paw (right) mechanical threshold, expressed in grams. Data represent the withdrawal threshold value before cyclophosphamide administration (basal); after cyclophosphamide and before treatment (pre); after cyclophosphamide and after treatment (post). Data are expressed as Mean ± S.E. ***$p<0.001$ versus basal values; °°$p<0.01$, °°°$p<0.001$, versus pre-treatment values (one way ANOVA with Tukey's test).

1.2.1.2. Safety Pharmacology

Study Type/Species/ Route/GLP Status	Dose Levels	Results
Neurobehavioral study/Rat/Single dose oral/GLP	50 mg/kg	---
	100 mg/kg	---
	150 mg/kg	NOEL
Respiratory function study/Rat/Single dose oral/GLP	50 mg/kg	---
	100 mg/kg	NOEL
	150 mg/kg	↑ respiratory rate (14.5%) at 1-3h post dose.
Effects on the hERG channel in HEK cells/in vitro/GLP	10 µM	93.09 ± 6.66 % of control
	50 µM	98.52 ± 10.2 % of control
	100 µM	80.90 ± 1.47 % of control
	300 µM	91.50 ± 1.06 % of control
	1000 µM	89.18 ± 2.74 % of control
Cardiovascular effect in anaesthetised Guinea pigs after IV administration/non-GLP .	1 mg/kg	---
	3 mg/kg	---
	10 mg/kg	NOEL
	30 mg/kg	↓ transient diastolic BP (non-significant)
Cardiovascular and body temperature Telemetry study/ Dog/Single dose oral/GLP [26]	30 mg/kg	---
	75 mg/kg	NOEL
	125 mg/kg	↑ heart rate (up to 52%), ↑ body temperature (up to 0.7 °C), ↓ systolic (3-8h post dose), ↓ arterial pressure, ↓ pulse pressure, ↓ RR, ↓PR and ↓ QT.

Abbreviations: --- = no noteworthy findings seen; ↑ = increased; ↓ = decreased; HEK = Human Embryonic Kidney; hERG = human ether-a-go-go-related gene; NOEL = No-observed-effect-level.

1.2.1.3. Pharmacokinetics

Pharmacokinetics of DF2755A were studied in rats and mice after single i.v. and oral administration; furthermore, pharmacokinetics were also evaluated after multiple oral administrations in rats and dogs to assess the toxicity. Besides, the absorption, distribution, metabolism and excretion after i.v. and p.o. of [^{14}C]DF2755A has been studied in rats and, after p.o. administration in dogs.

DF2755A is almost completely absorbed after oral administration in rats and the absolute bioavailability in both rats and mice is higher than 80% .

No gender differences in pharmacokinetic profile were observed in all animal species.

Following intravenous administration, the systemic clearance ranged from 2-8 % of the hepatic blood flow in all species .The volume of distribution at steady state (Vss) was 0.3-0.4 L/Kg in all species.

After oral administration, plasma levels generally reached maximal values within 2h in rats and then declines in a mono or biphasic manner with terminal elimination half-lives of 3-8h. Plasma half-lives were similar after intravenous and oral administration, suggesting that the absorption did not interfere with the elimination rate of DF2755A.

In the male and female fertility and embryo-fetal development combined study with DF2755A monohydrate in rats by oral gavage, the plasma exposure was measured in females on day 17 post-coitum.

At all dose levels the peak plasma concentration, C_{max}, was reached at 1 hour after dosing. For all groups t_{last} was 24 hours, as this was the time point on which the last sample was taken.

On Day 17 post-coitum, the C_{max} values and the systemic exposure, in terms of AUC_{last}, increased with increasing dose in a largely dose-proportional manner.

Table 1: Toxicokinetic parameters of DF2755Y in female pregnant rats

PK Parameters	Group numbers		
	Group 2 (50 mg/kg)	Group 3 (100 mg/kg)	Group 4 (150 mg/kg)
t_{last} (h)	24	24	24
t_{max} (h)	1	1	1
C_{max} (µg/mL)	189	245	284
C_{max}_D (kg·µg/mL/mg)	3.79	2.45	1.90
AUC_{last} (h·µg/mL)	2320	3000	4340
AUC_{last}_D (h·kg·µg/mL/mg)	46.5	30.0	29.0

Preliminary in vitro protein binding studies showed that DF2755A is highly bound to plasma proteins and varies according to DF2755A concentrations tested and seems to be saturable: 97.48% in human, 96.12% in rat and 92.7% in dog.

Radiolabelled compound [^{14}C]DF2755A is completely absorbed after oral administration and radioactivity is mainly excreted in faeces (Table 2). Elimination from plasma occurs with a $t_{1/2}$ from 1h to 4h after p.o or i.v. administration in rats with no substantial differences in PK profile between male and female (Figure 1).

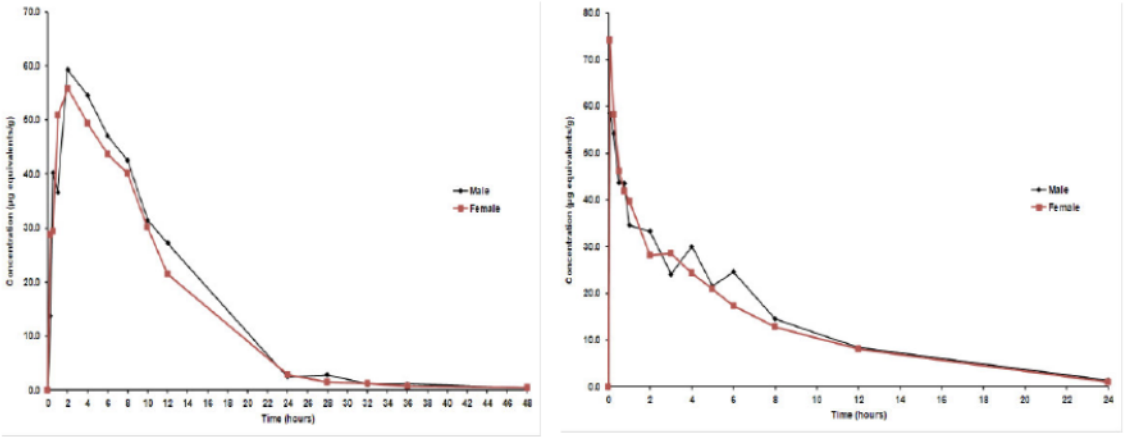

Figure 3: Representative profile showing concentrations of radioactivity in plasma obtained from male and female albino rats following a single oral (20 mg/kg) and intravenous administration (10 mg/kg) of [14C]DF2755A.

Table 2: Recovery of radioactivity (% of dose) from male albino rats over 168 hours following a single oral dose administration of [^{14}C]-DF2755A at a nominal rate of 20 mg free acid/kg

Sample	Cumulative excretion in rats (% radioactive dose) after oral administration of 20 mg/kg free compound of [^{14}C]DF2755A				
	Collection time (h)	Male		Female	
		Mean	SD	Mean	SD
Urine	0 - 168	21.53	5.28	24.13	0.47
Faeces	0 - 168	72.7	7.61	77.10	7.32
Expired air CO2 Trap 1	0 - 168	0.03	0.02	0.03	0.02
Expired air CO2 Trap 2	0 - 168	0.04	0.02	0.04	0.02
Cage Wash	0 - 168	0.13	0.07	0.26	0.16
Carcass	0 - 168	0.54	0.16	0.51	0.15
Total recovery	**0 - 168**	**94.97**	**2.09**	**102.06**	**6.99**

Pharmacokinetic profile in dog after single oral doses of [^{14}C]DF2755A showed that the maximum mean plasma total radioactivity concentrations of 116 and 158 µg equivalents/g respectively, were measured 30 minutes post dose (Table 3). Radioactivity remained detectable in plasma at the final sampling time with 0.307 and 0.443 µg equivalents/g detected at the 168 hour timepoint in male and female animals respectively. The apparent terminal half-lives of radioactivity in plasma were estimated at 111 and 125 hours, in male and female animals respectively (Figure 4).

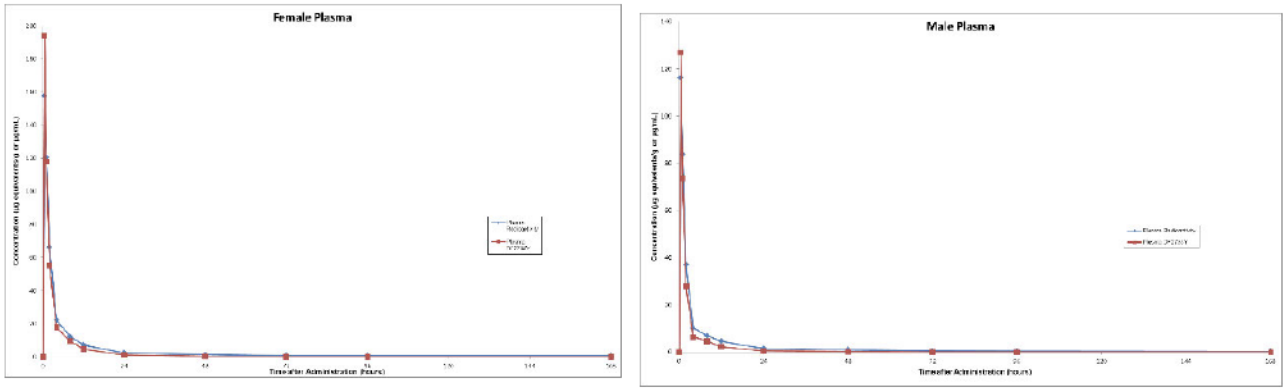

Figure 4: Representative profile showing concentrations of radioactivity in plasma obtained from female Beagle dogs following a single oral administration of [14C]DF2755A at a target dose rate of 40 mg free compound/kg

Concentrations of unchanged DF2755A (i.e. DF2755Y) in plasma were also determined. Maximum mean plasma concentrations of 127 and 194 µg/mL respectively, were measured 30 minutes post dose (Table 3).

Table 3: Pharmacokinetic parameters of radioactivity in plasma and DF2755 free compound (DF2755Y) in plasma following a single oral administration of [14C]DF2755A to male and female Beagle dogs at a target dose of 40 mg free compound/kg

Parameter	Male [a]		Female [a]	
	Radioactivity in plasma	DF2755Y in plasma	Radioactivity in plasma	DF2755Y in plasma
tmax (h)	0.5	0.5	0.5	0.5
C_{max} (µg equiv/g))	116.2	127	157.7	194
AUC_{0-t} (µg equiv·h/g)	357.783	219.637	557.693	398.941
AUC_{0-inf} (µg equiv·h/g)	406.816	220.530	637.277	399.159
k (h–1)	0.00626	0.0519	0.00557	0.0622
t½ (h)	110.7	13.4	124.5	11.1

a Results are based on a mean of 3 animals per sampling time point

Note: Radioactivity values are expressed as µg equivalents/g and the units for DF2755Y plasma concentrations are µg/mL

The difference between the two measurements of total radioactivity and DF2755A is due to the metabolite load.

Excretion of administered radioactivity was predominantly via the faecal route, with means of 57.75% and 56.44% of dose observed from 0 to 168 h post-dose, for males and females, respectively. Over the same period, radioactivity recovered in urine accounted for means of 26.43% and 31.94% of dose, for males and females, respectively. An additional 2.8% of radioactivity was measured in the cage washings in both male and female groups (Table 4).

Table 4: pharmacokinetic parameters of radioactivity in plasma and DF2755 free compound (DF2755Y) in plasma following a single oral administration of [14C]DF2755A to male and female Beagle dogs at a target dose of 40 mg free compound/kg.

Sample (0 – 168 h)	Mean recoveries (% dose) ± SD	
	Male (n=3)	Female (=3)
Urine	26.43 ± 2.18	31.94 ±3 .47
Faeces	57.75 ± 2.82	56.44 ± 2.14
Cage wash	2.82 ± 0.61	2.84 ± 0.86
Total	87.00±3.81	91.22±1.92

A proposed metabolic pathway in rat and dog of DF2755A is identified following single oral administrations of radiolabelled compound [^{14}C]DF2755A.

From the data in vivo in rats and dogs it is possible to see that in plasma, the largest radio-component circulating was parent drug (DF2755A) accounting for >83% of the sample radioactivity at all times up to 24 hours in the rat and >66% of the sample radioactivity at all times up to 24 hours in the dog. No metabolite represented >10% of the sample radioactivity in the rat plasma.

One Phase II metabolite was observed in the dog plasma – M8 (a glucuronide conjugate), which accounted for 2-10% of sample radioactivity.

In urine, only one single component, in both rat and dog, represented >10% of the administered radioactivity (M2 in the female dog (total 10.7% administered radioactivity). Both species would appear to contain many metabolites, with the rat urine containing small amounts of parent DF2755A in addition to several Phase I metabolites M2 (oxidation then hydrolysis), M3 (hydrolysis) and M4 (oxidation), all accounting for >10% of the sample radioactivity. The dog urine contained very small concentrations of parent DF2755A in addition to four metabolites, each representing > 10% of the sample radioactivity; two Phase I metabolites - M2 (oxidation then hydrolysis) and M3 (hydrolysis) and two Phase II metabolites M7 (taurine conjugate) and M8 (glucuronide conjugate).

In rat faeces, the largest radio-component was M1 (a sulphate conjugate of a Phase I hydrolysed metabolite) which accounted for approximately 50% of the total administered radioactivity. Parent drug (DF2755A) was also observed, at concentrations of >12% of total administered radioactivity. The other notable species, accounting for > 10% of administered and sample radioactivity was a Phase I metabolite M2 (oxidation then hydrolysis). The largest component in dog faeces was a Phase II metabolite M7 (taurine conjugate), accounting for >25% of the total administered radioactivity (>60% of the sample radioactivity). The only other component representing >10% of sample radioactivity was parent drug (DF2755A) which accounted for approximately 10% of administered radioactivity.

There are some similarities between the two species, in terms of the metabolites observed across the different matrices. Both species also generate both Phase I and Phase II metabolites. It would appear that the rat generates more Phase I metabolites and the dog generates some species-specific Phase II conjugates.

Quantitative whole body autoradiography (QWBA) studies show that [^{14}C]DF2755A is fairly rapid distributed into tissues when administered orally to both albino and partially pigmented male rats and widespread in the body , and no melanin binding is observed by the compound and/or its metabolites. The maximum concentrations of radioactivity in the vast majority of tissues were achieved at 2h and the largest proportion of dose was recovered in CCI and CCI at 24h. Very low concentration were found CCI ranging from 0.996 1.79 µg/eq DF2755A/g of tissue after 2h of administration in albino and partially pigmented rats. Indicating that the compound do not CCI ratio were 0.03 in abino rats and 0.04 in partially pigmented rats).

Maternal tissue distribution of the radiolabelled compound [^{14}C]DF2755A into pregnant female rats is comparable with tissue distribution of not radiolabelled compound DF2755A in male and female rats. The maximum concentrations of radioactivity were visible in foetal tissues at 6h after the administration and excreted also into milk, meaning that pups would be exposed to the compound.

The toxicokinetic of DF2755A has been studied after repeated administration (28 days) of DF2755A in rat at the dose of 30, 60 and 150 mg/kg b.i.d. corresponding to 60, 120 and 300 mg/kg/day.

The plasma concentration time curves displayed a clear double peak pattern associated with the twice daily dosing of DF2755A (figure 5). The plasma concentrations of DF2755A increased rapidly after the first and second dosing. The peak plasma concentration was determined after the first and the second administration. After the first dosing the peak plasma concentration was reached 1 or 2h after dosing, after the second dosing the peak plasma concentration was reached 4h after dosing, i.e. 12h. For all

groups t_{last} was 24h, as this was the time point on which the last blood sample was taken. No consistent changes in t_{max} in time, dose or sex were observed.

Time-dependent change in exposure was evaluated by comparing the C_{max} values after the first and second administration and the AUC values in time. In general no clear differences were noted between the C_{max} values after the first and second administration in time. At all dose levels AUC_{0-8}, AUC_{8-24}, and AUC_{last} values were comparable on Day1 and Week4 in both male and female animals, indicating no accumulation or induction occurred at the dose levels used in this study (Table 5).

