16. APPENDICES

16.1 Study Information

16.1.1 Protocol and Protocol Amendments

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Via Campo di Pile
67100 - L'Aquila - Italy
TEL. 08623381 FAX 0862338219

Via Santa Lucia, 6
20122 - Milan – Italy
TEL. 02583831 FAX. 0258383324

INVESTIGATOR'S BROCHURE

REPARIXIN

Solution for i.v. administration

Tablets for oral administration

Reparixin Final Version 12 – 9 March , 2015

Replaces previous edition:

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CONFIDENTIAL

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List of abbreviations

§	section
95% CI	95% confidence interval of ld_{50}
Ae	cumulative urinary excretion
AE(s)	adverse event(s)
ALT	alanine aminotransferase
AP	alkaline phosphatase
APA	action potential amplitude
APD_{50}/APD_{90}	action potential duration at 50% repolarization / action potential duration at 90% repolarization
AST	aspartate amino-transferase
AUC	area under the curve
AUCextra %	area under the concentration-time curve extrapolated from last sampling time to infinity in % of the auctot
AUClast	area under the concentration-time curve from zero up to the last sampling time
AUCtot	area under the concentration-time curve from zero up to infinity with extrapolation of the terminal phase
BAL	bronchoalveolar lavage
BBB	blood brain barrier
BOS	bronchiolitis obliterans syndrome
BW	body weight
C	coefficient in the sum of exponential
C0	extrapolated concentration at t 0
C_{24h}	the concentration value obtained at 24 h sampling time
C5a	complement protein c5a
C_{5min}	the concentration value obtained at 5 min sampling time
C_{672h}	the concentration value obtained at 672 h sampling time
CAPD	continuous ambulatory peritoneal dialysis
Cb	concentration in the brain
CBR	Clinical Benefit Rate
CCR_{0-8h}	combined 0-8 h concentration ratio for (metabolite1+metabolite2)/parent
Cinitial	initial plasma concentration at the end of the constant rate infusion
CL	total plasma clearance
Clast	the concentration value obtained at last sampling time
CLIN	clinical
CLM	confidence limits of mean
Cmax	maximum concentration
Con Med	concomitant medication
Cp	Concentration in the plasma
CPB	cardiopulmonary bypass
CSC	cancer stem cells
Css	concentration at steady state
CT	computed tomography imaging
CTC	Circulating Tumor Cells
CV	Cardiovascular
CXCL1	chemokine (CXC motif) ligand 1
CXCL8	chemokine (CXC motif) ligand 8 (interleukin-8)
CXCL8R I	CXCL8 receptor I
CXCL8R II	CXCL8 receptor II
CXCR	CXC chemokine receptor
DAB	German Pharmacopoeia
DF 1681A	reparixin D,L-lysine salt (International Nonproprietary Name) [formerly repertaxin D,L-lysine salt]
DF 1681B	reparixin L-lysine salt (International Nonproprietary Name) [formerly reperaxin L-lysine salt]
DF 1681Y	reparixin (International Nonproprietary Name) [formerly repertaxin]
DGF	delayed graft function
DMC	data monitoring committee
DRF	dose range-finding
ECG	electrocardiogram
EFF	efficacy
ER	excretion ratio
ER_{COOH}	excretion ratio for carboxytolbutamide/tolbutamide;
ER_{OH}	excretion ratio for hydroxytolbutamide/tolbutamide;

ESRD	end stage renal disease
F_{abs}	absolute bioavailability
FEV1	forced expiratory volume in one second
FiO_2	fraction of inspired oxygen
fMLP	n-formylmethionyl-leucyl-phenylalanine
fu	free fraction
FVC	forced vital capacity
GCP	good clinical practice
GFR	glomerular filtration rate
GI	gastrointestinal
GLP	good laboratory practice
GRO-α	growth-regulated oncogene-alpha
HB	haemoglobin
HTC	Haematocrit
IAT	islet auto transplantation
ICU	intensive care unit
IEQ	islet equivalent
i.v.	intravenous
IC_{50}	50% inhibitory concentration
IL-8	interleukin-8 (CXCL8**)**
INT	interaction
I/R	ischemia/reperfusion
ITT	intent to treat
KC	mouse homologue of human MCP-1
Kel	elimination rate constant from central compartment
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, 50% kill
LDL_0	minimal lethal dose
LLOQ	lower limit of quantification
LLT	lowest level term (meddra)0
LOQ	limit of quantitation
LSMEANS	least square means
mPFS	Median Progression-Free Survival
MCP-1	monocyte chemoattractant protein-1
MDZ	midazolam
MedDRA	medical dictionary for regulatory activities
MET	metabolism
MIP-2	macrophage inflammatory protein-2
MLD-STZ	multiple low doses streptozotocin
MMF	mycophenolate mofetil
MMTT	mixed meal tolerance test
MR	metabolic ratio defined as AUCtot 1-hydroxymidazolam/AUCtot midazolam
MR_t	metabolic ratio between defined as AUClast 1-hydroxymidazolam/AUClast midazolam
MRI	magnetic resonance imaging
MRT	mean residence time
MSA	methanesulfonamide
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
NK	natural killer
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NOS	not otherwise specified
NQ	not quantifiable
ORR	Objective Response Rate
OS	Overall Survival
p.o.	per oral
p.v.	paravenous
PaO2	partial pressure of arterial oxygen

PBL	peripheral blood lymphocytes
PBMC	peripheral blood mononuclear cells
PGD	primary graft dysfunction
P-gp	P-glycoprotein
PFS	Progression-Free Survival
PHA	phytohaemagglutinin
PK	pharmacokinetic(s)
PMN	polymorphonuclear (neutrophil) cells
PP	per protocol
PVP	polyvinylpyrrolidone
Rac	Accumulation ratio
RBC	red blood cell
RPM	resting membrane potential
RPT	reparixin
s.c.	subcutaneous
SCI	spinal cord injury
SD	standard deviation
SEM	standard error of the mean
SS	steady state
t½	half-life of elimination
T1D	type 1 diabetes
TK	toxicokinetics
tlast	the last sampling time at which a quantifiable concentration is found
TLB	tolbutamide
Tmax	time to reach maximum concentration
TNBC	Triple Negative Breast Cancer
TNF	tumor necrosis factor
TOL	Tolerability
TC	tumor cells
TTM	Median Time to New Metastasis
TTP	Time to Progression
UDS	unscheduled DNA synthesis
Vc; Vz	volume in the central compartment; apparent volume of distribution during the terminal phase
V_{max}	maximum upstroke velocity
Vss	apparent volume of distribution at steady state
λ_z	apparent terminal phase rate constant

2. SUMMARY

Clinical Objectives

Reparixin is a novel, potent and specific inhibitor of the chemokine CXCL8 [interleukin (IL)-8]. Based on its mechanism of action, early pre-clinical characterization of reparixin was specifically targeted to inhibition of PMN recruitment and prevention of Ischemia/Reperfusion (I/R) injury. Consequently, clinical development of reparixin was originally focused on the prevention of DGF (kidney transplantation) and primary graft dysfunction (lung transplantation).

Reparixin has received the orphan drug designation in EU in September 2001 and in USA in January 2003 for prevention of DGF after solid organ transplantation. More recently orphan drug designation has been granted in EU (September 2011) for the "prevention of graft loss in pancreatic islet transplantation" and in the US (September 2012) for the "prevention of graft loss in islet cell transplantation".

Reparixin has shown its ability to improve graft outcome after pancreatic islet transplantation in both syngeneic and allogeneic models in mice.

Moreover, oncology studies have shown that CXCR1 blockade with reparixin selectively targets human breast cancer stem cells *in vitro* and in xenografts. Experimental evidence of the role of IL-8 in tumor metastasis and in tissue damage induced by chemotherapeutic agents supports the clinical development of the compound in oncology.

The current clinical objective is to evaluate the safety and efficacy of reparixin to oncology patients and the safety and efficacy in type 1 diabetes patients undergoing pancreatic islet transplantation and liver transplantation.

The intended drug administration in oncology is by the oral route, whereas in type 1 diabetes patients is by IV route.

In agreement with the development in islet transplantation, a pilot phase 2 trial was conducted in intra-hepatic islet transplantation in type 1 diabetes patients (T1D).

The initial studies in oncology have begun in patients with breast cancer. A phase 1b study with the combination of reparixin + paclitaxel in patients with metastatic breast cancer began enrolment in February 2012 and has completed enrolment to all 3 dosing cohorts (400 mg t.i.d., 800 mg t.i.d., and 1200 mg t.i.d.). An expansion cohort at the 1200mg t.i.d. dosing level was also initiated and is also now completed with enrolment. All patients are now off treatment and the data is currently being collected and analyzed. A pilot "window of opportunity" study for preoperative early stage breast cancer began enrolment in April 2013 and continues in the enrolment phase.

Preliminary promising results in type 1 diabetes patients have led to a registration trial.

A phase 3 study to assess the efficacy and safety of reparixin in pancreatic islet transplantation is now ongoing. It is being conducted at 8 sites across four EU countries and one site located in the US.

In addition, a phase 2/3 study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation in patients undergoing total pancreatectomy is being conducted at 6 centers in the US. A Canadian center is going to be opened. To date, 34 patients have been enrolled and transplanted.

Product Description

Reparixin, formerly referred to as repertaxin or DF1681Y, is a new chemical entity. The chemical name is:

R(-)-4-Isobutyl-alpha-methylphenylacetyl-methanesulfonamide.

The investigational product is a 33 mg/mL concentrated solution of reparixin for infusion. The concentrated solution to be diluted is filled in clear glass vials. Each vial

contains 250 mL of the investigational product.

When given by oral route, the investigational product consists of immediate release tablets containing 100, 300, 500 or 600 mg of reparixin. The tablets are packed in PVDC/PVC/Aluminium blisters (or PVDC/PE/PVC/Aluminium blisters for 600 mg tablets only).

Both liquid and tablet forms of reparixin must be stored at a temperature not higher than 30°C (86°F).

Non-Clinical Pharmacology

Mechanism of action studies on reparixin showed that it is a non-competitive allosteric inhibitor of the CXCL8 receptors CXCR1 and CXCR2 inhibiting the intracellular signal transduction events activated by binding of CXCL8 to CXCR1/2.

In vitro chemotaxis experiments have shown that reparixin in the low nanomolar range inhibits human PMN migration induced by human CXCL8.

In vivo, reparixin prevented PMN recruitment in a mouse model of auricular inflammation induced by cantharidin. Moreover, reparixin prevented PMN infiltration into the transplanted kidney and lung, improving transplantation outcome. Furthermore, reparixin prevented PMN infiltration and tissue damage in other animal models of ischemia/reperfusion injury of liver, brain, intestine, heart and spinal cord.