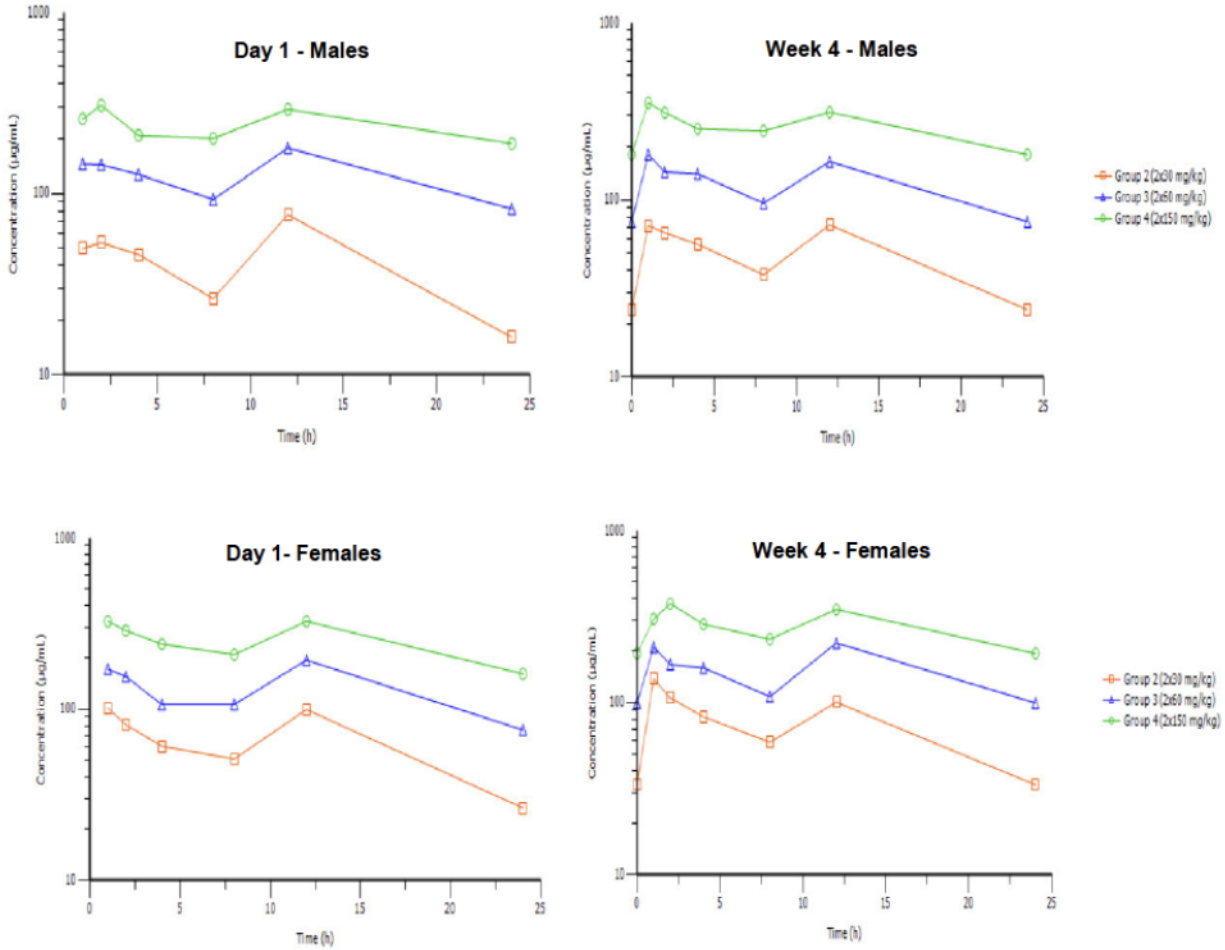

Figure 5: Mean plasma concentration-time profiles of DF2755A in Day 1 and Week4- after 30, 60 and 150 b.i.d. in male and female.

Table 5: Toxicokinetic parameters in plasma following a single and multiple oral administration in rats at different doses

PK Parameters	Group2 30mg/kg - b i.d.		Group3 60mg/kg b i.d.		Group4 150mg/kg - b i.d.	
	Male	Female	Male	Female	Male	Female
Day 1						
t_{last} (h)	24	24	24	24	24	24
$t_{max,1}$ (h)	2	1	1	1	2	1
$t_{max,2}$ (h)	12	12	12	12	12	12
$C_{max,1}$ (µg/mL)	53.5	101	145	171	306	325
$C_{max,2}$ (µg/mL)	76.3	99.4	178	193	291	325
AUC_{0-8} (µg·mL/h)	321	507	928	935	1750	1890
AUC_{8-24} (µg·mL/h)	759	1060	2100	2210	3860	3980

PK Parameters	Group2 30mg/kg - b i.d.		Group3 60mg/kg b i.d.		Group4 150mg/kg - b i.d.	
	Male	Female	Male	Female	Male	Female
AUC_{last} (µg·mL/h)	1080	1560	3030	3150	5600	5870
#AUC_{last} (µg·mL/h)	18.0	26.1	25.3	26.2	18.7	19.6
AUC_{∞} (µg·mL/h)	1210^	1930^	4130^	4560*	8680^	9000^
#AUC_{∞}(h·kg·µg·mL/mg)	20.2^	32.2^	34.5^	38.0^	28.9^	30.0^
$t_{1/2}$	5.70^	9.64^	9.29^	13.0^	11.3^	13.5^
Vz/F	407^	432^	389^	492^	565^	649^
Week4						
t_{last} (h)	24	24	24	24	24	24
$t_{max,1}$ (h)	1	1	1	1	1	2
$t_{max,2}$ (h)	12	12	12	12	12	12
$C_{max,1}$ (µg/mL)	71.5	137	180	210	353	375
$C_{max,2}$ (µg/mL)	72.3	100	164	222	311	347
AUC_{0-8} (µg·mL/h)	426	679	1040	1200	2150	2290
AUC_{8-24} (µg·mL/h)	798	1120	1960	2590	4060	4400
AUC_{last} (µg·mL/h)	1220	1800	3000	3790	6210	6680
#AUC_{last} (µg·mL/h)	20.4	30.0	25.0	31.6	20.7	22.3
$t_{1/2}$ (h)	7.53^	7.16^	9.67^	9.25^	19.9^	9.26^

t_{max1}, t_{max2}, C_{max1} and C_{max2}, are the t_{max} and C_{max} (peak concentration) after respectively the first and second dosing
:dose-normalized to 1mg/kg; ^:approximation

The toxicokinetic of DF2755A has been also studied after repeated administration (28 days) of DF2755A in dogs at the dose of 20, 50 and 100 mg/kg b.i.d. (corresponding to 40, 100 and 200 mg/kg/day)

Blood was sampled for toxicokinetic evaluation on Day1 and in Week 4.Evaluation of the toxicokinetic behavior of DF2755A resulted in the following observations: The plasma concentration time curves display a clear double peak pattern associated with the twice daily dosing of DF2755A (Figure 6).

The peak plasma concentration was determined after the first and the second administration, and was reached 1 or 2h after dosing. For all groups t_{last} was 24h. Generally, exposure increased in a dose proportional manner over the dose range. Exposure to DF2755A did not change importantly upon repeated dosing and no gender differences in exposure were observed during the study.

Table 6: Toxicokinetic parameters in plasma following a single and multiple oral administration in dogs at different doses

PK parameters	DF2755A					
DOG	**M**	**F**	**M**	**F**	**M**	**F**
DAY1						
DOSE BID(mg/kg)	**20**		**50**		**100**	
Tmax (h)[1]	1	1	1-2$	1-2$	1	1
Tmax (h)[2]	9-10$	9-10$	9-10$	9	9-10$	9
Cmax (µg/mL)[1]	17.1	29.0	77.9	43.3	168	128
Cmax (µg/mL)[2]	21.9	22.8	36.7	73.3	123	160
AUC_{0-8}(µg·mL/h)	45.2	55.5	174	115	366	291
AUC_{8-24}(µg·mL/h)	80.9	109	328	357	712	779
AUC_{last}(µg·mL/h)	126	165	501	471	1080	1070
AUC_{inf}(µg·mL/h)	128*	167*	515*	539*	1090*	1080*
$T_{1/2}$(h)	1.92	2.66*	2.38*	2.57*	1.62*	1.94*
V_{ss}(L/kg)	968*	961*	696*	732*	443*	600*
WEEK 4						
Tmax (h)[1]	1	1-2$	1-2$	1-2$	1	1-4$
Tmax (h)[2]	9	9	9-10$	9	9-10$	9-10$
Cmax (µg/mL)[1]	18.8	17.4	48.1	58.8	120	130
Cmax (µg/mL)[2]	19.2	16.7	37.9	34.3	100	86.6
AUC_{0-8}(µg·mL/h)	41.0	50.1	132	145	268	279
AUC_{8-24}(µg·mL/h)	77.2	86.3	308	255	554	599
AUC_{last}(µg·mL/h)	118	136	440	399	822	878

PK parameters	DF2755A					
DOG	M	F	M	F	M	F
$T_{1/2}$(h)	2.65	4.04*	2.22	6.30*	2.11*	2.40*

*:approximation; $:range ,$tmax^1$,$tmax^2$,$Cmax^1$,$Cmax^2$, are the tmax and Cmax (peak concentration)after respectively the first and second dosing

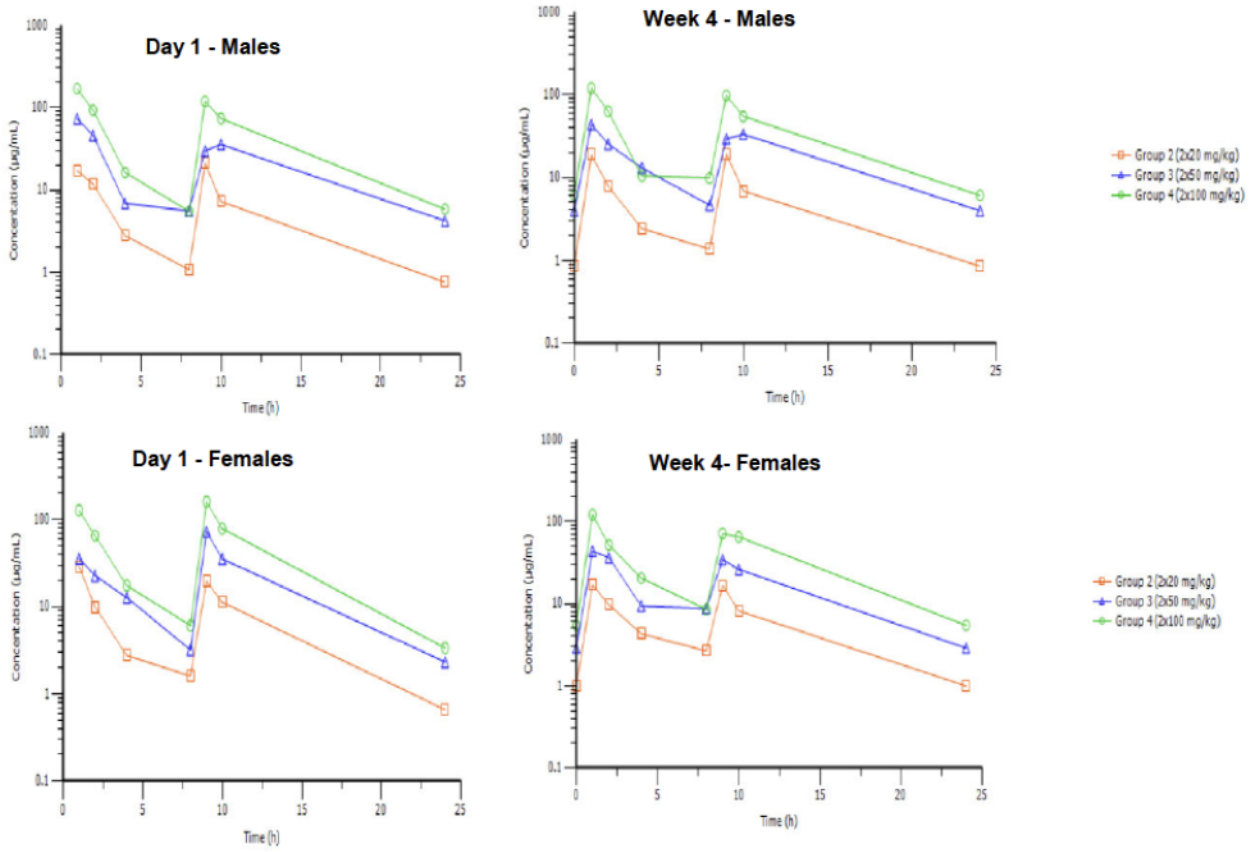

Figure 6: Mean plasma concentration-time profiles of DF2755A in Day 1 and Week4- after 20, 50 and 100 b.i.d. in male and female in dogs.

DF2755A showed moderate permeability in Caco-2 and MDCK cells without evidence for active efflux.

In vitro protein binding studies show that DF2755A is highly bound to plasma proteins in rat, dog and human species.

Table 7 DF2755A protein binding to plasma proteins in rat, dog and human.

DF2755A tested concentration (expressed as DF2755Y)	% Protein binding in plasma		
	Rat	Dog	Human
10 µM (3.383µg/mL)	96.12		97.48
48.5 µg/mL		92.7	

In vitro DF2755A potential direct inhibitory effect on the major human cytochrome P450 enzyme isoforms was evaluated (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) and it appeared that it is minimal or negligible at concentrations ≤ 30µM suggesting a low potential risk of in vivo direct inhibition effect tested P450 isoforms.

1.2.1.4. Toxicology

No effects were observed in a single-dose safety pharmacology neurobehavioral study in the rat up to the highest tested dose of 150 mg/kg, which was considered the NOEL. In a respiratory study in the rat, an increase of the respiratory rate and minute volume without affecting the tidal volume was observed. The NOEL for respiratory effects was therefore 100 mg/kg. No inhibition of the I_{Kr} channels was observed at concentrations up to 1 mM and no meaningful changes were seen in terms of blood pressure,

body temperature, heart rate or ECG intervals following oral doses up to 75 mg/kg in conscious Beagle dogs, while the highest dose of 125 mg/kg produced transient increase in heart rate and body temperature along with lower systolic-, mean arterial-, pulse pressure as well as shorter PR, RR, and QT. The dose of 75 mg/kg was the NOEL for cardiovascular function in the dog.

Oral gavage toxicology studies have been conducted with DF2755A for up to 4 weeks duration with 2 week recovery periods in rats and dogs. Range-finding studies of 7 days duration were performed in rats and dogs at dose levels of up to 800 and 900 mg/kg/day, respectively. Findings in rats and dogs were similar; in the rat, at the dose of 800 mg/kg/day, subdued behaviour, piloerection, bradypnoea and rales were observed along with decreased food intake. Microscopic effects included mixed cell infiltration and oedema of the glandular mucosa of the stomach, periportal inflammation in the liver and degenerative changes in the proximal tubules, mainly at the higher dose level. Biochemistry findings included increases in transaminases, lipids and inorganic phosphorus while haematology findings included increased platelet count, mean corpuscular volume and relative reticulocyte counts. Notable findings in dogs at the 900 mg/kg/day dose level included sporadic instances of haemorrhage (discoloured ears and eyes; dark or red faeces), vomiting, diarrhoea, tremors and lethargy. Food intake and body weight were also reduced. Microscopic findings at the higher dose included oedema, congestion and inflammatory infiltration and degeneration of the fundus mucosa, duodenum and ileum. In the liver, diffuse or centrilobular hepatocellular hypertrophy, single cell necrosis or minimal focal necrosis and diffuse hepatocellular vacuolation and periportal inflammation were observed. In the kidneys, tubular degeneration and regeneration and tubular dilation were observed. Biochemical findings included increased transaminases, total bilirubin, urea, creatinine and cholesterol and decreased electrolytes, glucose, total protein and albumin. Haematology findings included WBC parameter alterations and decreased platelet counts and decrease of the prothrombin time.

In the 28-day repeated dose studies in rats and dogs, toxicity findings were consistent with those observed in the range-finding studies. In the rat, DF2755A was administered at dose levels of 60 to 300 mg/kg/day (BID). No mortality occurred during the study and no treatment-related effects were noted in terms of food consumption, ophthalmoscopy or urine parameters. At 300 mg/kg/day, hunched posture and slight salivation were seen together with a lower body weight gain. The same dose level produced slight increases in transaminases. Decreased total protein, total globulin and glucose were seen along with increased lipids. Increased inorganic phosphorus and decreased potassium and calcium were also seen, while decreased urea and creatinine concentrations were observed in females. At histopathology, mixed cell infiltration, oedema, haemorrhage and erosions were noted in the GI tract but showed no dose-dependency and were seen also in control animals. Changes reminiscent of metabolic adaption were seen in the liver and kidneys correlated with increased organ weight and were fully reversible at recovery. Slight atrophy of the thymus, diffuse hypertrophy of the adrenal cortex and reduced secretion in the prostate, coagulating gland and seminal vesicles were also seen at 300 mg/kg/day. Other findings at 300 mg/kg/day included increased incidence of splanchnic haematopoiesis and increased bone marrow cellularity, correlating with decreased RBC count, haemoglobin, haematocrit and mean corpuscular haemoglobin and increased relative reticulocytes; all these changes were reversible at recovery. Slight increases in WBCs correlated with the inflammatory changes seen in the stomach. In the dog, repeated administration of DF2755A at dose levels in the range 40 to 200 mg/kg/day was generally well tolerated and no effects on body weight, food consumption, ophthalmoscopy, ECG examination or clinical pathology parameters were seen at any dose. Macroscopic findings included discoloration of the ileocaecal valve and reddish foci in the colon, rectum and pylorus. Upon microscopic examination, haemorrhage or congestion in the ileocaecal valve and/or colon as well as haemorrhage, erosion, mixed cell infiltration, and congestion of the pylorus were observed. Centrilobular hepatocellular hypertrophy was observed in females at mid and high dose and correlated with increased liver weights, but was not associated with any degenerative or inflammatory changes. In both species, the GI effects are similar to effects seen with other propionic acid derivative and likely a class effect, while the liver and kidney changes are considered to represent an adaptive metabolic phenomenon and of limited toxicological concern. The dose of 120 mg/kg/day was considered to be the NOAEL for repeated 4-week oral administration in the rat, while the NOAEL in the dog was 40 mg/kg/day.

Electron microscopy investigation in the liver of rats indicated the presence in the hepatocytes of areas of low electron-density and homogeneous appearance interspersed among the mitochondria and

rough endoplasmatic reticulum. However, no indication of cellular or organelles alterations were observed.

The *in vitro* genetic toxicity test for bacterial mutations (Ames assay) and chromosomal aberrations in human lymphocytes revealed no genotoxic potential for DF2755A. No evidence of clastogenicity or aneugenicity was found in the rat micronucleus test following administration of DF2755A by oral gavage up to the maximum tolerated dose level of 300 mg/kg/day.

A male and female fertility study combined with embryo-fetal development study with DF2755A Monohydrate was done in rats by oral gavage. Ninety-six male and ninety-six main female Wistar Han rats were assigned to four dose groups. The test item was administered once daily by oral gavage at doses of 50, 100 and 150 mg/kg (Groups 2, 3 and 4 respectively). The rats of the control group received the vehicle, water, alone. In addition, five satellite animals (females only) per treatment group and three satellite females in the control group were exposed and used for toxicokinetic blood sampling on Day 17 post-coitum. Males were exposed for 43-46 days, i.e. 2 weeks prior to mating, during mating, and up to termination. Mated females were exposed for 33-46 days, i.e. during 2 weeks prior to mating, during mating, and continuing until Day 17 post-coitum, inclusively. Females without evidence of mating were dosed until one day prior to euthanasia.

No reproduction and developmental toxicity was observed up to a dose level of 150 mg/kg. A slight, treatment related decrease in body weight gain was observed in males treated at 150 mg/kg from Day 4 of treatment onwards, resulting in approximately 5% lower absolute body weights at the end of treatment. As this effect was very small and no concurrent effect on food consumption was noted, this was considered not to be adverse.

No maternal toxicity was observed in females at any dose groups.

In conclusion the NOAEL to be considered for the calculation of the maximum recommended starting dose (MRSD) in humans are:

TOX 4 week (28 day) and corresponding exposure levels

RAT:	NOAEL 120 mg/kg (60 mg/kg b.id.)	$Cmax_{ss}$	193 µg/mL	$AUCss_{(0-24)}$	2275 h*µg/mL
DOG:	NOAEL 40 mg/kg (20 mg/kg b.id.)	$Cmax_{ss}$	18.00 µg/mL	$AUCss_{(0-24)}$	81.75 h*µg/mL

1.3. Summary of the potential risk and benefits to human subjects

Study subjects will not receive any direct therapeutic benefit from taking part in this study.

The subjects enrolled in the present study being healthy are not expected to derive any benefit from participating in the present study. DF2755A is being developed for a chronic condition, IC/BPS, that as of today lacks effective treatment. A growing body of evidence suggest that molecules affecting the IL-8 – CXCR1/2 axis like DF2755A could represent a novel treatment options. DF2755A exhibits a non-clinical safety and pharmacokinetic profile compatible with chronic treatment, and therefore satisfies all criteria to qualify as a candidate for clinical studies in patients with IC/BPS. As to the present phase I clinical trial, adequate safety margins based upon toxicology studies in rats and dogs, a staggering approach to dosing subjects at each dose level, close monitoring of clinical conditions of participants and a safety monitoring committee to be convened between each dose level to review the data of the previous cohort and confirm or select the next dose for evaluation, represent adequate safety measures to ensure the safety of trial participants.

1.4. Description and justification for the dosage regimen

Doses were calculated using the body surface area conversion. The calculated maximum recommended starting dose (MRSD) is 117 mg in rats and 130 mg in dogs. Based on data available for the rat, the proposed MRSD is 50 mg/subject, 23 times lower than the HED at NOAEL. As general rule the human equivalent efficacy dose is higher than the efficacy dose obtained in animals.
A prediction of the human PK parameters and the human equivalent pharmacological dose of DF2755A using allometric scale simulation has been carried out using the information on efficacy in rats.

Pharmacologic HED was calculated using the estimated (scaled) human clearance and the AUC that resulted in pharmacological activity. Pharmacologic HED ranged from 77 to 3202 mg BID (i.e. 144 -6404 mg) depending on the rat efficacious exposure and projected CL used. Considering these results, the dose of 50 mg could be considered the suitable first dose in human due to the fact that it is lower than the projected human pharmacological equivalent dose by a factor >7 or >10 if 7 or 10 mg/kg are considered the efficacy doses in rats, respectively.

These results are in agreement, see above, with the HED calculated from the 4-week oral toxicity study in rat were applying a safety factor of 23-fold the starting dose results to be 50 mg.

To preserve the safety of the subjects after the first cohort (PK parameters permitting) a PK simulation to determine the predicted PK parameters at the higher dose/s will be performed to predicted the expected exposure.

The following single doses are planned to be administered in Part A:

Table 8: Doses for administration in Part A

Proposed Doses	**SAD**			
Human Equivalent dose (HED) considering the more sensitive species (RAT) mg/kg	**Dose mg/subj (60kg)**	**Dose mg/kg**	**Safety margin**	**Ratio**
19.44	**50**	0.83	23.33	
	150	2.50	7.78	3
	450	7.50	2.59	3
	700	11.67	1.67	1.55

The maximum dose of 700 mg is in the range of the pharmacological HED and it is in the range of the exposure covered by the toxicological studies.

The first MAD cohort will be initiated only after the authorization, from ANSM of a substantial amendment that will be presented in order to notify the results of SAD part of the study.

The anticipated doses tested in Part B are the following:

Table 9: Doses for administration in Part B

Proposed Doses	**MAD (proposed 14 days) b i.d.**				
Human Equivalent dose (HED) considering the more sensitive species (RAT) mg/kg	**Dose mg/subj (60kg)**	**Total dose day mg/subj**	**Dose mg/kg**	**Safety margin**	**Ratio**
19.44	**100**	200	3.33	5.83	
	200	400	6.67	2.92	2
	300	600	10.00	1.94	1.5

The administered dose in part B could be modified according to the results of the part A.

1.5. Ethical considerations

The study will be performed according to the local regulations, the recommendations on Good Clinical Practice (GCP) "ICH Topic E6, CPMP/ICH/135/95", July 1996 including post Step 4 errata, status September 1997 and post Step errata (linguistic corrections), July 2002 and according to the relevant guidelines of the Declaration of Helsinki.
Dose Calculation: Guidance for Industry - Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers - U.S. Department of Health and Human Services - Food and Drug Administration - Center for Drug Evaluation and Research (CDER) July 2005

The clinical study will start upon receipt of the approval of both the Ethics Committee "Comité de Protection des Personnes" (CPP) and the French Health Authorities "Agence Nationale de sécurité du médicament et des produits de santé" (ANSM).

1.6. Description of the population to be studied

Part A: Eight healthy male and female subjects (6 active and 2 placebo) will be included in the four dose level. At least two healthy female subjects will be included in each cohort. One of them should be allocated to placebo. . In total, 32 subjects will be enrolled.

Part B: Twelve healthy male and female subjects (9 active and 3 placebo) will be included in each dose level. Between three and six healthy female subjects will be included in the cohort. At least one of them should be allocated to placebo. .
In total, 36 subjects will be enrolled.

For both parts, subjects will be recruited from volunteers' database of the clinical unit. Newspaper advertisements, radio spots, posters, mailing, specific press inserts, broadcast message or clinical unit recruitment website may be used. Only study-specific recruitment tools approved by EC will be used.

1.7. References

- Directive 2001/20/EC of the European Parliament and of the Council on the approximation of laws regulations and administrative provisions of the members states relating to the implementation of GCP in the conduct of clinical trials on medicinal products for human use;

- Declaration of Helsinki, 18th World Medical Assembly, Helsinki, Finland 1964, as modified in Fortaleza (2013);

- Code de la Santé Publique (CSP);

- ANSM guidelines "Estimation of the starting dose, definition of dose progression and protocol administration to volunteers" September 2006.

2. STUDY OBJECTIVES AND PURPOSE

2.1. Principal objective

To evaluate the tolerability and safety of ascending single and repeated doses of DF2755A in healthy adult male and female volunteers.

2.2. Secondary objectives

- To determine the pharmacokinetics parameters of DF2755A
- To establish a dose concentration-response relationship over a wide range of doses in order to select a narrower range of doses and dosing regimen to be subsequently studied in patients after single and multiple administration
- To evaluate the effects of ascending single and repeated doses on the pharmacodynamics parameters.
- To compare metabolites pathway in human with the one observed in animals

3. STUDY DESIGN

3.1. Evaluation criteria

3.1.1. Primary endpoint

AE, vital signs, 12-lead ECG, laboratory exams.

3.1.2. Secondary endpoints

Measurement of pharmacokinetics parameters: C_{max}, t_{max}, λ_z, $t_{1/2}$, $AUC_{0\text{-last}}$, AUC_{inf}, V_z/F, CL/F, $Ae_{0\text{-last}}$, CL_R, total amount in faeces, parent:metabolite(s) ratio.

Measurement of Cytokine Inflammation Markers [CXCL1(Gro alfa), CXCL2(Gro beta); CXCL5(ENA-78), CXCL6 (GCP-2), CXCL8 (IL-8)] in part B and Leukocyte subsets (CD11b and CD18) in part A, except for 1st cohort and part B.

3.2. Design

The study is a phase I, single center, double-blind, placebo controlled, randomized, ascending single and repeated doses study in healthy male and female volunteers.

The design consists of a double blind comparison of the test compound versus placebo in which the dose is increased in successive treatment periods.
The Multiple Ascending Dose (MAD) has to be started only after the end of all the cohorts of Single Ascending Dose (SAD). Besides in order to decide to move forward from SAD to MAD, the pharmacokinetic data for all doses administered during SAD will be evaluated (i.e. plasma: quantitative determination of DF2755A bound and unbound; qualitative and preliminary quantitative determination of known metabolites; urine and faeces: qualitative and quantitative determination of parent compound and qualitative and preliminary quantitative investigation of known metabolites).
Dose will be escalated in order to achieve enough safety information on an interval of doses possibly encompassing both the effective dose and the maximum tolerated dose (defined as the highest t dose devoid of any clinical signs/symptoms).

Part A: Single doses of 50 mg oad, 150 mg oad, 450 mg oad or 700 mg oad of DF2755A are planned to be tested in healthy volunteers.

Part B: Repeated doses of 100 mg bid, 200 mg bid or 300 mg bid of DF2755A are planned to be tested in healthy volunteers.

The study plan is shown in Figure 7 and 8.

Study procedures are detailed in Section 6 and 7.

Figure 7 - Study plan - Part A

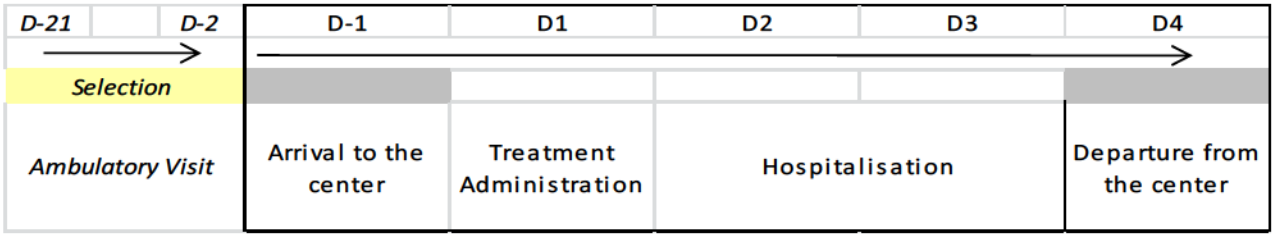

Figure 8 - Study plan - Part B

D-21 D-2	D-1	D1	D2-D14	D15	D16	D17
Selection	Treatment Administration					
Ambulatory Visit	Arrival to the center	Randomization	Hospitalisation			Departure from the center

3.3. Description of the measures taken to minimize and avoid bias

3.3.1. Randomization

The randomization list will be provided by Eurofins Optimed.

The randomization list will be prepared to permit that one of the two first subjects treated per each cohort, will receive a placebo.

The treatments will be allocated at D1 for both parts.

3.3.2. Blinding

The following measures are taken to avoid bias:

- Double Blind Study,
- The capsule containing active drug and placebo will be indistinguishable in appearance.

3.3.3. Risk assessment, study conduct and method of dose escalation

3.3.3.1. Number of subjects receiving the study drug simultaneously

Based on clinical data, no major specific safety issues related to acute toxicity with DF2755A is expected at the doses planned to be tested. However, to mitigate the risk, the administration of the investigational product within each dose group cohort will be conducted according to a staggering approach. For the 2 first subjects of a dose level, the administrations will take place with a lag time of 1h during which the Investigator will carefully monitor for adverse events (AEs) or reactions, if any. For the subsequent subjects of a dose level the administrations will take place with a lag time of 30 minutes.
For example: for Part A: 2 subjects on the first day, 3 subjects on the second day, and the remaining 3 subjects on the third day. And for Part B: 2 subjects on the first day, 3 subjects on the second day, 3 subjects on the third day, and the remaining 4 subjects on the fourth day.

3.3.3.2. Dose Escalation

The dose escalation is designed to progress from the first to the highest dose up to the occurrence of relevant events supporting Maximal Tolerated Dose (MTD) determination.

At the end of each dose level, an interim safety report will be issued by the Investigator. A dose escalation teleconference meeting will be held between the Investigator and the Sponsor representatives, and the decision on how to proceed (e.g. next higher dose) will be taken on the basis of a blind safety and pharmacokinetic data review.

The following safety parameters will be reviewed for both part, for all the subjects:

- Any adverse events reported;
- All laboratory parameters outside of ranges;

- Concomitant therapy;
- Physical examination abnormalities;
- Vital signs abnormalities;
- ECG abnormalities.

For dose escalation, Pharmacokinetic data on plasma (quantitative determination of DF2755A bound and unbound; qualitative and preliminary quantitative determination of known metabolites), urine and faeces recovery (qualitative and quantitative determination of parent compound and qualitative and preliminary quantitative investigation of known metabolites) will be considered by using, for each new dose level, the pharmacokinetic results of the previous dose level in order to re-estimate the safety margin based on the animal-human exposure ratio. This re-estimation must be taken into account when deciding whether to proceed to the next dose level.
The exposure does not exceed the AUC of 1000 µg·h/mL.

Identification of non-acceptable risks or significant proportion of AEs with common pathological pattern can justify the modification of the dose escalation design.

After mutual agreement between the Sponsor and the Investigator and if it is considered useful for the selection of the next dose level, the treatment of a specific subject and/or a cohort may be unblinded before the next dose level of DF 2755A is administered.

At the end of the teleconference meeting, the following decisions can be taken:

- Dose escalation will continue as scheduled;
- An intermediate dose between the current dose and the following dose will be administered to the next cohort;
- A lower intermediate dose, between the current dose and the dose administered in the previous cohort will be administered to the next cohort;
- The current dose will be repeated in the next cohort;
- The study will be stopped.

According to the conclusion of this meeting, the formal agreement/disagreement to move to a next cohort will be signed by both the Sponsor and the Investigator.

3.4. Expected duration of subject participation

3.4.1. Description and duration of trial periods

Part A:

Screening within 21 days prior to the first administration,
Hospitalization approximately for 4 days (D-1 morning to D4 morning). Consideration should be given to the hospitalization after the last dose, in fact it could be reviewed according to PK parameters of the compound after each dose of SAD.

Expected duration: approximately 4 weeks for each participating subject.

Part B:

Screening within 21 days prior to the first administration,
Hospitalization approximately for 17 days (D-1 morning to D17 morning). Consideration should be given to the hospitalization after the last dose, in fact it could be reviewed according to PK parameters of the compound after each dose of MAD.

Expected duration: approximately 6 weeks for each participating subject.

3.4.2. Duration of follow-up

During the last visit, subjects will undergo a complete clinical and biological examination, identical to the examination at the start of the study. AEs will be recorded, and if they are on-going a further follow-up will be arranged; follow-up will continue until the event is resolved or the condition is unlikely to change or the subject is lost to follow-up (see section 8.5).

3.5. Stopping rules

3.5.1. Stopping rule applicable at any time

In case of occurrence of a SAE at least possibly related to the study drug administration, the safety review committee will decide to put the study on hold, and will ask for unblinding for the subject. If the subject was treated by placebo, the study will be continued as planned per protocol. If the subject was receiving DF2755A, the study will be immediately interrupted and depending on the nature and SAE resolution it should be potentially re-started after submission and approval by both EC and CA of a substantial amendment.

3.5.2. Stopping rule for a subject

During the treatment period, the safety and tolerability will be evaluated on an ongoing basis, the following criteria will apply to stop a subject:

- The subject experiences an AE that prevents him from continuing in the study,
- Significant increase (i.e. > 3N) of ALT (N being the upper limit of the normal value),
- Abnormal laboratory results with simultaneous increases of total bilirubin (> 2 N), ALT (> 2 N) and alkaline phosphatases (> 1.5 N),
- The subject develops a serum creatinine ≥ 2 times the ULN,
- Sustained QTcF value of > 500 msec confirmed by at least one repeat ECG,
- The subject withdraws consent,
- At the request of the Sponsor or at the Investigator's request (for example if the Investigator considers that the subject's health is compromised by remaining in the study or the subject is not sufficiently cooperative).

See also Section 3.6 “Removal of Subjects from Therapy or Assessment”.

3.5.3. Stopping rules within a group

During the treatment period, the safety and tolerability will be evaluated on an ongoing basis, the following criteria will apply to stop the cohort **:**

- 2 subjects experiencing severe non serious AEs considered as at least possibly related to the study drug,
- 2 subjects experiencing a significant increase (i.e. > 5N) of ALAT (N being the upper limit of the normal value),

3.5.4. Stopping rule for dose escalation

The safety review committee will decide to stop the dose escalation as planned per protocol in case of occurrence of:

- 2 subjects experiencing severe non serious AEs of the same organ class and nature related to the study drug,
- 4 subjects (for SAD) and 8 subjects (for MAD) experiencing study drug related moderate non serious AEs (that are considered directly related to the drug effect unless they resolved despite the continuation of the treatment),
- 2 subjects of the group experience a significant increase (i.e. > 3 N) ALAT,
- 2 subjects of the group experience simultaneous increases of total bilirubin (> 2 N), ALAT (> 2 N) and alkaline phosphatases (> 1.5 N),

- 2 subjects has a sustained QTc value > 500 ms (confirmed by a second ECG under strict resting position),
- One subject presents a C_{max} or $AUC_{(0-24h)}$ values respectively above 150 µg/mL and 1000 µg/mL*h,
- The mean C_{max} and $AUC_{(0-24h)}$ leads to believe taking into account the dose increase that C_{max} or $AUC_{(0-24h)}$ values of 125 µg/mL and 600 µg/mL*h will be overpass with the following planned higher dose,
- In MAD part the mean at the steady state C_{max} and $AUC_{(\tau)}$ leads to believe taking into account the dose increase that C_{max} or $AUC_{(0-24h)}$ values of 125 µg/mL and 800 µg/mL*h will be overpass with the following planned higher dose,

In one of the cases listed above, the dose group in progress will be stopped and the blind could be broken. Regarding laboratory and QTc abnormalities described above, if unblinding confirms that at least two subjects presenting these abnormalities were receiving the active drug the dose escalation will be stopped.