Preliminary results in syngeneic and allogeneic models of intrahepatic islet transplantation in diabetic mice indicated that reparixin may improve islet engraftment, In the allogeneic model, reparixin appears to delay the time to rejection, prolonging the normoglycaemia. In parallel, reparixin prevented the hepatic infiltration of PMNs. In a model of multiple low dose streptozotocin-induced (MLD-STZ) diabetes in mice, the treatment with reparixin delayed diabetes development.

In vitro assays on breast cancer cells showed that reparixin reduces metabolic capability and leads to a reduction in cell number. Reparixin has also been investigated in a NOD/SCID mouse model of breast cancer. This model explored the effect of reparixin treatment, alone or in combination with docetaxel or paclitaxel, on tumor growth *in vivo*, using SUM159 cells or xenografts of primary human breast cancers which were transplanted orthotopically into mice. Tumorigenicity of ALDEFLUOR+ CXCR1+ and ALDEFLUOR+ CXCR1– SUM159 cells was assessed in the mice. Reparixin was demonstrated to be able to specifically target and reduce the CSC population. Docetaxel and paclitaxel targeted the differentiated tumor cells and in association with reparixin were capable of reducing both the bulk of the tumor mass as well as the resident CSC population.

Furthermore, reparixin effects were investigated in a mouse model of brain metastasis induced by human MDA-MB-231 breast carcinoma cells. First of all, reparixin was demonstrated to be able to cross the blood-brain barrier (BBB) in presence of brain metastasis in mice. After that, the effects of reparixin as single agent or in combination with paclitaxel, were characterized and quantified by MRI in the same experimental model. When reparixin was given from the day of tumor cell injection for 14 days, it was able to inhibit both the number and the volume of brain metastasis. Reparixin effect was similar to the effect of paclitaxel administered alone and to the effect of the combination treatment. Due to the severity in the temporal progression of the metastasis development, when reparixin or paclitaxel or their association were given 7 days after tumor cells injection until 21 days, the inhibitory effects of all the different treatment options showed a tendency to decrease. Nonetheless, under these experimental conditions reparixin and paclitaxel alone were still able to inhibit the total metastasis volume and the effect on this parameter was higher when the drugs were given in combination. In addition the combination of reparixin and paclitaxel demonstrated a statistically significant inhibitory effect on metastasis number.

The effects of reparixin on renal, CV and respiratory systems were investigated in rat and dog safety pharmacology studies. The results suggested that reparixin has a safe profile at doses higher than those intended for human use.

The carboxy-reparixin (DF 2243Y), the major metabolite in rats and humans,was active *in vitro* (inhibition of CXCL8 induced PMN migration) but far less potent than the native compound, while it was ineffective in animal models of I/R injury.

Pharmacokinetics and product metabolism

The PK of reparixin was evaluated after i.v. or p.o. single dose in rats, after i.v. single dose in dogs, after continuous infusion in rats and dogs and after 14 days by oral route in rats and by iv route in dogs. The continuous infusion and the repeated studies were part of toxicity studies.

Overall, PK studies by i.v. injection revealed that reparixin is very rapidly eliminated in rats ($t_{1/2}$ 0.5-3hrs) whereas elimination is slower in dogs (12-28hrs). Absolute bioavailability in rats after single p.o. administration accounted for more than 80%.

In both species, the compound showed a low volume of distribution and a low plasma clearance. The distribution of total radioactivity into tissues was generally much smaller than those in plasma. Total radioactivity crossed the placenta in pregnant rats.

[^{14}C] reparixin was highly bound to plasma protein (> 98.9%), mainly to albumin and more distributed in plasma than in blood.

The reparixin PK was linear in rats and in dogs after 28 days infusion in the range of 100-900 mg/kg/day and 14 days infusion at dose ranges 0.4-10.8 mg/kg/day, respectively.

In dogs, after 14 days continuous reparixin infusion, a possible induction of its metabolism was observed after administration of 100, 200 or 400 mg/kg/day.

After 14 days of repeated oral administration of reparixin in rats, exposure in females was slightly higher than in males. Possible gender differences in dogs after i.v. infusion were also seen. However, the data are not consistent and no definite conclusions can be drawn.

After 13 weeks of repeated oral administration of reparixin in rats, exposure in females was higher than in males. The duration of treatment did not induce accumulation of the parent compound DF1681Y or its metabolite DF1674Y in both sexes on day 93. Apparent half-life was not influenced by the doses.

The presence of an enterohepatic recirculation was possible in dogs.

Unchanged reparixin was poorly or not excreted in the urine of rats and dogs,.

Reparixin was extensively metabolised in rats, lesser in human, and slightly in dog hepatocytes. Three main metabolic pathways were identified: hydroxylation, carboxylation and hydrolysis.

In human liver microsomes, metabolism was catalysed by CYP2C9 and to a lesser extent by CYP2C19 to give two hydroxylated metabolites named DF 2188Y and DF 2260Y.

Qualitative identification of reparixin L-lysine salt metabolism indicated the presence of 8 and 10 metabolites in rats and dogs plasma, respectively. Eight metabolites were detected in rat urine and some of these, such as ibuprofen, DF 2260Y, DF 2239Y and DF 2243Y, were the same found in the plasma.

Exposure to ibuprofen after administration of reparixin 2.77 mg/kg/h for 48hrs (the highest dose tested in humans) was similar or lower than that obtained after a standard therapeutic single dose of ibuprofen (300mg).

Reparixin L-lysine salt *in vitro* resulted as a minor inhibitor of the isoenzymes CYP3A4 (IC_{50} = 8 µM), CYP2C9 (IC_{50} = 79 µM) and of CYP2C19 (IC_{50} = 868 µM).