3.6. Removal of Subjects from Therapy or Assessment

In accordance with the Declaration of Helsinki, subjects will be free to withdraw from the study at any time if they wish so, for any reason specified or unspecified.

The following reasons will be accepted for study discontinuation:

- Withdrawal of subject consent, or loss to follow-up, or inability to remain under medical observation including post study examination,
- Non-compliance or major deviation from the protocol,
- Appearance of a serious AE (SAE),
- New significant information (French regulation : "fait nouveau"), any new fact concerning the research or the study product which may jeopardize the participants' safety, and which leads the Sponsor or Investigator to take appropriate urgent security decision,
- Any other situation where, in the opinion of the Investigator, continuation of the study would not be in the interest of the subject,
- Discontinuation of the study by the Sponsor.

Should any of the subjects be withdrawn from the study, the Sponsor's representative and Investigator will discuss the possibility of replacement. All subjects withdrawing for reasons unrelated to the investigational product will have to be replaced. The reason for withdrawal will have to be recorded in the e-CRF for all withdrawn subjects.

The e-CRF has to be completed up to the time of drop out. All drop outs after the first intake of investigational product should be given a post-study assessment as appropriate. The premature termination form in the e-CRF must be completed for all drop outs.

All data, including any drug concentrations from any withdrawn subject, has to be included in the final report. These data will only be evaluated if both the Sponsor and the Investigator agree that it is valid to do so.

Subjects withdrawn because of adverse experiences will undergo a physical examination and laboratory tests planned at the follow-up visit. A follow-up of AEs will also be undertaken for withdrawn subjects.

3.7. Blind and procedures for unblinding

This study is a double blind study.

Randomization will be performed at D1.

3.7.1. Coding list

The analytical centre as well as the Investigator and the team and the subjects will be in blind conditions.

For each subject, randomization envelopes, containing the identification of the treatment (emergency envelopes) will be supplied by Eurofins Optimed and kept in a safe place on site during the whole clinical study period.

In the case of a pharmaceutical preparation is required, the decoding system used is a sealed coding list to be given to the EUROFINS-OPTIMED pharmacist. The sealed coding list should be kept in a safe place and accessible to any person authorised to unblind.

The investigator, CRO staff (except the Pharmacist and pharmacy assistant in charge of the IP final packaging) and Sponsor's clinical trial team members will not have access to the randomization (treatment/product) code except under certain circumstances.

3.7.2. Breaking the blind

The code for any study participant should only be broken by the Investigator or authorised person if it is absolutely necessary.

In case of emergency, for a subject in particular, the code may be broken to ascertain the type of treatment given to study participant concerned. If so, the Investigator (or a person designated from his team) must write his name, signature, date, the number of the participant concerned, the reason for code breaking and the Sponsor must be notified within 24 hours and a full written explanation must be provided.

The code may also be broken by the Clinical Quality Assurance (CQA) representative if this information has to be provided to the physician in charge of subjects treatments (i.e. at the hospital). In this case, the code will be broken using the sealed coding list if available or sealed envelope. The information resulting from code-breaking (i.e., the subject's group assignment) will not be communicated to either the Investigator or the Sponsor.

A copy of the emergency envelopes will be supplied also to the Sponsor. While the Investigator (PI) will operate under blind conditions until the end of the study, the Sponsor may open the code for a single cohort, but only after the study cohort has been completed and the joint (together with the PI) blinded decision, about whether or not to continue with the planned dose escalation, has been taken.
The randomization envelopes should not be opened by the Investigator at the end of the study.

In case of Suspected Unexpected Serious Adverse Reaction (SUSAR), the Sponsor Drug Safety should break treatment codes before reporting on an expedited basis the SUSAR to the Competent Authorities and to the Ethics Committee concerned. The blinding code should be broken only for the subject concerned with that SUSAR and treatment code shall not be disclosed to Investigator staff and to Sponsor Clinical Department. An additional copy of the Individual envelopes identified with the subject assignment number is stored by Dompé Drug Safety. Dompé Drug Safety will unmask a patient treatment only for safety reason or to assess regulatory reportability of a potential SUSAR and will document envelope opening.

4. STUDY POPULATION

4.1. Subject inclusion criteria

For eligibility into the trial, subjects must meet all the following inclusion criteria :

1- Healthy male subject, aged between 18 and 55 years inclusive;

2- Healthy female subject infertile or in post menopause for at least two years, aged between 18 and 60 years inclusive;
3- Body Mass Index (BMI) between 18.5 and 29.9 kg/m^2 inclusive and weight ≤ 90kg;
4- Considered as healthy after a comprehensive clinical assessment (detailed medical history and complete physical examination);
5- Normal Blood Pressure (BP) and Heart Rate (HR) at the screening visit after 10 minutes in supine position:
    - 90 mmHg ≤ Systolic Blood Pressure (SBP) ≤ 140 mmHg or 150mmHg for subject > 45 years,
    - 50 mmHg ≤ Diastolic Blood Pressure (DBP) ≤ 90 mmHg,
    - 40 bpm ≤ HR ≤ 100 bpm,

      Or considered NCs by investigators;
6- Smoker < 5cigarettes per day who stop totally during the study
7- Normal ECG recording on a 12-lead ECG at the screening visit:
    - 120 ≤ PR < 210 ms,
    - QRS < 120 ms,
    - QTcf ≤ 430 ms for male and ≤ 450 ms for female,
    - No sign of any trouble of sinusal automatism,

      Or considered NCs by investigators;
8- Normal oral temperature
    - 36.3°C ≤ oral body temperature < 37.5°C;
9- Laboratory parameters within the normal range of the laboratory (haematological, blood chemistry tests, urinalysis). Individual values out of the normal range can be accepted if judged clinically non relevant by the Investigator;
10- Normal dietary habits;
11- Able to communicate well with the Investigator, understand and comply with the requirements of the study, and understand and sign a written informed consent prior to selection;
12- Covered by Health Insurance System and / or in compliance with the recommendations of National Law in force relating to biomedical research.

4.2. Subject non-inclusion criteria

Subjects meeting any of the following criteria will not be included into the trial :

1- Subject has had a clinically significant illness in the six weeks before screening in the opinion of the Investigator;
2- Subject has had a serious adverse reaction or significant hypersensitivity to any drug, has a known clinically significant allergy to anti-inflammatory drugs or chemically related compounds or has a clinically significant allergy to drugs, foods or other materials (in the opinion of the Investigator). However, subjects with mild hayfever may be included in the study.
3- Subject has used prescription medication in the 14 days prior to dosing or over-the-counter preparations for 7 days prior to dosing (including vitamin supplements and herbal remedies), with the exception of paracetamol which will be allowed during the study (maximum 500 mg per administration, total daily dose maximum 2 grams).
4- Subject has a significant history of drug/solvent abuse or a positive drugs of abuse (DOA) test at any time during the study.
5- Subject has a history of alcohol abuse in the last 5 years or currently drinks in excess of 21 and 14 units per week for males and female, respectively, or has a positive alcohol breath test (ABT) at any time during the study.
6- Subject is not willing to refrain from caffeine/xanthine containing products in the 48 hours prior to admission to the clinical unit on Day -1 and for the duration of the residential period.
7- Subject who has a positive human immunodeficiency virus (HIV) screen, Hepatitis B screen or Hepatitis C screen.
8- Subject has donated blood or blood products (e.g., plasma or platelets) within the three months prior to screening.

9- Subject who is not suitable to participate in the study in the opinion of the Investigator;
10- Subject who has participated in any clinical study with an investigational drug/device within three months prior to the first day of dosing.
11- Subject who is in the exclusion period from another study.
12- Administrative or legal supervision;
13- Subject who would receive more than 4500 euros as indemnities for his participation in biomedical research within the 12 last months, including the indemnities for the present study.

4.3. Subject Identification

4.3.1. Screening number

The screening number will be S and 3 digits, for example: S003. It will be a chronological number. The screening number will be used throughout the screening period, until subjects are randomized.

4.3.2. Inclusion number

The inclusion number will be composed of 7 digits, 3 for the number of centre (001) and 4 for the inclusion number: from 1001 to 1032 for part A and from 2001 to 2036 for part B. It will be a chronological number.

4.3.3. Randomization number

The randomization number will be the same as the inclusion number and the treatment number.

4.4. Subject withdrawal criteria

4.4.1. Definitive or temporary stop of a person's participation in the research

4.4.1.1. List of withdrawal criteria

The criteria of withdrawal could be serious adverse events (SAE) or AEs

4.4.1.2. Reasons for withdrawal

The subjects may withdraw from the study for the following reasons:

- the subject withdraws consent at any time and irrespective of the reason.
- the subject incurs a significant protocol violation that puts the safety of the subject or the integrity of the data at risk (the definition of significant is made contemporaneously by the Investigator).
- at the Investigator's request (for example if the Investigator considers that the subject's health is compromised by remaining in the study or the subject is not sufficiently cooperative).
- at the request of the Sponsor.

4.4.2. Definitive or temporary stop of a part or the totality of the research

4.4.2.1. Decided by the Sponsor

The Sponsor may decide premature discontinuation of the study in the following cases:

- The study is not conducted in accordance with the procedures defined in the approved protocol (i.e. low rate of recruiting - protocol deviations - failure to ensure the quality of the data collected);
- Information on the Investigational Medicinal Product (IMP)/Study Product that might change the current benefit-risk profile of the IMP or that would be sufficient to require changes in the IMP administration or in the overall conduct of the trial;

- Left at the discretion of the Sponsor.

4.4.2.2. Decided by the Investigator

The Investigator may suspend or stop the research if in his judgment the participating subjects are exposed to risks that are not ethically or scientifically justifiable and must notify in writing the Sponsor of this decision providing the reason thereof.

If the research is suspended for safety reasons, the Health Authorities must be immediately notified of the suspension. In this case, the study can resume after an amendment is approved by the Health Authorities.

If the study is stopped, Ethics Committee and Health Authorities should be informed by Sponsor, within 15 days after the decision is taken.

4.5. Premature discontinuation of subject

4.5.1. Data to be collected, and time of recording of these data

Each participant is free to discontinue from the study at any time, for any reason. If a participant discontinues from the study (regardless of the reason for a participant's discontinuation and regardless of the participant's status as evaluable or not evaluable), the Investigator will indicate the reason for discontinuation on the appropriate eCRF page. A follow-up visit will be planned by the Investigator. The exams scheduled for the end-of-study visit will be performed.

In case of premature discontinuation due to AEs, the Investigator will indicate that on the AE Form of the eCRF and in the SAE Form, if appropriate. Participants will be monitored until resolution.

4.5.2. Methods of replacement

A subject who prematurely ends his/her study period after the start of the baseline period and who received treatment will be replaced.

The replacement subject will undergo the complete study. He/she will be given a number corresponding to the number of the subject being replaced plus 100 (i.e. for the subject number 1004, the replacement subject will receive the number 1104).

Subjects who have been withdrawn from the study cannot be re-included in the study. Their inclusion and treatment number must not be re-used.

4.5.3. Methods of follow-up of premature discontinuations

In case of premature discontinuation due to AEs/SAEs, follow-up will continue until the event is resolved or the condition is unlikely to change or the subject is lost to follow-up (see section 8.5).

4.6. Exclusion period

The subjects included in this study will be prohibited from participating simultaneously in other research.

The exclusion period planned at the end of the research will be 3 months.

5. STUDY PRODUCT/TREATMENT

5.1. Description of the treatment(s)

5.1.1. Pharmaceutical form

Name of the compound:	DF 2755A
Pharmaceutical form:	Capsules of 50 mg and 200 mg
Dose per administration:	Part A: 50 mg oad, 150 mg oad, 450 mg oad or 700 mg oad Part B: 100 mg bid, 200 mg bid or 300 mg bid
Number of capsules / administration:	1, 2, 3 or 6 capsules per administration according to the dose level
Timing for administration:	Part A: Single oral dose administration on D1 according to the randomization. The administration will take place at around 8:00 a.m with 200 ml of tap water, in sitting position, in fasting conditions. Part B: Repeated oral administration from Day 1 to Day 14. The administration will take place at around 8:00 a.m and at around 8:00 p.m with 200 ml of tap water, in sitting position, in fasting conditions.

Name of the compound:	Placebo
Pharmaceutical formulation:	Matching capsule of 50mg and 200mg
Dose per administration:	NA
Number of tablets/capsules / administration:	1, 2, 3 or 6 capsules per administration according to the dose level
Timing for administration:	Part A: Single oral dose administration on D1 according to the randomization. The administration will take place at around 8:00 a.m with 200 ml of tap water, in sitting position, in fasting conditions. Part B: Repeated oral administration from Day 1 to Day 14. The administration will take place at around 8:00 a.m and at around 8:00 p.m with 200 ml of tap water, in sitting position, in fasting conditions.

5.1.2. Unit form, manufacture, packaging and labelling

The manufacture of bulk DF2755A capsules and bulk matching placebo capsules and the packaging is carried out, in accordance with Good Manufacturing Practice at:

SYNERLAB DEVELOPPEMENT
CS 70002 1, rue Charles Coulomb
45077 Orléans Cedex 2 - France
SYNERLAB DEVELOPPEMENT is a sub-contractor of EUROFINS OPTIMED.

IMP (DF2755A and matching placebo capsules) will be shipped to the EUROFINS OPTIMED Pharmacy.

Labelling storage and dispensation of DF2755A capsules and matching placebo capsules, are conducted in accordance with the applicable Good Clinical Practices at EUROFINS OPTIMED Pharmacy

OPTIMED EUROFINS
1 rue des Essarts
38610 GIERES

The API is manufactured by AMSA on behalf of Dompé Farmaceutici S.p.A.

AMSA
Via g. di Vittorio, 6
22100 Como, ITALY

A tripartite quality agreement between Dompé Farmaceutici (the contract giver),EUROFINS OPTIMED and SYNERLAB DEVELOPPEMENT (both contract acceptor) will be in place for the GMP management of any IMP manufacturing activities.
DF2755A and matching placebo capsules will be packaged into blister of 10 capsules. Depending of the doses, capsules will be places in envelope, each envelope containing: Part A: 1, 3, 3 or 6 capsules of DF2755A or matching Placebo; Part B: 2, 1 or 2 capsules of DF2755A or matching Placebo.
Label for blister:

- **DF2755A**

BPS0115 / OP099516.DOM
EudraCT N° 2016-003629-41
Promoteur: **DOMPE FARMACEUTICI S.P.A**
Via Santa Lucia 6 – 20122 Milano – Italy
Tel.: + 39 0258 38 31 – Fax: +39 02 5838 33 24
Investigateur : Dr Yves DONAZZOLO
Eurofins Optimed Clinical Research
1 rue des Essarts – 38610 GIERES France

Traitement : **DF2755A – gélules de *XX* mg**
1 blister de 10 gélules
Lot n° : *XXXXXXXXXX* Date de péremption : MM/YYYY

Pour administration orale
A utiliser conformément aux indications du protocole
A conserver à une température ne dépassant pas 30 °C
Pour recherche sur la personne humaine uniquement

- **PLACEBO**

BPS0115 / OP099516.DOM
EudraCT N° 2016-003629-41
Promoteur: **DOMPE FARMACEUTICI S.P.A**
Via Santa Lucia 6 – 20122 Milano – Italy
Tel.: + 39 0258 38 31 – Fax: +39 02 5838 33 24
Investigateur : Dr Yves DONAZZOLO
Eurofins Optimed Clinical Research
1 rue des Essarts – 38610 GIERES France

Traitement : **DF2755A PLACEBO – gélules de *XX* mg**
1 blister de 10 gélules
Lot n° : *XXXXXXXXXX* Date de péremption : MM/YYYY

Pour administration orale
A utiliser conformément aux indications du protocole
A conserver à une température ne dépassant pas 30 °C
Pour recherche sur la personne humaine uniquement

Labelling operations performed by the EUROFINS OPTIMED Pharmacist, will be in compliance with EudraLex - Volume 4, EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use - Annex 13.

Labels for unitary doses will bear the following information's:

BPS0115/OP099516.DOM
Dompé farmaceutici s.p.a
Dr Yves Donazzolo
Traitement : DF2755A ou placebo
N° du sujet : 001-XXX
Dose : XXmg soit X gélules
Voie d'administration : orale
A utiliser conformément aux indications du protocole
n°: PREP201_ _ _ _
Date de péremption : _ _/_ _/_ _ _ _
Administration de **DXTX :** _ _/_ _/_ _ _ _
Conserver à une température ne dépassant pas 30°C
Pour recherche sur la personne humaine uniquement

The address and telephone number of the main contact for information on the product, clinical trial and for emergency contact will be displayed on study cards that subjects will have been instructed to keep in their possession at all times.

5.1.3. Route and mode of administration

For Part A, DF2755A will be administered orally, at T0h (around 8:00 a.m), in sitting position, in fasting conditions, with 200 ml of tap water.

For Part B, repeated administrations will be performed at T0h (around 8:00a.m) and at T12h (around 8:00p.m). For each subject, these administrations will be performed with an authorised window of ±15 minutes.

Treatments will be administered under the supervision of the Investigator, in the Clinical Pharmacology Unit EUROFINS OPTIMED.

The actual time of drug administration will be documented in the source documents and reported in individual eCRF.

5.2. Accountability procedures for the investigational product(s)

5.2.1. Responsibilities

The Investigator, the pharmacist or other personnel allowed to store and dispense IMP will be responsible for ensuring that the Investigational Treatment used in the study is securely maintained as specified by the Sponsor and in accordance with the applicable regulatory requirements.

The IMP will be dispensed in accordance with the Investigator's prescription and it is the Investigator's responsibility to ensure that an accurate record of Investigational Product issued and returned is maintained.

5.2.2. Accountability

Details of the quantities of each medication dispensed will be entered onto the accountability form. At the end of the study, the amount of each product retained (if required) in the Clinical Unit and the remaining amount (if any), will be returned to the Sponsor or destroyed after a final reconciliation (if agreed with the Sponsor). A copy of the form will then be sent to the Sponsor, together with any remaining medication (if agreed) (see section 5.2.3).

Specific procedures for the IMP preparation, dispensation, storage and destruction when required will be detailed in the "pharmacy manual for study OP099516.DOM". This document will be supplied by the pharmacist of Eurofins Optimed and approved by the sponsor, apart from the study protocol.

5.2.3. Return and /or destruction of IMP

Investigational medicinal product reconciliation must be performed at the site by the Investigator (or the pharmacist) and the monitoring team using the appropriate form countersigned by the Investigator (or the pharmacist) and the monitoring team. Test materials (including investigational products and placebo) remaining at the end of the study will be returned to the Sponsor or their representative, or destroyed on behalf of the Sponsor after a final reconciliation of investigational medicinal product.
In case of destruction, a written authorization for destruction will be given by the clinical trial team once the investigational medicinal product reconciliation is achieved. This destruction will be performed by the pharmacist or delegated person and a certificate of destruction will provided.

5.3. Medication(s)/treatment(s) permitted and no permitted before and/or during the trial

No concomitant therapy (prescribed or non-prescribed drug included OTC) will be allowed during the study, except paracetamol (maximum 500mg per administration, total daily dose maximum 2g). However, in case of intercurrent illness or emergency, the investigator is allowed to use any needed medication.
This must be done with a particular attention to the available pharmacological knowledge of the given medication and possible interaction(s) with the study drug. In case of intake of any concomitant medication during the study, the following information must be noted in the relevant section of the eCRF:

- Name of the treatment and its form,
- Reasons for prescription,
- Date and time of start,
- Route of administration,
- Daily dose,
- Duration of treatment.

The decision to withdraw the subject may be made by mutual agreement between the Investigator and the Sponsor.

5.4. Procedures of monitoring subject compliance

Administration will be performed under medical supervision. A mouth control will be performed immediately after the administration.

6. ASSESSMENT OF PHARMACOKINETIC

Derivation of PK parameters will be carried out by PhinC Development using WinNonlin® Professional software (Version Phoenix 6.4 – Pharsight Corporation – Moutain View, California – USA). A non-compartmental analysis approach will be performed.

The statistical analysis will be carried out by PhinC development using the SAS® package (release 9.4).

In each part of the study, a first interim PK analysis with nominal time points will be run after completion of each dose level. After database lock, a final analysis will be run with actual time points. For the interim analysis, no formal statistics will be presented.

After completion of the bioanalytical part of the study, an electronic copy of the quality checked and validated data will be provided to PhinC Development.

6.1. Pharmacokinetic endpoints

For the calculation of the PK parameters and characteristics the following rules will be applied:

- All the plasma concentrations validated by the bioanalytical laboratory and provided to the pharmacokineticist will be used for the PK analysis.
- The actual blood sampling time points related to the preceding administration will be used if available. Otherwise, nominal blood sampling times will be taken into account.
- At time points in the lag-time between time zero and the first concentration equal or above the limit of quantification (LOQ), concentrations below LOQ will be set to zero (0). Concentrations below LOQ between 2 concentrations equal or above LOQ will be considered as missing data. Trailing concentrations below LOQ will not be used in calculations.
- For plasma concentration above the upper limit of quantification (ULOQ) and reported as above the limit of quantification (ALQ) in the final plasma concentration tables, ALQ will be replaced by the first measurement for the PK analysis.
- Not reported concentration (NR) will be excluded from the PK analysis.
- If pre-dose concentration is less than or equal to 5% of C_{max} value in a PK profile, the subject's data can be included in all PK measurements and calculations without any adjustments. If the pre-dose value is greater than 5% of C_{max}, the subject will be dropped from all statistical evaluations (applicable only at the predose on day 1).

According to the study part and the day of PK assessment, the following PK parameters of DF2577Y bound and unbound (or related metabolites) will be derived from DF2577Y (or related metabolites) plasma concentrations for each subject on Day 1 (SAD and MAD parts) and on Day 14 (MAD part):

C_{max}/$C_{ss,\,max}$ The observed maximum plasma concentration of DF 2577Y (or metabolites) measured in a subject after dosing identified by inspection of the plasma drug concentration *versus* (*vs.*) time data by Phoenix WinNonlin.

$C_{ss,\,min}$ The observed plasma concentration of DF 2577Y (or metabolites) measured in a subject at 12 h post-dose just before the second daily dosing *versus* (*vs.*) time data by Phoenix WinNonlin (Day 14 in MAD part only).

$C_{ss,av}$ The average concentration at steady-state defined as the ratio $AUC_{0-\tau/\tau}$ (Day 14 in MAD part only).

t_{max}/$t_{ss,\,max}$ The time at which C_{max} was apparent, identified by inspection of the plasma drug concentration *vs.* time data by Phoenix WinNonlin.

t_{lag} The time before occurrence of the first quantifiable plasma concetration will also be obtained directly from the concentration-time data; it is the time point immediately prior to the first quantifiable plasma concentration.

λz The terminal plasma elimination rate-constant will be estimated from log-linear regression analysis of the terminal phase of the plasma concentration *vs.* time profile (SAD part and MAD part Day 14 only). For the slope of the terminal elimination phase to be accepted as reliable, the following criteria will be imposed:

- a coefficient $r^2 \geq 0.90$,
- a minimum of 3 data points, including the last measured data point and excluding C_{max}, are available for the regression.

If these 2 criteria could not be met, the slope of the terminal elimination phase will be considered as not calculable and all the parameters derived from this value ($AUC_{0-\infty}$, $t_{1/2}$, …) will be reported as not calculable (NC).

$t_{1/2}$ $t_{1/2}$ will be calculated according to the following equation: $t_{1/2} = \ln 2 / \lambda z$ (SAD part and MAD part Day 14 only).

$AUC_{0-\tau}$ The area under the concentration *vs.* time curve over the dosing interval will be calculated using a linear trapezoidal method (*i.e.* from time zero (pre-dose) to 12 h post-dose in MAD part only).

AUC_{0-24} The area under the concentration *vs.* time curve from time zero (pre-dose) to 24 h post-dose will be calculated using a linear trapezoidal method (SAD part only).

AUC_{0-t} The area under the concentration *vs.* time curve from time zero (pre-dose) to the time of last quantifiable concentration will be calculated using a linear trapezoidal method.

$AUC_{0-\infty}$ The area under the serum drug concentration *vs.* time curve from time zero to infinity: [$AUC_{0-\infty} = AUC_{0-t} + (C_t/ke)$, where C_t = the observed concentration of drug for the last sample on the PK profile in which drug was detected, and ke as defined above. The percentage of extrapolation of $AUC_{0-\infty}$ should normally not exceed 20% to consider the value as reliable (SAD part only).

fu Fraction of unbound in plasma

Cl/F The apparent volume of the central compartment cleared of drug per unit time will be estimated using the formula (DF 2577Y only in SAD part only).

$$Cl/F = Dose / AUC_{0-\infty}$$

Vd/F The apparent volume of distribution based on the terminal elimination phase. The estimate does not account for the bioavailability (F, as a fraction of 1) and is therefore nominally divided by this value when drug is given via extravascular routes (DF 2577Y only in SAD part only).

$$Vd/F = Cl/F / ke$$

PTF The peak trough fluctuation will be calculated on Day 14 as the following ratio:

$$[(C_{ss,\,max} - C_{ss,\,trough})/C_{ss,\,av}]$$

where $C_{ss,trough}$ is the pre-dose concentration measured on the day of assessment.

In the MAD part, accumulation ratios will be calculated for C_{max} and $AUC_{0-\tau}$ between Day 14 and Day 1 for each compound.

Parent to metabolite ratios will be calculated for C_{max} and all AUCs.

The following PK parameters of DF 2577Y (or related metabolites) will be derived from DF 2577Y (or related metabolites) urine concentrations for each:

Ae Amount of DF 2577Y (or related metabolites) measured in a given urine fraction:

Ae = DF 2577Y (or related metabolites) concentration measured in the fraction × Urinary volume of the fraction

fe Percentage of the dose excreted in the urine (DF 2577Y only) determined over various time intervals (24 h post-dose and t_{last} in SAD part; 12 and 24 h post-dose on Day 1 in MAD part).

Cl_R Renal clearance determined as the ratio Ae_{0-t}/AUC_{0-t} with t = 24 h post-dose and t_{last} in SAD part and t = 12 and 24 h post-dose on Day 1 in MAD part

6.2. Specification of pharmacokinetic parameters

Part A:

The following pharmacokinetic parameters will be determined from DF2755Y (the acid form of DF2755A and for metabolites if any):

- C_{max} (ng/mL);
- t_{max} (h);
- λ_Z;
- $t_{1/2}$(h)::;
- AUC_{0-24} (h×ng/mL);
- AUC_{0-t} (h×ng/mL);
- $AUC_{0-\infty}$ (h×ng/mL);
- fu
- V_Z/F (DF2577Y only);
- CL/F (DF2577Y only);
- Ae_{0-24} and AUC_{0-last};
- fe_{0-24} and $fe_{0-tlast}$;
- CL_R;
- Total amount in faeces;
- Parent:metabolite(s) ratio for C_{max} and AUCs..

Part B:

The following pharmacokinetic parameters will be determined from DF2577Y (the acid form of DF2755A and for metabolites if any):

Day 1

- C_{max} (ng/mL);
- t_{max} (h);
- t_{lag} (h);
- λ_Z;
- $t_{1/2}$ (h);
- AUC_{0-12} (h×ng/mL);
- Ae_{0-12} and Ae_{0-24};
- fu;
- fe (%);
- CL_R: renal clearance;
- Parent:metabolite(s) ratio for C_{max} and AUCs.

Day 14

- $C_{ss,max}$ (ng/mL);
- $t_{ss,max}$ (h);
- t_{lag} (h);$C_{ss,min}$ (ng/mL): measured plasma concentration at the end of the dosing interval;
- λ_Z: terminal phase rate constant;
- $t_{1/2}$ (h): terminal half-life;
- $AUC_{0-\tau}$ (h×ng/mL);
- $C_{ss,av}$ (ng/mL);
- fu;
- $Ae_{0-tlast}$;
- Total amount in faeces;
- Parent:metabolite(s) ratio;
- Accumulation ratios for C_{max} and AUC_{0-12}.

6.3. Methods and timing for assessing, recording, and analysing pharmacokinetic parameters

6.3.1. Collection, treatment and storage of blood samples

Blood sampling will be performed for DF 2755Y concentration measurements at the exact time-points with an authorised time-window described in the table below.
When ECG, vital signs and PK are required at the same time, this specific order will be followed: ECG first, then vital signs and blood sample for PK at the end.

Part A :

Day	Sampling time	Sample N°	Time window(min)
1	T0 (pre-dose)	P00	
	T0h30min	P01	+/-1
	T1h00	P02	+/-3
	T2h	P03	+/-3
	T3h	P04	+/-5
	T4h	P05	+/-5
	T5h	P06	+/-5
	T6h	P07	+/-5
	T7h	P08	+/-5
	T8h	P09	+/-5
	T9h	P10	+/-5
	T10h	P11	+/-5
	T12h	P12	+/-5
	T16h	P13	+/-5
2	T24h	P14	+/-15
	T36h	P15	+/-15
	T40h	P16	+/-15
3	T48h	P17	+/-15
	T60h	P18	+/-15
4	T72h	P19	+/-15

After the determinations of DF 2755Y in plasma samples during cohort 1 and according to results obtained, the blood sampling times and the collection periods could be reviewed to optimize the collection times with respect to the PK profile of DF 2755Y.

Part B :

Day	Sampling time	Sample N°	Time window(min)
1	T0 (pre-dose)	P00	
	T0h30min	P01	+/-1
	T1h00	P02	+/-3
	T2h	P03	+/-3
	T3h	P04	+/-5
	T4h	P05	+/-5
	T5h	P06	+/-5
	T6h	P07	+/-5
	T7h	P08	+/-5
	T8h	P09	+/-5
	T9h	P10	+/-5
	T10h	P11	+/-5
	T12h	P12	+/-5
	T16h	P13	+/-5
2	Pre-dose/T24h D1	P14	+/-15
5	T0 (pre-dose)/ T24h D4	P15	+/-15
	T_{Cmax}[1]	P16	+/-15
	T12h	P17	+/-15
	T_{Cmax}[1]	P18	+/-15
6	Pre-dose/T24h D5	P19	+/-15
10	T0 (pre-dose)/ T24h D9	P20	+/-15
	T_{Cmax}[1]	P21	+/-15
	T12h	P22	+/-15
	T_{Cmax}[1]	P23	+/-15
11	Pre-dose/T24h D10	P24	+/-15

Day	Sampling time	Sample N°	Time window(min)
14	T0 (pre-dose)/ T24h D13	P25	+/-15
	T0h30min	P26	+/-15
	T1h	P27	+/-15
	T2h	P28	+/-15
	T3h	P29	+/-15
	T4h	P30	+/-15
	T5h	P31	+/-15
	T6h	P32	+/-15
	T7h	P33	+/-15
	T8h	P34	+/-15
	T9h	P35	+/-15
	T10h	P36	+/-15
	T12h	P37	+/-15
	T16h	P38	+/-15
15	T24h D14	P39	+/-15
	T36h	P40	+/-15
	T40h	P41	+/-15
16	T48h	P42	+/-15
	T60h	P43	+/-15
17	T72h	P44	+/-15

[1]This time (T_{Cmax}) will be determined thanks to part A results.

Blood handling procedures:

A cannula or direct venepuncture will be used to collect blood samples. 6 mL of blood for assay of DF 2755Y and metabolites (if applicable) will be taken into lithium heparin vacutainers. Blood samples will be centrifuged within 15 minutes of collection at 1500 g at 4°C for 10 minutes. Around 3 mL of plasma will be obtained and, immediately after the centrifugation, divided equally into two aliquots. Plasma samples will be stored immediately at approximately -20°C into plus screwed polypropylene tubes.

Blood samples Transport:

Samples will be sent to Dompé farmaceutici S.p.A Bioanalytical Laboratories for analysis. The shipment will be done in a dry ice or under controlled temperature between -15°C and -25°C by a specialized carrier.

Temperatures will be monitored using data logger during all transport. The first set will be sent frozen separately to the second one.

Dompé farmaceutici S.p.A Bioanalytical Laboratories

Via Campo di Pile

s.n.c L'Aquila, Italy

attention to Dr. ssa Maria Teresa Massucci.

6.3.2. Collection, treatment and storage of urine samples

Part A:
Urine collection will be performed for DF 2755Y and two metabolites urine concentration measurements, at the following time points:]D-1T-24h – D1T0h(pre-dose)],]D1T0h – D1T6h],]D1T6h – D1T12h],]D1T12h – D2T24h],]D2T24h – D3T48h],]D3T48h – D4T72h].

Part B:
Urine collection will be performed for DF 2755Y and two metabolites urine concentration measurements, at the following time points:]D-1T-24h – D1T0h(pre-dose)],]D1T0h – D1T12h],]D1T12h – D2T24h],]D5T0h – D6T24h],]D10T0h – D11T24h],]D14T0h – D14T12h],]D14T12h – D15T24h],]D15T24h – D16T48h],]D16T48h – D17T72h].

Urine handling procedures:

Urine will be collected for PK analysis from 24 hours before dosing on Day 1 until 72 hours post-dose in Cohorts 1, 2, 3 and 4 (SAD, PART A) and from 24 hours before dosing on Day 1 until 408 h after the first dose in Cohort 5, 6 and 7 (MAD, PART B). The urine collection intervals are detailed above. Subjects will be asked to void their bladders prior to the start of each collection interval and the start and stop time of each collection interval will be recorded in the CRF.

Urine collections for PK analysis will be collected into bottles without preservative. Following completion of each urine collection period, urine pH will be measured and the weight of each collection will be recorded. The collection will be thoroughly mixed and one 5 mL aliquot will be removed from each collection to determine creatinine excretion (Eurofins laboratory). Then two aliquots of 5 mL will be taken for PK analysis and stored at approximately -20°C in stopped polypropylene tubes.

Urine samples Transport:

Samples will be sent to Dompé farmaceutici S.p.A Bioanalytical Laboratories for analysis. The shipment will be done in a dry ice or under controlled temperature between -15°C and -25°C by a specialized carrier. Temperatures will be monitored using data logger during all transport. The first set will be sent frozen separately to the second one.