Reparixin has some potential *in vitro* for a uncompetitive inhibition of the human hepatic

enzyme CYP3A4 that is involved in the metabolism of cyclosporine A, tacrolimus and rapamycin. However, since inhibition is evident at concentration far higher than the free plasma concentration of reparixin at steady state in humans, it was predicted that the clinical relevance of such inhibition is remote.

Reparixin and DF 2243Y did not affect P-gp activity, also in presence of cyclosporine A, sirolimus and tacrolimus. Besides, reparixin is not a substrate for P-gp.

Toxicology

Reparixin under the form of the L-lysine salt (DF1681B) or as free acid (DF1681Y) was tested for toxicity in rodent and non-rodent animal species after single or repeated administrations either by IV or oral, according to the human foreseen administration route.

The general toxicological profile of reparixin L-lysine salt is characterised by a low toxicity after single i.v. or oral route to mice (LD_{50} 609 mg/kg i.v.; >3 g/kg p.o.) and to rats (LD_{50} 348 mg/kg i.v.; 1303 mg/kg p.o.). In rats, DF1681B and DF1681Y administered bid by oral route were very well tolerated up to a daily dose of 2000 mg/kg.

The i.v. infusion to rats for 28 days resulted in a safe dose of 1000 mg/kg/day (NOAEL); while in dogs for 2 weeks resulted in a safe dose of 60 mg/kg/day, even if at the dosage of 50 mg/kg/day for 2 weeks one male animal showed a mucosal ulceration in the fundic area of the stomach.

Reparixin acid (DF1681Y) was administered orally (gavage) twice a day (bid) to rats for a period of 14 days where resulted to be safe and well tolerated with a NOEL of 400 mg/kg/bid in males and with a NOAEL of 400 mg/kg/bid in females.

DF1681Y was administered twice daily (8 hrs apart) via gavage to SPF-bred Wistar rats at dose levels of 100, 200 or 400 mg/kg bw/bid for a period of 13 weeks. The treatment did not result in mortality, relevant clinical signs, body weight, food consumption, hematology, ophthalmoscopic examination or urinalyses. Minor changes in blood chemistry, organ weights and in the morphology of liver and of male kidneys at 400 mg/kg/bid were of no toxicological relevance and reversed after 4-week of recovery period. The NOAEL was settled at 400 mg/kg/bid.

The local tolerability of reparixin L-lysine salt assayed in the rabbit, lateral ear vein model, indicated that the compound is well tolerated at 7.5 mg/mL.

An immunotoxicity study in rats by infusion for 28 days up to 1000 mg/kg/day resulted in no treatment effect on splenic lymphocyte numbers or the Natural Killer cell activity of the splenocytes.

I.v. infusion of reparixin to male and female rats at doses up to 1000 mg/kg/day did not induce any significant adverse effects on mating performance and fertility.

At high and cytotoxic concentrations after metabolic activation with S9, reparixin had a clastogenic activity in human lymphocytes chromosome aberration assay. However, considering the high concentrations, this activity is unlikely to be reproduced *in vivo*. All other tests performed (Ames test, micronucleus in rats, DNA repair in rat liver, UDS test) were devoid of any genotoxic activity. Therefore, reparixin poses no genotoxic hazard for humans.

DF 2243Y, a major metabolite of reparixin, was assessed for its toxicological potential in a 2-week i.v. infusion study in rats at 600 and 900 mg/kg/day without any relevant adverse effect**s**.

Effects in Humans

A total of 405 subjects have been involved in phase 1 and phase 2 completed (CSR issued) clinical studies. Among these, 266 subjects have been exposed to reparixin.

Clinical pharmacokinetics were studied in four Phase 1 trials.

Reparixin was characterised by a low clearance a low volume of distribution and a short terminal half-life (about 1 h). Up to the dose of 8mg/kg given as 30 min infusion, the

pharmacokinetics appeared to be dose independent, whereas when given as 48 h continuous infusion, plasma concentrations seemed to increase less than in direct proportion with the dose.

Reparixin was highly bound to plasma proteins; the free plasma concentrations accounted for about or less than 1%. As observed in *in vitro* studies, the percentage of free fraction appeared to increase at high concentrations suggesting a saturation of binding.

The excretion of unchanged reparixin in urine was absent and low in faeces.

Reparixin was extensively metabolized: in urine fourteen different metabolites were detected. The main metabolites were ibuprofen, methansulfonamide, DF 2243Y and DF 2188Y (the hydroxylated and carboxylated metabolite, respectively). The total recovery of reparixin and its metabolites in urine accounted for 60-75% of the administered dose.

The pharmacokinetics of methansulfonamide, ibuprofen and DF 2243Y was elimination-rate limited, whereas the pharmacokinetics of DF 2188Y was formation-rate limited.

No clinically significant PK interaction between reparixin and midazolam/ tolbutamide (probe substrates for CYP3A4 and CYP2C9, respectively) was observed.

The comparison of the pharmacokinetic profile between normal renal function and renal impairment, indicated that the PK profile of reparixin is not influenced, as expected, by the degree of renal function. Viceversa, renal function influenced the pharmacokinetic profile of the major metabolites that progressively accumulate in plasma.

The safety and efficacy of reparixin was evaluated in nine completed sponsor-initiated clinical studies.

In the first of six phase 1 clinical studies conducted to date, reparixin L-lysine salt 1 to 16 mg/kg was administered by short infusion (30min). The compound was well tolerated at all doses, with minor and unspecific AEs which were not dose-related.

In the second study, the compound was administered as 48h i.v. infusion targeting to reparixin Css of 10, 20 and 30 µg/mL. Reparixin resulted well tolerated; again AEs were minor and not dose related. The local reactions were observed to subside by administering a more diluted solution at a higher infusion rate.