Dompé farmaceutici S.p.A Bioanalytical Laboratories
Via Campo di Pile
s.n.c L'Aquila, Italy
attention to Dr.ssa Maria Teresa Massucci

6.3.3. Collection, treatment and storage of faeces samples

Part A:
Faeces collection will be performed for DF 2755Y and two metabolites faeces concentration measurements, at the following time points:]D-1T-24h – D1T0h(pre-dose)],]D1T0h – D2T24h],]D2T24h – D3T48h],]D3T48h – D4T72h].

Part B:
Faeces collection will be performed for DF 2755Y and two metabolites faeces concentration measurements, at the following time points:]D-1T-24h – D1T0h(pre-dose)],]D1T0h – D2T24h],]D5T0h – D6T24h],]D10T0h – D11T24h],]D14T0h – D15T24h],]D15T24h – D16T48h],]D16T48h – D17T72h].

Faeces handling procedures:

Faeces will be collected for PK analysis from 24 hours before dosing on Day 1 until 72h post dose in Cohorts 1, 2, 3 and 4 (SAD) and from 24 hours before dosing on Day 1 until 408 h after the first dose in Cohort 5, 6 and 7 (MAD). The faeces collection intervals are detailed above.

After the collection of each faeces samples, the sample will be weighed and homogenized within 30 minute. Two 10 g aliquots of each homogenised sample will be taken. Each 10 g aliquot will be mixed withPhosphate buffered salin (PBS) at PH = 7.4. The faeces/buffer ratio should be 1:2 (e.g., 10 g faeces mixed with 20 mL buffer). The samples (2 aliquots) will be stored at approximately -20°C in stopped polypropylene tubes.

Faeces samples Transport:

Samples will be sent to Dompé farmaceutici S.p.A Bioanalytical Laboratories for analysis. The shipment will be done in dry ice or under controlled temperature between -15°C and -25°C by a specialized carrier. Temperatures will be monitored using data logger during all transport. The first set will be sent frozen separately to the second one.

Dompé farmaceutici S.p.A Bioanalytical Laboratories

Via Campo di Pile

s.n.c L'Aquila, Italy

attention to Dr.ssa Maria Teresa Massucci

6.4. Analytical methods

Plasma, urine and faeces samples will be assayed to determine the concentrations of DF2755Y (bound and unbound plasma sample) according to Dompé farmaceutici S.p.A Bioanalytical Laboratories study plans. The samples will be assayed with validated analytical methods. The metabolic pathway will be also studied to identify the potential metabolites.

7. ASSESSMENT OF PHARMACODYNAMIC

The Assessment of Pharmacodynamic will be performed in Part A (only for leucocyte subsets) starting from the second ascending dose and in Part B of the study.

7.1. Cytokine Inflammation Marker analysis

Inflammation Cytokines:

1. CXCL1 (Gro alfa)
2. CXCL2 (Gro beta) (Biorad)
3. CXCL5 (ENA-78)
4. CXCL6 (GCP-2)
5. CXCL8 (IL-8)

will be analyzed from blood samples.

The method (Elisa) that will be used for the determination of cytokines will be discussed in the final report.

Part B:

Day	Sampling time	Sample N°	Time window(min)
1	T0 (pre-dose)	Il100	NA
5	T0 (pre-dose)	Il101	+/-15
14	T0 (pre-dose)	Il102	+/-15
16	T48h	Il103	+/-15
17	T72h	Il104	+/-15

Blood handling procedures: At each time point indicated in the table, a 10 mL blood sample should be drawn into K2 EDTA tube. This volume is in addition with the one of the assessment of pharmacokinetic described previously. After collection, gently mix the blood by inverting the tube few time for complete mixing with anticoagulant. Store vacutainer tubes upright at 4ºC until centrifugation. Blood samples should be centrifuged within four hours of blood collection. Centrifuge blood samples for 15 minutes at 1000 g at 4°C. After centrifugation carefully transfer the plasma layer with an appropriate transfer pipette without disturbing the buffy coat layer to a clean polypropylene tube. To complete remove platelets and precipitates, centrifuge again for 10 min at 10000g at 4°C. Aliquot plasma into 5 pre-labelled polypropylene tubes. Each tube will contain approximately 700 µL of plasma. All sample tubes must be clearly and appropriately labelled. Tubes will be capped immediately from each time point and the plasma will be frozen in an upright position at approximately –80°C for storage.

Blood samples Transport: Samples will be sent to laboratory Hôpital Lyon Sud for analysis of secondary objective. The shipment will be done in a dry ice by a specialized carrier. Temperatures will be monitored using data logger during all transport. Four sets will be sent frozen separately to Hôpital Lyon Sud and one will be kept at EUROFINS OPTIMED.

7.2. Leukocytes subset blood samples

Leukocytes subset: CD11b and CD18 will be analysed on PMN from blood samples.

Part A:

Day	Sampling time	Sample N°	Time window(min)
1	**T0** (pre-dose) T_{Cmax} (time will be set after the results of the first dose administred) T_{Cmax} + **3h** (time will be determined after the results of the first dose administred) **T12h**	CD00 – RS00 CD01 – RS01 CD02 – RS02 CD03 – RS03	NA +/-5 +/-5
2	**24 h**	CD04 – RS04	+/-15
3 or 4	**48 h or 72 h** **(**time will be determined after the results of the first dose administred)	CD05 – RS05	+/-15

Part B:

Day	Sampling time	Sample N°	Time window(min)
1	**T0** (pre-dose) T_{Cmax}	CD10 – RS10 CD11 – RS11	 +/-5
Window from 5 to 7	T0 (pre-dose)/ T_{Cmax}	CD12 – RS12 CD13 – RS13	+/-15 +/-15
14 (Last dose)	T0 T_{Cmax}	CD14 – RS14	+/-15
15	T24h	CD15 – RS15	+/-15

Blood handling procedures:
- CD11b & CD18: at each time point indicated in the table, a 6 mL blood sample should be drawn into a K3 EDTA tube. This volume is in addition with the one of the assessment of pharmacokinetic and for cytokines described previously.

Sample will be treated at Eurofins-Optimed laboratory (detailed will be discuss in the final report) and will be read at immunology laboratory CHU de Grenoble by a flow Cytometer.

Samples Transport: samples will be shipped to Immunology laboratory CHU de Grenoble at 4°C protected from light.

8. ASSESSMENT OF SAFETY

8.1. Specification of safety parameters

8.1.1. Clinical parameters

8.1.1.1. Blood pressure and heart rate

Vital signs consist of systolic (SBP) and diastolic (DBP) blood pressures and heart rate.

The measurements should be made after at least 10 minutes rest in the supine position and after 2 minutes in the standing position, before venepuncture when times of each coincide (except pre-dose).

8.1.1.2. Physical examination

A physical examination including evaluation of main body systems/regions, including: skin and mucous, ears/nose/throat, pulmonary, cardiac, gastro-intestinal and neurological systems.

In case of abnormality, a comment will be recorded in the eCRF.

8.1.1.3. Body weight and height

Measurements of body weight and height (at screening only), will be done during physical examination (when time of body weight measurement coincides with physical examination)

8.1.1.4. Electrocardiogram (ECG)

Twelve-lead ECGs will be recorded after at least 10 minutes in supine position using a Cartouch Cardionics® Device.

ECGs should always be recorded before the PK sampling (if any), except pre-dose.

Each ECG consists of a 10 second recording of the 12 leads simultaneously, leading to a12-lead ECG (25 mm/s, 10mm/mV) print-out with HR, PR, QRS, QT, QTc automatic correction evaluation, including date, time, number of the subject, signature of the research physician, and at least 3 complexes for each lead. The Investigator medical opinion and automatic values will be recorded in the eCRF. This print-out will be retained at the site level.

8.1.2. Biological parameters

8.1.2.1. Routine laboratory observations

Before the subject is allowed to enter the study a full biological test will be obtained and the absence of clinically significant abnormalities confirmed by the Investigator.

The full biological test comprises:

Haematology:. These tests will be performed as follow: Haemoglobin, haematocrit, Red Blood Cells (RBC), White Blood Cells (WBC), differential count, platelet count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC).

Hemostasis: PT, aPTT.

Biochemistry:. These tests will be performed as follow: Creatinine, fasting blood glucose, total proteins, albumin, globulin (albumin/globulin ratio), total cholesterol, triglycerides, LDH, measured LDL, HDL, electrolytes (sodium, potassium, chloride, calcium, phosphore), uric acid, alanine serine transferase (AST), alanine leucine transferase (ALT), Gamma Glutamyl Transferase (GGT), alkaline phosphatases, urea, creatine phosphokinase (CPK), total and conjugated bilirubin.

8.1.2.2. Urinalysis

The urinalysis tests will be performed by Eurofins-Optimed. Appearance and colors of urine must be reported in source documents.

Semi-quantitative ("dipstick") analysis will be performed for the following parameters: pH, ketone bodies, proteins, glucose, blood, leukocytes, urobilinogen, bilirubin, nitrite and specific density. If "dipstick" shows a positive result for any of these parameters, a quantitative result will be performed with supplementary analysis: erythrocytes, epithelial cells cells, epithelium renal cells, crytals, cylinders, mucus and bacteria.

8.1.2.3. Drug of abuse screening tests

Drug of abuse screening tests will be performed, at Eurofins Optimed, on urine samples.

Screened drugs are methamphetamines/amphetamines, cannabis, cocaine, opiates, ecstasy.

8.1.2.4. Serologies

It will consist of the determination of: HBs antigen (hepatitis B antigen), anti HCV antibody, anti HIV 1 and 2 antibodies.

8.1.3. Other parameters

8.1.3.1. Alcohol breath test

An alcohol breath test will be performed at the centre, at screening and D-1 for each part. The test will be performed using the alcohol breath device ref 7410 plus Drager.

8.2. Study Procedure

8.2.1. Screening procedures

Screening procedures occur within 3 weeks (D-21 to D-1) before starting study medication.

Subject enrolment – Screening visit

Subjects will be screened within subject's panel of Eurofins Optimed or recruited via advertisements if necessary (in this case, the advertisement has to be submitted to the Ethics Committee for approval before use).

Dedicated recruitment officers will propose subjects to participate in this study. They will be first informed verbally about the study. Then an appointment will be scheduled at the clinical centre (selection visit).

Before any screening assessment is performed, complete and detailed information about the aim, the consequences and the constraints of the trial will be given by a physician, both verbally and by reviewing the information leaflet and consent form. If subject agrees to perform the study, he/she will sign the Informed Consent form and a copy of the information leaflet and consent form will be given to subjects.

At the screening visit, each subject will undergo a complete medical history and a physical examination including blood pressure and heart rate measurements. The subject will undergo the following tests and procedures:

- A medical examination including age, ethnic origin, alcohol, caffeine and nicotine consumptions, previous medication usage, surgical and medical history;
- A complete physical examination including height (cm), weight (kg) and BMI (kg/m^2);
- An alcohol breath test;
- Blood pressure (SBP and DBP) and heart rate in both supine position (after at least 10 minutes rest) and standing position (after 2 minutes), using an automatic sphygmomanometer;
- ECG,
- A biological screening test, including:

- Serology test: HBsAg, anti-HCV antibody, anti-HIV 1 and 2 antibodies,
- Urine drug screen: metamphetamines/amphetamines, cannabis, cocaine, opiates and ecstasy,
- Haematology,
- Haemostasis,
- Biochemistry,
- Urinalysis (qualitative).

8.2.2. Description by type of visit

Part A:

D-1

- Subject admission;
- Physical and medical examination;
- Body weight
- Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
- AE/Prior medication check;
- Alcohol breath test;
- Urine test for drug abuse;
- ECG;
- Vital signs;
- Urine collection;
- Faeces collection.

D1

- *Before administration*

- Vital signs;
- ECG;
- AE/Concomitant medication check;
- Blood sampling (Pre-dose, PK, leukocytes subsets (except for 1st cohort)).
- Urine collection
- Faeces collection

- *oral administration in fasting condition.*

- AE/Concomitant medication check (any time);
- ECG;
- Vital signs;
- Blood sampling (PK, leukocytes subsets (except for 1st cohort));
- Urine collection;
- Faeces collection.

D2

- Vital signs;
- Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
- AE/Concomitant medication check (any time);
- Blood sampling (PK, leukocytes subset (except for 1st cohort));
- ECG;
- Urine collection;
- Faeces collection.

D3

- AE/Concomitant medication check (any time);
- Blood sampling (PK, leukocytes subset (except for 1st cohort));
- ECG;
- Urine collection;
- Faeces collection.

D4 end of study visit

- Physical and medical examination;
- Body weight
- Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
- ECG;
- Vital signs;
- Blood sampling (PK, leukocytes subset, if not done on D3(except for 1st cohort))
- Urine collection;
- Faeces collection;
- AE/Concomitant medication check;
- Subject discharge.

Part B:

D-1

- Subject admission;
- Physical and medical examination;
- Body weight
- Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
- AE/Concomitant medication check;
- Alcohol breath test;
- Urine test for drug abuse;
- Vital signs;
- ECG;
- Urine collection;
- Faeces collection.

D1

- *Before administration*
  - Physical and medical examination;
  - Vital signs;
  - ECG;
  - AE/Concomitant medication check;
  - Blood sampling (PK, cytokine plasma concentration and leukocytes subset);
  - Urine collection;
  - Faeces collection.

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time);
  - ECG;
  - Blood sampling (PK and leukocytes subset);
  - Urine collection;
  - Faeces collection.

D2

- *Before administration*
  - AE/Concomitant medication check (any time);

- ECG;
- Blood sampling for PK;
- Urine collection;
- Faeces collection.

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time);
  - ECG;

D3

- *Before administration*
  - AE/Concomitant medication check (any time);
  - ECG;

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time);
  - ECG;

D4

- *Before administration*
  - AE/Concomitant medication check (any time);
  - ECG;

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time);

D5

- *Before administration*
  - Physical and medical examination;
  - Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
  - Vital signs;
  - AE/Concomitant medication check;
  - Blood sampling (PK, cytokine plasma concentration and leukocytes subset);
  - Urine collection;
  - Faeces collection.

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time);
  - ECG;
  - Blood sampling (PK and leukocytes subset);
  - Urine collection;
  - Faeces collection.

D6

- *Before administration*
  - AE/Concomitant medication check (any time);
  - ECG;
  - Blood sampling (PK and leukocytes subset if the case)
  - Urine collection;
  - Faeces collection.

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time).

- Blood sampling (leukocytes subset if the case)
-

D7

- *Before administration*
  - Physical and medical examination;
  - Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
  - Vital signs;
  - AE/Concomitant medication check.
  - Blood sampling (leukocytes subset if the case)
  -

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time);
  - ECG.
  - Blood sampling (leukocytes subset if the case)

D8

- *Before administration*
  - AE/Concomitant medication check;
  - ECG.

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time).

D9

- AE/Concomitant medication check;

D10

- *Before administration*
  - Physical and medical examination;
  - Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
  - Vital signs;
  - AE/Concomitant medication check;
  - Blood sampling for PK;
  - Urine collection;
  - Faeces collection.

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time);
  - ECG;
  - Blood sampling for PK;
  - Urine collection;
  - Faeces collection.

D11

- *Before administration*
  - AE/Concomitant medication check (any time);
  - ECG;
  - Blood sampling for PK;
  - Urine collection;
  - Faeces collection.

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time).

D12 – D13
- AE/Concomitant medication check;

D14

- *Before administration*
  - Physical and medical examination;
  - Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
  - Vital signs;
  - AE/Concomitant medication check;
  - Blood sampling (PK, cytokine plasma concentration);
  - Urine collection;
  - Faeces collection.

- *oral administration in fasting condition.*
  - AE/Concomitant medication check (any time);
  - ECG;
  - Blood sampling (PK);
  - Urine collection;
  - Faeces collection.

D15
- AE/Concomitant medication check;
- ECG;
- Blood sampling (PK and leukocytes subset)
- Urine collection;
- Faeces collection.

D16
- AE/Concomitant medication check (any time);
- Blood sampling (PK, cytokine plasma concentration);
- Urine collection;
- Faeces collection.

D17 end of study visit
- Physical and medical examination;
- Body weight
- Laboratory safety (haematology, biochemistry, haemostasis, urinalysis);
- AE/Concomitant medication check;
- Vital signs;
- ECG;
- Blood sampling (PK, cytokine plasma concentration);
- Urine collection;
- Faeces collection;
- Subject discharge.

8.2.3. Sampled blood volume

The total amount of blood collected during the study will be approximately:

Part A (Cohorts: 1) Tests	Number of Samples	Volume (mL)	Total (mL)
Screening biology	1	18.7	18.7
Study biology	3	10.2	30.6
Pharmacokinetics (SAD)	20	6	120

Total volume in Part A cohort 1 169,3 mL

Part A (Cohorts: 2, 3, 4) Tests	Number of Samples	Volume (mL)	Total (mL)
Screening biology	1	18.7	18.7
Study biology	3	10.2	30.6
Pharmacokinetics (SAD)	20	6	120
Pharmacodynamics CD11b&CD18	6	6	36

Total volume in Part A cohort 2, 3, 4 205.3 mL

Part B (Cohorts: 5, 6 and 7) Tests	Number of Samples	Volume (mL)	Total (mL)
Screening biology	1	18.7	18.7
Study biology	6	10.2	61.2
Pharmacokinetics (MAD)	45	6	270
Pharmacodinamics cytokine	5	10	50
Pharmacodinamics CD11b&CD18	6	6	36

Total volume in Part B 435.9 mL

8.3. Evaluation of Adverse Event and Safety Information

Adverse Event (AE), is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment/product. 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 (investigational) product, whether or not related to the medicinal (investigational) product.