In the third study (interaction study), co-administration of midazolam/tolbutamide (probe substrates for CYP3A4 and CYP2C9) with reparixin did not raise safety concerns.

The fourth study was performed in subjects with different degree of renal impairment. The i.v. infusion of 2 mg/kg/h of reparixin L-lysine salt was safe and well tolerated both in patients with different degree of renal impairment and in subjects with normal renal function. Very few AEs were reported, the majority of which were mild in intensity and unlikely due to reparixin.

Two studies were performed in the cantharidin blister model to assess if reparixin can reduce the influx of PMNs inflammatory mediators, during acute inflammation. In the first study, there was a very high variability in the response to cantharidin. Only two out of 8 subjects were evaluable. The analysis did not detect any significant difference between treatments (reparixin versus placebo) and unfortunately the blister volumes were not sufficient to evaluate the differences in the inflammatory mediators. The second of these two studies was to evaluate the effect of dexamethasone and reparixin on inflammatory mediators in this model. There were no statistically significant differences observed in the inflammatory mediators from the two groups. Due to errors in sample handling with flow cytometry samples, no conclusion could be made regarding these data. The safety of reparixin was confirmed in both of these studies.

Three Phase 2 studies have been completed to date. Two studies investigating the

efficacy of reparixin in the prevention of PGD/DGF in lung/kidney transplantation were conducted with reparixin administered by continuous intravenous infusion, up to a maximum of 48h. Neither of the two studies was able to show a statistically significant effect of reparixin on short- and long- term functional and clinical outcomes after transplantation. In the lung study, a total of 7 patients died, all in the placebo group. The AE profile was similar for both placebo and reparixin groups. Twenty-eight patients experienced SAEs; 8 SAEs in 6 patients were judged possibly related to the study drug. During the renal transplant study, one patient in the reparixin intermittent infusion group and one patient in the placebo group died. The AE profile was similar for both reparixin and placebo groups. SAEs were reported in 21 patients. SAEs possibly related to study drug were reported for only 2 patients, both in the reparixin continuous infusion group. An assessment performed by DMC on a possible study-related higher incidence of thrombosis, excluded the potential relationship of reparixin with these events.

A phase 2 study evaluating the efficacy of reparixin in improving transplant outcome in T1D patients undergoing pancreatic islet transplantation was conducted with reparixin administered by intravenous infusion over 7 days. This study shows a clinical benefit of reparixin in terms of improved β-cell function and islet transplant clinical outcomes, and provides a preliminary clinical proof of its potential in pancreatic islet transplantation in patients with T1D. Data obtained from this pilot trial further support the safety of reparixin in this clinical setting. In patients treated with a 7 day course of reparixin a few AEs were reported which were judged to be at least possibly related to reparixin treatment. There were 11 reports of SAEs in 3 patients taking reparixin compared with 1 report in 1 patient in the control group. The safety profile was in line with previous clinical experience and there were no safety issues that would preclude further development of reparixin in islet transplantation.

Due to encouraging data obtained in the pilot trial, a phase 3 study has been implemented and is ongoing in EU/ US (one site only) to further evaluate the efficacy of reparixin in pancreatic islet transplantation. To date, 49 patients have been randomized, and 44 have gone on to treatment and transplant. 23 patients have received a second islet infusion. 13 patients have completed one year follow-up after last islet infusion.

In addition, a phase 2/3 is being conducted at 6 centers in the US to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation. A Canadian center is going to be opened. To date, 34 patients have been randomized and transplanted.

A phase 1b study evaluating the safety, PK profile, and effects on Cancer Stem Cell (CSC) markers of reparixin oral tablets in combination with paclitaxel in metastatic breast cancer was conducted. At the time of data cut-off for first analysis, enrolment was completed and 7 patients were still actively receiving treatment. All patients are now off treatment and data is being collected and analyzed. The study shows that oral reparixin administered with paclitaxel was safe and well tolerated in patients with HER-2 negative metastatic breast cancer. Several objective responses were recorded; however, due to the small numbers of patients within each treatment group and considering that single agent weekly paclitaxel has established activity against metastatic breast cancer, the response rate should be interpreted with caution. Nonetheless, given the clear safety data and good tolerability of the combination, coupled with a sizeable response rate and some interesting long term responders, further investigation of the combination is warranted.

One study is ongoing in breast cancer patients, and another is in the process of start up. The first is a pilot “window of opportunity” clinical study in operable breast cancer patients where reparixin is administered as single agent in the time period between clinical diagnosis and surgery. This study aims to evaluate the effects of orally administered reparixin on CSCs in the primary tumor and the tumoral microenvironment in an early breast cancer population. Enrolment to this study began in April 2013 and continues in the enrolment phase.

The second is a randomized, double-blind, placebo-controlled phase 2 study of the

combination of reparixin and paclitaxel versus paclitaxel alone as front-line therapy for Metastatic Triple-Negative Breast Cancer (TNBC). The primary objective of the study is to evaluate Progression-Free Survival (PFS) in the two treatment groups. Secondary objectives include: determining median PFS (mPFS) and Overall Survival (OS), evaluating Objective Response Rates (ORR), and assessing the safety of the combination treatment. Additional exploratory objectives include: determining median Time to New Metastasis (TTM) and proportion of patients progressing with new metastatic lesions; comparing the incidence and severity of peripheral neuropathy between the two arms, and evaluating CSCs in metastatic tissue. The study is expected to activate in April 2015, and will be conducted in the US and EU.