Adverse Drug Reaction (ADR), all untoward and unintended responses to an IMP reasonably related to any dose administered i.e. any AEs judged by either the Investigator or the Sponsor as having a reasonable causal relationship with the IMP. The expression "reasonable causal relationship" means to convey in general that there is evidence or argument to suggest a causal relationship. The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

Serious Adverse Event or Reaction, a Serious Adverse Event (SAE) or Reaction (SAR) is any untoward medical occurrence, including intercurrent pathology, or effect that at any dose:

- Results in death (a cause of death shall always be specified when known);

- Is life-threatening (at the time of the event);

  NOTE: Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include an event which hypothetically might have caused death had it occurred in a more severe form.
- Requires in patient hospitalization or prolongation of existing hospitalization;
  NOTE: In general, hospitalization means that the individual remained at the hospital or emergency ward for observation and/or treatment (usually involving an overnight stay) that would not have been appropriate in the physician's office or an out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred, the event should be considered serious.
- Results in persistent or significant disability or incapacity;
  NOTE: This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions, but do not constitute a substantial disruption.
- Is a congenital anomaly or birth defect;
- Is an important medical event: an important medical reaction, that may not result in death, be life-threatening or require hospitalization, may be considered a SAE when, based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above. *Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in patient hospitalization, or the development of drug dependency or drug abuse.*

Pre-planned hospitalization or hospitalization, lasting less than 24h, not associated with any deterioration in condition are not considered to be serious adverse events. These events must be recorded in the AE page of the CRF where a variable will be ticked to indicate that they are not SAEs.

Unexpected adverse reaction, an unexpected adverse reaction is an adverse reaction, whose nature, severity or outcome is not consistent with the applicable medicinal product information i.e. the Investigator's Brochure (Reference Safety Information) for an unauthorized IMP or the Summary of Product Characteristics (SmPC) for an authorized product which is being used according to the terms and conditions of the marketing authorization. Events that are mentioned in the Investigator Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation are considered unexpected.

Suspected unexpected serious unexpected adverse reaction (SUSAR) is defined as an adverse reaction that is both unexpected (not consistent with the applicable product information) and meets the definition of a Serious Adverse Reaction. The determination of expectedness should be made on the basis of the IB (Reference Safety Information).

New event (*faits nouveaux*) is defined as new safety data that may lead to a re-assessment of the benefit/risk balance of the investigational medicinal product or of the clinical study or that may be sufficient to lead to changes in the IMP administration, study conduct or study related documents.
In clinical study involving healthy volunteers, fatal adverse events and events leading to hospitalization, potentially related to the study drug, of a subject enrolled in the study is qualified as new event. The new event shall immediately lead to discontinuation of study treatment administration to any enrolled participants until their safety is guaranteed (through urgent safety measures). Subjects' informed and written consent must be obtained before re-starting the trial. The new event can correspond to SUSAR suspicion. In this case, the event will be declared twice : as a SUSAR and as a new event.

Urgent safety measure (*mesures urgentes de sécurité*) is a decision taken, by the Sponsor or the Investigator, following the occurrence of a new event likely to impact on the safety of the study subjects. It may include the immediate discontinuation of a clinical trial or immediate action on the study conduct, in order to protect subjects from any safety danger.

8.3.1. Recording of adverse events

Any AE (including laboratory test abnormalities, intercurrent illnesses or injuries, and/or study procedures related AE) reported spontaneously by the subjects, or observed by the Investigator, will be recorded according to the procedures in force at EUROFINS OPTIMED.
Any untoward medical event, which occurs from the time of signed Informed Consent to the time of IMP administration, will be classified as "pre-dose event".
Any AE (non-serious and serious) which occur during the course of the study will be recorded in the CRF. Any pre-existing medical conditions or signs/symptoms present in a patient prior to the start of the study (i.e., before informed consent is signed), should be entered in the baseline history section of the CRF.
Any untoward medical event which occurs within 30 days after the completion of the clinical trial and that is possibly reported by the Investigator to EUROFINS OPTIMED will be classified as a "post-study event".
Laboratory, vital signs or ECG abnormalities are to be recorded as AE only if:

- Symptomatic, and/or
- Requiring either corrective treatment or clinically significant , and/or
- Leading to IMP/non IMP discontinuation or modification of dosing, and/or
- Fulfilling a serious criterion, and/or

In line with ICH E2A provisions, although the Investigator does not usually need to actively monitor subjects for adverse events once the trial has ended, if the Investigator becomes aware of a serious adverse event occurring to a subject after the treatment of that subject has ended, the SAE should be reported by the Investigator to the Sponsor. Such "cases" should be regarded for expedited reporting purposes as though they were study reports. Therefore, a causality assessment (by the Investigator and by Dompé) and determination of expectedness (by Dompé) are needed for a decision on whether or not expedited reporting is required.

8.3.2. Analysis of adverse events

The Investigator will evaluate the seriousness of any reported adverse event.

The Investigator will also evaluate each event with regard to its severity. The severity of the AEs will be determined in the following manner:

Mild	Grade 1 - Does not interfere with subject's usual function (awareness of symptoms or signs, but easily tolerated [acceptable]).
Moderate	Grade 2 - Interferes to some extent with subject's usual function (enough discomfort to interfere with usual activity [disturbing]).
Severe	Grade 3 - Interferes significantly with subjectt's usual function (incapacity to work or to do usual activities [unacceptable])

The Investigator will also evaluate the causality of the study treatment/product and any other treatments for each AE, and will transmit the result of this evaluation to the Sponsor. The possible relationship between the AE and the study product/treatment will be quoted as following:

None (Intercurrent Event)	An event that is not and cannot be related to the investigational product, e.g. patient is a passenger in a road traffic accident or surgical intervention performed during the study, but planned before patient enrolment into the study
Unlikely (remote)	Relationship is not likely e.g. a clinical event including laboratory test abnormality with temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals or underlying disease provide plausible explanations
Possible	Relationship may exist, but could have been produced by the patient's condition or treatment or other cause
Probable	Relationship is likely, the AE abates upon discontinuation of investigational product and cannot be due to the patient's condition
Highly Probable	Strong relationship, the event abates upon discontinuation of investigational product and, if applicable, re-appears upon repeat exposure
Not Assessable	Not possible to assess, because of insufficient evidence, conflicting data or poor documentation.

Also the Sponsor will evaluate serious adverse events which are reported by the Investigator, assessing causality with the study drug and any other treatments for each SAE and expectedness.

SAEs for which the Investigator or the Sponsor consider that a causal link with the study product could reasonably be envisaged will be considered to be suspected adverse effects. Should the evaluations of the Sponsor and the Investigator differ with regard to causality, then both will be reported in the declaration of suspected adverse effect.
SAE considered "Possible", "Probable" and "Highly Probable" related to the IMP treatment, if considered serious and unexpected, will be reported to appropriate Regulatory Authorities.

8.4. Procedures in place for the recording and notification of serious adverse events, SUSAR and new events

Reporting procedure from Investigator to Sponsor

The Investigator will notify the Sponsor without delay on the day of awareness of any SAE. In addition, the Investigator will notify the Sponsor of AEs and abnormal analysis results defined in the paragraph "Description of safety evaluation parameters".

The Investigator must:

- **note** in the participant's medical file the date on which he/she becomes aware of the event (at a follow-up visit or a telephone contact with the participant or a third person, etc);
- **immediately inform** by email, the person responsible at SPONSOR (Pier Adelchi Ruffini, MD mobile +39 346 874 5908, pieradelchi.ruffini@dompe.com) and by fax or e-mail the SPONSOR PHARMACOVIGILANCE office, at the following addresses: Fax number : +39 02 36026913 email: farmacovigilanza@dompe.com;
- complete the SAE form and send it by fax or email to the persons responsible designated above, immediately (within 24 hours) after of being informed of this event, without waiting for the results of the clinical outcome or of additional investigations;
- provide the persons designated above, as it becomes available, with any relevant information (together with translation into English language) that could contribute to the clarification of the SAE and to the assessment of potential risk for the study subjects and with anonymised copies of the documents which provide additional useful information, such as hospital admission reports, reports of further consultations, laboratory test reports, reports of other examinations aiding diagnosis (where possible, the results from pre-treatment assessments should be appended for

comparison with the results obtained under treatment), or the autopsy report, if autopsy is performed;
- inform the persons designated above of the outcome, if not previously reported, and other relevant follow up information of the SAE as soon as possible, through a SAE form clearly marked as Follow Up;

The Investigator must also report all SAEs/SARs in the eCRF by filling in the AE form.
If the SAE is the reason of subject drop-out from the study, the Investigator will detail the reason for such a statement in the comment section of the form.

The minimum criteria to be reported are as follows:
- a suspected investigational medicinal product;
- an identifiable subject (at least study subject identification code number, gender, age, but no subject initials);
- an AE assessed as serious;
- an identifiable reporting source;
- an unique clinical trial identification;
- the opinion of the Investigator about the causal relationship between the event and the IMP.

The outcome of the SAE shall be classified as following:
- recovered/resolved;
- recovering/resolving;
- recovered/resolved with sequelae;
- not recovered/not resolved;
- fatal;
- unknown.

Details should be given for the latter four categories.

Regulatory reporting procedure from Sponsor to Regulatory Authorities

The Sponsor will submit to the Competent Authority (ANSM) and to the Ethics Committee which gave its approval for the research to take place, all unexpected SARs which took place during the research and likely to be due to the tested products (SUSAR), new event or urgent safety measures.SUSAR shall also be electronically transmitted to EudraVigilance CT Module (EVCT).

- **SUSAR declaration**

In case of SUSAR regarding a clinical trial on medicinal products involving healthy volunteers

- Each SUSAR must to be reported in an individual electronic email message
- It should be sent to : declarationsusars@ansm.sante.fr and to the EC (CPP)
- The subject line should be written as follows : EC-VS SUSAR_yyyymmdd_name of the substance (or trial code number)_ Worldwide unique case identification number_CT
- The following document should be attached to the message : CIOMS form (PDF format)/file name labelled as follows : yyyymmdd_name of substance (or trial code number)_worldwide unique case identification number_CT_C
- An acknowledgement of receipt will be automatically sent by return email

Treatment will be unmasked by Sponsor Drug Safety prior to submission of a SUSAR to Regulatory Authorities and only cases referred to active treatment will be considered expeditable for regulatory reporting, in line with law requirements.

Declarations must be done :

- Without delay (and in no case later than seven days) : if the event is fatal or life threatening; to be followed by any relevant information within eight days.

- No later than fifteen days if the event is serious but neither fatal nor life threatening; to be followed by any relevant information within eight days.

The same timelines apply for SUSAR electronic submission via EVCT.

- **New event declaration**

- It should be sent to : aec-essaiscliniques@ansm.sante.fr and to the EC (CPP)
- The subject line should be written as follows and in French : Fait nouveau/volontaires sains/n° essai/DCI ou code substance
- The text message (in English) should include:
  - EudraCT number,
  - Trial title,
  - Trial code number,
  - New event summary,
  - If applicable, urgent safety measures taken, all relevant information

A new event may be a fatal event or an event leading to subject's hospitalization, potentially related to the drug study. It may also be a SUSAR: in this case, the corresponding event must be reported twice, both as New Event and as SUSAR, to relevant involved parties.

Declarations must be done :

- **Without delay** and to be followed by any relevant information within eight days.

The Sponsor will ensure that subjects involved in the study will be informed of New Events as applicable.

The new event shall immediately lead to discontinuation of study treatment administration to any enrolled participants until their safety is guaranteed (through urgent safety measures). After the New Event evaluation by the Sponsor, if subject's safety is guarantee, the clinical study is re-started and subjects' informed and written consent must be obtained prior to IMP administration.

- **Urgent safety measure declaration**

- First by phone to ANSM: gastroenterology department: Nathalie Dumarcet: +33(0)1 55 87 36 77
- Then by email to: ams-essaiscliniques@ansm.sante.fr and to the EC (CPP)
- The subject line should be written as follows and in French : MUS/n° essai/DCI ou code substance
- The text message (in English) should include the details of the urgent safety measure and the action plan

Declarations must be done :

- Verbally **without delay** and followed by a report within fifteen days
- The declaration without any delay has to be followed by :
  - ✓ The submission of a substantial modification of the clinical study within fifteen days after the initiation of the urgent safety measure (in order to specify a temporary cessation or to make substantial modification)

Or

  - ✓ Declaration of an anticipated end of study, within fifteen days.

Periodical Reporting to Regulatory Authorities and Investigators

Dompé Drug Safety shall be responsible to prepare and submit periodical safety reports (Development Safety Update Report – DSUR) to relevant Regulatory Authorities and Ethic Committee, as applicable.

Periodical (six monthly) line listing of SUSARs will be provided to Investigators as well, maintaining treatment information blinded, as applicable.

The Sponsor will answer to any request from the Competent Authority or Ethics Committee concerning such reactions.
The Sponsor Pharmacovigilance is responsible for all declarations referred to safety to Ethics Committee and Health Authorities.

8.5. Type and duration of the follow-up of subjects after adverse events

All AEs will be monitored by the Investigator until a satisfactory outcome is obtained (i.e. the event is resolved or the condition is unlikely to change or the subject is lost to follow-up). All clinical and biological examinations judged necessary by the Investigator will be continued until return to normal. The Investigator will provide the Sponsor with copies of all examination results and treatments linked to the follow-up of AEs.

8.6. PREGNANCY IN THE CLINICAL TRIAL

Prior to enrolment in the clinical trial, volunteers 'partners with childbearing potential must be advised of the importance of avoiding pregnancy from the time of informed consent signature up to 90 days after the last IMP administration-and of the potential risks associated with an unintentional pregnancy.

A male participating to the study have to agree to use a condom with spermicide, on the time of informed consent signature, up to 90 days after the last IMP administration. The Principal Investigator or a delegate must inform a female sexual partner to use at least one of the following reliable methods of contraception:

a. Hormonal oral, implantable, transdermal, or injectable contraceptives
b. A non-hormonal intrauterine device [IUD] or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide

During the trial and within 30 days after the last IMP administration, female subjects are to be instructed to contact the Investigator immediately if they suspect they might be pregnant. In the same way, male subjects who become aware that the partner might be pregnant, are to be instructed to contact the Investigator immediately.

The Investigator must report every pregnancy on a pregnancy report form as soon as possible (within 24 hours of learning of the pregnancy to the Dompé Drug Safety contacts reported at Paragraph 8.4, even if no AE has occurred, and follow it to term. If, however, the pregnancy is associated with an SAE (eg, if the mother is hospitalized for an adverse event), in addition to the pregnancy report form, a separate SAE report form must be filed as described in Section 8.4 with the appropriate serious criterion (eg, hospitalization) indicated on the SAE report form. Miscarriage, stillbirth and any malformation/disease must be reported as a SAE.

A form prepared by Dompé will be utilized to capture all pregnancy-related information until the birth of the child.

8.7. OVERDOSE

Cases of overdose (accidental or intentional) which may or may not result in serious adverse reactions are to be reported to Sponsor Drug Safety, following the same procedure for SAE, within 24 hours from the Investigator's knowledge of its occurrence. This includes reports related to drug intake with suicidal intentions and consequent drug overdose.

Overdose, and shall be reported, even if not associated with adverse reactions, within 24 hours, in a SAE form, to Dompé PHARMACOVIGILANCE contacts reported at Paragraph 8.4 and to Dompé Medical Expert, who should be contacted to discuss corrective treatment, if necessary.

9. DATA MANAGEMENT

9.1. Definition of source data

All evaluations that are reported in electronic Case Report Form (eCRF) must be supported by appropriately signed identified source documentation related to but not limited to:

- Subject identification, last participation to a clinical trial, medical history, previous and concomitant medication;
- Physical examination, blood pressure and heart rate, body weight, BMI, subject habits;
- Dates and times of study drug administration;
- Pharmacokinetic time points;
- Laboratory assessments, meals;
- AEs.

9.2. Source document requirements

According to the guidelines on GCP, the Monitoring Team must check the eCRF entries against the source documents, except for the pre-identified source, data directly recorded/enclosed in/to the CRF. The Informed Consent Form will include a statement by which the subject allows the Sponsor's duly authorized personnel, the Ethics Committee, and the regulatory authorities to have direct access to source data which supports the data on the eCRF (e.g. subject's medical file, appointment books, original laboratory records, etc.). These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

9.3. Use and completion of the eCRF and additional request

9.3.1.1. Data collection

All clinical data will be reported electronically by the Investigator or authorised designee on a web-based eCRF. This eCRF is specifically designed for the study and developed by the Data Management Department of Eurofins Optimed using Viedoc version 4., a validated Electronic Data Capture system, 21 CFR Part 11 compliant.

Should a correction be made, the corrected information will be entered in the eCRF and the initial information will be tracked in the audit trail.

9.3.1.2. Responsibilities

The Investigator or authorised designee is responsible for the timeliness, completeness, and accuracy of all observations and other data pertinent to the clinical investigation in the eCRFs.

The Investigator will ensure that all data are entered promptly (within 2 days) after the evaluation has occurred, in accordance with source documents and specific instructions accompanying the eCRFs, designed specifically for the study.

The Data Management Department of Eurofins Optimed will provide all tools, instructions, and training necessary to complete the eCRF, and each user will be issued a unique username and password.
The data management of Eurofins Optimed will be responsible for data processing, in accordance with the CRO data management procedures.