Two academic independent clinical trials were conducted by the Medical University of Vienna. The results of the first study indicated that LPS-induced neutrophilia was not significantly affected by reparixin in human volunteers. No AEs were reported. The second study was conducted in patients undergoing elective coronary artery bypass grafting with cardiopulmonary bypass. The rise of the neutrophil count after CPB was less marked in the reparixin group.

3. INTRODUCTION

CXCL8 [interleukin (IL)-8] is a member of a class of cytokines (usually termed chemokines) involved in leukocyte recruitment and activation in tissues. Two types of CXCL8 receptors are known (CXCR1 and CXCR2) that are expressed in neutrophils, the primary target cells of CXCL8 [1].

CXCL8 role has been claimed in a variety of inflammatory conditions [15]. Therefore the modulation or inhibition of CXCL8 activity is considered as a valid target for the development of innovative treatments of a variety of severe clinical conditions, still with an unmet medical need.

Dompé has identified a new chemical entity, reparixin, as a novel CXCL8 inhibitor. Results show that reparixin is *in vitro* a potent and specific inhibitor of CXCL8 induced recruitment of human PMN. Moreover, reparixin reduces both PMN recruitment and tissue damage in animal models of warm and cold Ischemia/Reperfusion (I/R). Thus, early clinical development of reparixin was originally focused on the prevention of DGF (kidney transplantation) and primary graft dysfunction (lung transplantation) as these clinical conditions can be considered as a paradigm of I/R damage. Reparixin received orphan drug designation in EU in September 2001 and in USA in January 2003 for such clinical conditions.

Furthermore, recent data reported CXCR1/2 as a key factor in modulating several tumor cell growth such as melanoma [2], colon cancer liver metastases [3] and gastric cancer [4].

CXCR1 was expressed in breast carcinoma sub-population represented by Cancer Stem Cells (CSC). Charafe-Jauffret's data [5] lead to the evaluation of reparixin effect on breast CSC population as a possible new strategy to improve current cancer therapy, since CSCs represent the forefront target for cancer therapy, as they are believed to drive tumorigenesis, but also tumor metastasis as well as tumour recurrence after treatment.

Ginestier and colleagues [6] demonstrated that reparixin selectively decreased the CSC population *in vitro* and in NOD/SCID xenograft models through a pathway involving the focal adhesion kinase/AKT/forkhead transcription factor FKHRL1. Furthermore in the NOD/SCID xenograft model they demonstrated that administration of reparixin retarded tumor growth and reduced the development of systemic breast cancer metastasis.

CXCL8 has also been shown to be expressed by human pancreatic islets and could play a crucial role in triggering the inflammatory reaction [7, 8] and, might represent a relevant therapeutic target to prevent early graft failure after islet transplantation.

Pancreatic islet transplantation has become a feasible option in the treatment of Type 1 Diabetes (T1D) which offers advantages over whole pancreas transplantation. However such a procedure is limited by a poor efficiency as multiple islet transplants are required to achieve insulin independency which is then lost over time [9]. The Instant Blood-Mediated Inflammatory Reaction is one of the earliest and crucial events contributing to overall outcome of islet transplantation and PMNs have been found to be the predominant cell types infiltrating *in vitro* the islets.

Reparixin tested in syngeneic and allogeneic models of intrahepatic islet transplantation in diabetic mice indicated that the compound may improve islet engraftment [10].

In addition, preliminary results in a pilot clinical trial in lung/kidney transplantation subjects show that reparixin treatment improves graft survival and pancreatic islet function; these data, coupled with the safety shown in human phase 1 and 2 studies supported further clinical development of reparixin in islet transplantation and prompted the conduct of a phase 3 registration trial [10].

The data obtained in NOD/SCID xenograft model and those obtained in the pilot clinical trial in lung/kidney transplantation subjects provide a rationale to support the clinical investigation of reparixin in oncology field and in type 1 diabetes pancreatic islet transplantation.

3.1. REFERENCES (SECTION 3)

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4. PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

NOTE 1 - The generic name of the study drug is "reparixin". The name reparixin attained in December 2004 the status of recommended International Nonproprietary Name (INN). The former generic name used until December 2004 was "repertaxin". Throughout this brochure, the study drug will be referred to by its new name "reparixin".

NOTE 2 - The study drug used in nonclinical and Phase 1 clinical studies was reparixin [repertaxin] L-lysine salt (DF 1681B) whose chemical properties and Phase 1 formulations are described in the § 8.1.

The study drug used in Phase 2 clinical studies is reparixin [repertaxin] whose chemical properties and formulation are described below. The reparixin formulation has the same final product composition of the previous formulations; in fact, it contains an equimolar amount of L-lysine as solubilizer ingredient and the same buffering system used in the previous formulations.

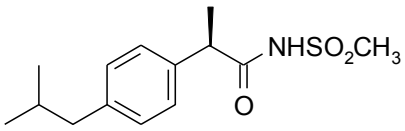
Conversion factor for reparixin L-lysine salt to reparixin equivalent:

dose of reparixin L-lysine salt x 0.66 = equivalent dose of reparixin

4.1. NOMENCLATURE

Recommended International Nonproprietary Name (INN):	Reparixin
Nonproprietary Name adopted by the USAN Council (USAN):	Reparixin
Former Generic Name (until December 2004):	Repertaxin
Chemical Name:	[R(-)-4-Isobutyl-alpha-methylphenylacetyl-methanesulfonamide]
Dompé Laboratory Code:	DF 1681Y (1174 AMSA code)
Relative Molecular Mass:	283.4 g/mole
Molecular Formula:	$C_{14}H_{21}NO_3S$
CAS (Chemical Abstracts Service) Number:	266359-83-5

4.2. MOLECULAR STRUCTURE



4.3. PHYSICAL AND CHEMICAL CHARACTERISTICS

Reparixin is a white or almost white powder. The specific optical rotation value is –84.0° to -88.0 calculated with reference to the anhydrous substance. It is freely soluble in ethanol, and practically insoluble in water (according to European Pharmacopoeia,). Reparixin possesses one chiral centre and exists as the pure enantiomer.