9.3.1.3. Data Management

During the study, through regular data collection and monitoring, clinical data will be reported in the eCRF. Computerised logic and/or consistency checks will be systematically applied in order to detect errors or omissions. Queries will be generated and submitted through the electronic data capture (EDC) system to the investigator sites for resolution (queries should be answered within 3 working days, except close to the database lock period, queries should be answered within 1 working day).
Correction will be made either automatically from the immediate completion or following the review of the data during the Eurofins Optimed monitoring. An audit trail, which will be initiated at the time of the first data entry, allows tracking all modifications.
The Data Management Department of Eurofins Optimed may generate additional requests to which the Investigator must respond electronically by confirming or modifying the data questioned. The requests with their responses will be implemented to the eCRFs.

Each step of this process will be monitored through the implementation of individual passwords and regular backups to maintain appropriate database access and to ensure database integrity.

When eCRFs are complete and all queries have been answered, the Investigator has to sign the eCRFs . Then eCRFs are locked and no modification is possible anymore.

After integration of all corrections in the complete set of data, the database will be locked and saved before being released for statistical analysis.

After database lock, a Patient Data Report (PDR) consisting of the creation of the entire casebook for a subject, including the audit trail, will be generated for each subject in .pdf format. The investigator and the Sponsor will receive the PDRs for archiving after the lock of the database. All Data Management activities will be described in a document, the Data Management Plan.

9.4. Study monitoring

EUROFINS OPTIMED will perform the study in accordance with this protocol, GCP and the applicable regulatory requirements and the contract with the Sponsor.

The Investigator is required to ensure compliance with the Investigational Product schedule, visit schedule and procedures required by the protocol. The Investigator agrees to provide all information requested in the CRF in an accurate manner according to the instructions provided and to ensure direct access to source documents to Sponsor representatives.

The Sponsor is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol compliance, integrity and validity of the data recorded on the Case Report Forms. Thus, the main duty of the Monitoring Team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

9.5. Access to data by the subjects

In conformity with the law, any subjects who so wish may access any data concerning them, at the end of the research. They should address their request in writing to the Investigator, and will obtain a response within 8 working days.

10. STATISTICS

10.1. Description of the statistical methods

The statistical analysis will consist in individual data listings and descriptive statistics performed by EUROFINS OPTIMED, using the SAS® computer program (release 9.3).

All individual data for all included subjects will be presented in data listings, sorted by subject within study product/treatment group.

Demographic and baseline characteristics data will be summarized by product/treatment, cohort and visit day where consistent.

For parameters with evaluation before dosing and in case of rechecked value(s) for one subject, only the last observation will be used in descriptive statistics and derivations of other parameter values. After dosing, only observations planned in the protocol will be used in descriptive statistics.

10.1.1. Descriptive statistics

Descriptive statistics for quantitative parameters will be provided using mean, Standard Deviation (SD), Standard Error of the Mean (SEM), minimum, median, maximum, and number of observations; descriptive statistics for qualitative parameters will be provided using frequencies (n) and percent frequencies (%). Percent will be calculated using as denominator the actual number of non-missing data.

10.1.1.1. Subject demographic characteristics, medical history and diagnoses

Continuous variables (age, height, weight, BMI and qualitative variables (race) will be summarized in descriptive statistics on the included subjects and/or pharmacokinetic population, if relevant.

Medical history will be listed and summarized by system organ class and preferred term, if relevant (Medical Dictionary for Regulatory Activity (MedDRA)). Abnormal physical findings at baseline will be listed.

10.1.1.2. Previous medications

Previous medications will be coded according to the World Health Organization-Drug Reference List (WHO-DRL). Subjects who took medications that were stopped before the first study drug dosing will be listed.

10.1.1.3. Baseline safety parameters

Individual safety data (clinical laboratory, vital signs) measured before the first drug administration will be checked for validity of entrance criteria, and abnormalities will be documented. Individual abnormalities before dosing will be flagged in data listings and presented along with post-dose measurements in the statistical appendices.

10.1.1.4. Study drug and concomitant therapy

Drug dispensing information and details of drug dosing (actual products/treatment received, actual dose received, date and time of drug intake) for each subject will be listed by product/treatment group.

Concomitant treatments will be coded according to the World Health Organization-Drug Dictionary (WHO-DD). Subjects who received concomitant treatments along with the study drug will be listed by study product/treatment group and subject. If relevant, concomitant medications will also be summarized by anatomic class and therapeutic class for each treatment group and overall subjects, presenting the frequency of subjects (n) taking a given medication and the number of occurrence of each medication.

10.1.1.5. Pharmacokinetics

All descriptive statistics will be presented independently for each part of the study.

In each part, individual DF2577Y bound and unbound (or related metabolites) plasma concentrations will be presented by dose level and nominal time points, when applicable.

Descriptive statistics for the plasma concentrations will be presented as number of available data (N), mean, standard deviation (SD) and will be calculated if at least 2/3 of the plasma values per time-point are above LOQ. For descriptive statistics calculations, concentrations below LOQ will be set to zero (0) if they are reported before the first quantifiable sample or considered as missing data if they are reported after the first quantifiable data.

Individual derived PK parameters will be presented by dose level (Escalating Dose Part).Descriptive statistics of the PK parameters will be presented as N, mean, SD, standard error (SE), coefficient of variation (CV%), median, minimum (Min), maximum (Max) values, and geometric mean (GM).

In the tables of individual PK parameters, all the deviations from planned analysis will be mentioned by flagging the abnormal results (*e.g.* $t_{1/2}$ determined with terminal elimination slope whose r^2 is lower than 0.90 will also be flagged).

Possible exclusion of flagged PK parameters could be performed if, in the judgment of the pharmacokineticist, they are deemed not to be "pharmacokinetically relevant". Exclusion of PK parameters will be discussed in the PK results section and also discussed with Sponsor. If data are excluded from the PK dataset, all subsequent statistical analyses will be performed twice, once using the complete available PK dataset and once using the final PK dataset as defined by the pharmacokineticist. Study conclusion will be *a priori* based on the final dataset as defined by the pharmacokineticist.

Individual measured plasma concentration *vs.* actual time curve will be produced in graphic for each subject, for each dose level on both linear/linear and log/linear scales where applicable. Similarly, mean plasma concentration *vs.* time curves will also be produced for each dose level.

10.1.2. Analysis of principal criteria

10.1.2.1. Adverse event (for each part)

AEs will be coded according to the Medical Dictionary for Regulatory Activity (MedDRA). They will be classified into pre-defined standard categories according to chronological criteria:

- Treatment emergent AEs (TEAE): AEs that occurs for the first time or if present before worsened during an exposure to drug(s).
- Non-treatment emergent AEs (NTEAE): AEs that occurs before the study drug administration (also called "pre-dose event" see paragraph 7.3.2).

An AE occurring during the run-out will be related to the last day of treatment administration received.

AEs will be individually listed per subject number, presenting: assigned treatment group (or treatment received), verbatim, MedDRA Primary System Organ Class, MedDRA Lowest Level Term, MedDRA Preferred Term, emergence (yes or no) date and time of onset, date and time of last study drug administration before AE, duration, time from onset since last study drug administration, frequency, severity and seriousness, relationship to study drug, the required action taken (e.g. corrective treatment, hospitalisation) outcome and if it is a reason for drop-out.

The non-treatment emergent AEs will be summarised by System Organ Class and Preferred Term for the safety set.

The treatment emergent AEs will be summarised by Primary System Organ Class, Preferred Term and treatment group for the safety set. It will consist in the evaluation of the number of AEs and the number of subjects reporting these AEs.

10.1.2.2. Physical examination, ECG and vital signs (for each part)

Physical examination, ECGs (including QTcF) and vital signs recorded during the study will be individually listed and quantitative parameters will be summarised by using descriptive statistics.

For vital signs and ECG parameters, values and clinically potentially significant abnormalities will be described by treatment and overall, at screening, inclusion, baseline (D1 pre-dose), each scheduled assessment during treatment phase and at the end of the study.

For vital signs and ECG parameters, change between the value at baseline and the value during at each scheduled assessment during treatment phase and at the end of study visit will be described for each parameter by treatment and overall.

In general, appropriate descriptive statistics according to the nature of the variable will be applied: categorical variables will be presented using counts and percentage, whilst continuous variables will be presented using mean, standard deviation (SD), median, minimum, maximum, coefficient of variation (CV(%)) and number of subjects.

Appropriate inferential statistics, based on variable characteristics, will be applied, if consistent.

10.1.2.3. Laboratory parameters (for each part)

All laboratory values recorded during the study will be individually listed and flagged for values outside reference ranges and for clinical relevance (assessed by investigator). Quantitative parameters will be summarised by descriptive statistics.

Values, position according to laboratory range and clinical assessment will be described at screening, baseline (D-1), during the treatment phase and at the end of study by treatment for studies and overall.

Change between the value at baseline and the value during treatment phase and at the end of study visit will be described for each parameter by treatment and overall.

All quantitative and qualitative urinary test results will be listed, sorted by treatment for studies in parallel groups or by sequence for crossover studies.

Serology, ABT and DOA test results will be listed for each subject.

10.1.1. Analysis of secondary criteria

10.1.1.1. Formal statistics for pharmacokinetic

For DF2577Y bound and unbound and related metabolites, the hypothesis that C_{max}, $AUC_{0-\infty}$ (SAD part) or $AUC_{0-\tau}$ (MAD part) are dose proportional will be formally tested using a power model approach. AUC and C_{max} values, for all dose levels, will be analyzed for dose proportionality using analysis of variance techniques.

Data will be fitted to the following model:

$$\log(\text{AUC or } C_{max}) = \mu + [\beta \times \log(\text{Dose})]$$

This is usually referred to as a power model because after exponentiation:

$$AUC_{0-\infty} \text{ or } C_{max} = \alpha \times \text{Dose}^{\beta}$$

Prior to the analysis, the assumption of a linear relationship between the log $AUC_{0-\infty}$ (C_{max}) and log-dose will be tested using analysis of variance by partitioning the sums of squares for treatments into those for linearity and departures from linearity. If the departures from linearity are significant then the hypothesis of dose proportionality is rejected and the power model analysis will not be performed.

In case, $AUC_{0-\infty}$ could not be considered as reliable in a majority of subjects (*e.g.* too large percentage of extrapolation, poor quality of ke determination), dose proportionality assessment will be performed on AUC_{0-t} values.

The estimate obtained for β is a measure of dose proportionality. The estimate of β together with its 90% confidence interval (CI) (β_l, β_u) will be presented to quantify the degree of non-proportionality.

The dose proportionality will be confirmed if the 90% CI of β (β_l, β_u) is contained completely within the following critical region:

$$[\Theta_L;\Theta_H]=\left[1+\frac{\log(0.8)}{\log(r)};1+\frac{\log(1.25)}{\log(r)}\right]$$

where *r*, defined as the dose ratio, is equal to h/l , *h* being the highest dose and *l* the lowest dose.

Assessment of the dose proportionality will be performed on the complete dose range. In case of departures from linearity or of negative conclusion of dose proportionality on the complete dose, further investigation using the same methodology might be done on a restrained dose range.

The statistical package SAS® v.9.4 will be used to perform all statistical analyses.

For each part of the study and day of PK assessment, the individual AUC and C_{max} values will be presented graphically by dose level. The mean AUC and C_{max} values along with the SD will also be displayed graphically by dose level.

10.1.1.2. Correlation between pharmacokinetic and pharmacodynamic parameters

The relationship between values of pharmacodynamic parameters time matched with DF2577Y (bound and unbound) plasma concentrations will be first evaluated by visual inspection of graphs relating pharmacodynamic parameters with DF2577Y (bound and unbound) plasma concentrations.
In case of reliable relationships, basic models, such as E_{max} model, will be first developed. Then, the selected models will be optimized by testing covariate such as BMI, age, etc.
Further model development might be done depending on the results of each modelling.

10.1.2. Calendar for analyses

NA

10.2. Sample size

No formal sample size calculation was made. The subjects treated with test product and the subjects treated with placebo are considered sufficient to detect drug effects and determine pharmacokinetic parameters before escalating to the next dose level.

SAD cohorts: Thirty -two (**32)** healthy volunteers (HV) + 4 reserve subjects (total 36) will be enrolled to have 4 groups. Three groups of eight (8) male subjects and one group of 4M +4 F.

MAD cohorts: MAD cohorts: Thirty -six (36) healthy volunteers (HV) + 4 reserve subjects (total 40) will be enrolled to have 3 groups. Two groups of twelve (12) male subjects and group of 6M+6 F.

10.3. Level of significance to be used

The level of significance will be fixed at 0.05.

Tests will be two-tailed with no multiplicity adjustment.

10.4. Interim Analysis

For each part of the study, an interim PK analysis on DF 2577Y will be performed after completion of each dose level. This analysis will be done on anonymized data provided by the bioanalytical center to prevent any violation of the double blind condition of the study.

10.5. Procedure for accounting, for missing, unused, and spurious data

Missing data values will not be replaced.

10.6. Procedures for reporting any deviation(s) from original statistical plan

This plan may be revised during the study to accommodate protocol amendments and to make changes to adapt to unexpected issues in study execution and data collection that could affect planned analyses. These revisions will be based on review of the data, and a final plan will be issued prior to database lock, if applicable.

10.7. Selection of subjects to be included in the analyses

In case of incorrect treatment assigned, subjects will be analysed in the treatment group they actually received.

11. QUALITY CONTROL AND ASSURANCE

11.1. Quality Assurance

The study will be carried out in conformity with legal conditions and French regulations, and with respect to GCP (ICH E6). The Quality Assurance system in force at EUROFINS OPTIMED will apply, except for any specific clauses added to the protocol or specified in writing by the Sponsor before the start of the study.

11.2. Quality Control

The main study stages (coherence between source and CRF for: eligibility criteria, main evaluation criteria, AEs) will be submitted to a quality control process.

11.3. Sponsor audits and inspections by regulatory agencies

The study may be subjected to on-site audit visit by the Sponsor and inspection by applicable Regulatory Authorities in order to verify the study is conducted in compliance with the principles of GCP and with the study protocol. The auditor/inspectors will have direct access to medical records, source documents, and all documents and facilities relevant to the clinical trial.

The Investigator agrees to allow the auditors/inspectors to have direct access to study records for review, being understood that this personnel is bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.

The confidentiality of the data verified and the anonymity of the subjects should be respected during these inspections.

12. ETHICS

12.1. Informed consent form

The persons participating in the study will be selected during a screening visit. During this meeting, the study objectives and methodology will be explained.
The volunteer subjects will receive a synthesis document explaining the requirements of research, the title and the objectives of the study, the detailed research protocol and the risks and constraints of the research.

Before being included in the study, each participant must give his/her written consent. The text of the consent is to be signed and dated and initialled on each page by the subject and dated and signed by the Investigator.

12.2. Ethics Committee and Competent Authorities

The study will be carried out in conformity with the principles of the Declaration of Helsinki as modified in Fortaleza (2013), and National Regulation.
This study will be undertaken after approval by the Ethics Committee and of the Competent Authorities.

12.3. Protocol amendments

Neither the Investigator nor the Sponsor can modify the protocol without agreement from the other party. Any protocol modifications must be the topic/matter of an amendment which will be dated and signed by the two parties and must be included as an addendum to the protocol.
Depending on the importance of the changes to the study conditions, the amendment may be sent to the Ethics Committee and/or Health Competent Authorities either for approval or for information.

A substantial protocol amendment must be submitted to the ANSM, in order to notify the results of SAD part of the study.

12.4. Protocol deviations

No deviations are systematically tolerated. Any protocol deviations will be notified to the Monitor/Sponsor on an ongoing basis, and no later than the date of the blind review, and will give rise to a discussion to define their status (minor – major).

13. DATA HANDLING AND RECORD KEEPING

13.1. Archival

All documents related to the study must be kept by the Investigator in appropriate files. The archives of the subjects, original informed consent forms, source documents, case report forms, inventory of study products, correspondence with the Sponsor and Ethics committee related to the study, must be filed.
The Investigator authorises direct access to the source documents for monitoring, audits and inspections.
The Investigator keeps a list identifying the subject names (with addresses and/or medical file numbers), their respective code number and the dates of entry into the study and end of study, in order to be able to verify the concordance between the data contained in the case report forms and those in the source documents .

These documents must be kept on the Investigator site until at least fifteen years. Even at the end of this period, no destruction can be achieved unless authorized in writing by a duly mandated Sponsor's representative.
If the Investigator/Institution is no longer able to be responsible for essential documents the Sponsor must be notified in writing of this change and informed as to whom the responsibility has been transferred.

13.2. Confidentiality

All information obtained during the study (except the informed consent form data) will be input onto computer by EUROFINS OPTIMED, subcontracted by the Sponsor in conformity with the "Information Technology and Liberty Law" (Article 40 of 6 January 1978) which respects the European Directive 95/46/CE.

13.3. Ownership of results

The Sponsor is the sole owner of the data and research results. He reserves the right to use them in any form whatsoever, to submit them to the Health Authorities of any country.

Should the study generate results likely to be patented, then only the Sponsor will be authorised to depose such a patent, in his name and at his costs.

14. FINANCING AND INSURANCE

The Sponsor certifies that it has taken out a liability insurance policy which covers the current research in accordance with local laws and requirements.

An insurance certificate will be provided to the Investigator in countries requiring this document.

15. PUBLICATION

All information issuing from the study will be considered to be confidential, and must not be divulged without the Sponsor's prior agreement.

The study results may be published or presented by the Investigator or analysis experts, in collaboration with the Sponsor, with the sponsor's written permission. The Sponsor may use the study results for any publication or communication, with the written agreement of the Investigator or the analysis experts if they are cited.

16. LIST OF APPENDICES

APPENDIX I : DECLARATION OF HELSINKI

APPENDIX I
DECLARATION OF HELSINKI

WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words,

"The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by

individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and

standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain

for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made

publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.