4.4. STRUCTURAL SIMILARITIES TO OTHER KNOWN COMPOUNDS

Reparixin is structurally related to the known anti-inflammatory drug ibuprofen. Specifically, reparixin is an acylmethanesulfonamide obtained starting from R(-) enantiomer of ibuprofen.

4.5. INVESTIGATIONAL PRODUCT

For oral administration, the investigational product consists of immediate release tablets containing 100 mg, 300 mg,500 mg or 600 mg of reparixin (DF 1681Y). The tablets are packaged in PVDC/PVC/Aluminium blisters for 100-300-500 mg while PVDC/PE/PVC/Aluminium for 600 mg
The different tablet strengths present the same active ingredient/excipients ranging around a similar ratio while differ for the tablet weights.
The composition of tablets for each dosage strength is reported in the table below along with information on the function and quality standard of each ingredient.

NAMES OF INGREDIENT	AMOUNT FOR 100 MG TABLET	AMOUNT FOR 300 MG TABLET	AMOUNT FOR 500 MG TABLET	AMOUNT FOR 600 MG TABLET	FUNCTION OF INGREDIENT	QUALITY
Reparixin (DF 1681Y)	100 mg	300 mg	500 mg	600.0 mg	Drug substance	In-house specification
Cellulose Microcrystalline	78.16 mg	234.45 mg	390.75 mg	141.48 mg	Diluent/ Disintegrant	EP, USP/NF current edition
Lactose monohydrate	56.77 mg	170.32 mg	283.87 mg	94.77 mg	Diluent	EP, USP/NF current edition
Croscaramellose	10.0 mg	30.00 mg	50.00 mg	36.00 mg	Disintegrant	EP, USP/NF current edition
Hypromellose	2.57 mg	7.73 mg	12.88 mg	20.07 mg	Binder	EP current edition
Colloidal silica anhydrous	1.25 mg	3.75 mg	6.25 mg	3.15 mg	Glidant	EP current edition
Magnesium stearate	1.25 mg	3.75 mg	6.25 mg	4.50 mg	Lubricant	EP current edition
Total	250 mg	750 mg	1250 mg	900 mg	---	---

For intravenous infusion administration, reparixin is provided as a concentrated solution for infusion packaged into 250 mL clear glass vials with the following composition per single (250 mL) unit:

Reparixin composition per single-dose 250 mL vial

NAME OF INGREDIENT	QUANTITY	FUNCTION OF INGREDIENT	QUALITY
Reparixin (DF1681Y)	8.25 g	Drug substance	In-housespecification
Sodium Dihydrogen Phosphate Dihydrate	1.96 g	Buffer	EP current edition
L-lysine monohydrate	4.78 g	Solubilizer	DAB current edition
Sodium hydroxide	qs to pH 8.0	Buffer	EP current edition
Water for injections	qs to 250 mL	Solvent	EP, USP/NF current edition

4.6. STORAGE AND HANDLING

For oral administration, the investigational product is an immediate release oral tablet, whereas for intravenous infusion is a photostable solution. Both liquid and tablet forms of reparixin should not have been frozen or esposed to temperatures higher than 30°C (86°F).
The storage conditions included in the product labelling are “Do not store above 30°C. Do not refrigerate or freeze.”

4.7. ADMINISTRATION

The investigational product will be administered as an immediate release oral tablet or after dilution by i.v. infusion to patients according to the clinical protocols.

4.8. REFERENCES (SECTION 4)

There are no references for this section.

5. NONCLINICAL STUDIES

5.1. NONCLINICAL PHARMACOLOGY

5.1.1. Summary

Reparixin is a novel, potent and specific inhibitor of the biological activity of the chemokine CXCL8.

Mechanism of action studies have shown that reparixin is a non-competitive allosteric inhibitor of the CXCL8 receptors CXCR1 and CXCR2. Interaction of reparixin with CXCL8 receptors inhibits the intracellular signal transduction events activated by binding of CXCL8 to CXCR1 and CXCR2. Chemical computational studies and alanine scanning mutagenesis have identified the interaction site between reparixin and CXCR1/2 in the transmembrane region of CXCR1 and CXCR2.

A number of *in vitro* and *in vivo* assays were performed*: in vitro* chemotaxis experiments demonstrated that reparixin inhibits human PMN migration induced by human CXCL8. Chemotaxis of rat PMN induced by the rat counterparts of human CXCL8 was also inhibited by reparixin.

The *in vivo* PMN recruitment was inhibited in a mouse model of auricular inflammation induced by cantharidin. Reparixin treatment decreased the extent of pro-inflammatory cyokine and effector cells (PMN and T lymphocyte).

Earlier investigations addressed the development of the drug in the prevention of DGF in kidney and lung transplantations.

Reparixin was investigated in rat models of DGF in kidney and lung transplantation. Treatment of rats with reparixin prevented PMN infiltration into the reperfused transplanted kidney and kidney damage, as assessed by an increase in serum creatinine levels. In the lung model, reparixin produced a marked improvement in isolated graft oxygenation, reduced pulmonary edema, and significantly reduced neutrophil infiltration into transplanted lung grafts with induced ischemia/reperfusion injury.

Moreover, reparixin prevented PMN infiltration and tissue damage in animal models of ischemia/reperfusion injury of liver, brain, intestine, heart and spinal cord.

The efficacy of reparixin was evaluated in syngeneic and allogeneic models of intrahepatic islet transplantation in diabetic mice.

According to preliminary results obtained in both models, reparixin appeared to improve islet engraftment. In the allogeneic model, reparixin also appeared to delay the time to rejection. In parallel, reparixin prevented the hepatic infiltration of PMNs. Additional experiments will be required to further confirm and characterize the effect of reparixin in islet transplant models in mice. Reparixin was also tested in a model of MLD-STZ diabetes in mice, where it was shown to significantly delay diabetes development and, more importantly, even after diabetes development, to reduce glycaemic levels, resulting lower in reparixin treated than in vehicle treated group.

A number of *in vitro* and i*n vivo* assays on recognized breast cancer models have been conducted in support of the planned clinical development in Oncology. An *in vitro* assay to assess the effect of reparixin on breast cancer cell growth showed that reparixin reduces metabolic capability and cell number. *In vivo* modeling studies of advanced breast cancer with CXCR1 inhibition in the presence of conventional chemotherapeutics (docetaxel and paclitaxel) demonstrated that reparixin specifically targets and reduces the CSC population. Both docetaxel and paclitaxel target the differentiated tumor cells: the association of docetaxel or paclitaxel with reparixin is capable of reducing both the bulk of the tumor mass as well as the resident CSC population.

Brain metastases confer significant morbidity and mortality in breast cancer patients. A mouse model of brain metastases of breast cancer using intracarotid injections of human MDA-MB-231 breast carcinoma cells was used in order to evaluate reparixin cerebral uptake and efficacy. Reparixin was found to be able to cross the BBB in the preclinical experimental model. On this basis, the activity of reparixin as single agent or in association with paclitaxel was assayed. In the presence of brain metastasis, when treatment was started at the time of tumor cell injection, reparixin alone or in combination with paclitaxel was able to reduce

metastasis numbers and volume. The treatment with paclitaxel as single agent showed effectiveness on the same parameters as well. When treatment started 7 days after tumor cell injection, the different treatment options showed a tendency to decrease their effectiveness. Nonetheless, reparixin and paclitaxel alone were still able to inhibit the total metastasis volumes although to a lesser extend, and the effect on this parameter was higher when the drugs were given in combination. In addition the combination of reparixin and paclitaxel demonstrated a statistically significant inhibitory effect on metastasis number.

The effects of reparixin and DF 2243Y (carboxy-reparixin major metabolite) on renal, cardiovascular and respiratory systems were investigated in rat and dog safety pharmacology studies. The results suggested that reparixin has a safe profile even at the highest doses tested.

Pharmacological studies conducted with reparixin L-lysine salt are summarised in the table below and detailed in the subsequent paragraphs.

Nonclinical Pharmacology Studies

Study Type	Species Number of animals Sex Cell type	Dose (mg/kg) Route Concentration Study design	Results	Reference
***In vitro* Assays**				
Effect of reparixin L-lysine salt on mouse and rabbit PMN chemotaxis	Mouse peritoneal PMN and peripheral rabbit PMN	Reparixin L-lysine Single concentration 1 nM-1 µM	Inhibition of mouse PMN chemotaxis induced by mouse KC and mouse MIP-2 (IC_{50} 1 nM). Slight reduction of rabbit PMN chemotaxis induced by CXCL8 (IC_{50} 1 µM).	A0321[2] (§ 1)
Inhibition by reparixin L-lysine salt of rat PMN chemotaxis induced by rat CXCL8 -related chemokines KC and MIP-2	Rat peritoneal PMN	Reparixin L-lysine salt Single concentration 1 nM-100 nM	Inhibition of rat PMN migration induced by the rat KC (IC50 1 nM) and rat MIP-2-induced chemotaxis of rat PMN.	A0212[2] (§ 2)
Inhibition by reparixin L-lysine salt of human PMN chemotaxis induced by CXCL8	Human peripheral PMN or monocytes	Reparixin L-lysine salt Single concentration 0.01 nM-1 µM	Inhibition of CXCL8 induced chemotaxis of human PMN (IC_{50} 1 nM). PMN chemotaxis induced by C5a, fMLP and TNF unaffected at 100 nM -1 µM. Chemotaxis of human monocytes induced by MCP-1 unaffected at 100 nM-10 µM.	A0142[2] A0212[2] (§ 3)
Inhibition by reparixin L-lysine salt of human PMN chemotaxis mediated through type I and type II CXCL8 receptors	Human peripheral PMN	Reparixin L-lysine salt Single concentration 1 nM-1 µM	Inhibition of PMN chemotaxis induced by CXCL8 (which acts through type I and type II CXCL8 receptors) and by GRO-α (which acts only through type II CXCL8 receptor).	A0142[2] (§ 4)
CXCL8 binding to cell CXCL8 receptors in the presence of reparixin L-lysine salt	Human peripheral PMN	Reparixin L-lysine salt Single concentration 100 nM-1 µM	No influence on binding of CXCL8 to PMN CXCL8 receptors.	A0142[2] (§ 5)
Inhibition by reparixin L-lysine salt of CXCL8 induced signal transduction in human PMN	Human peripheral PMN	Reparixin L-lysine salt Single concentration 100 nM-1 µM	Inhibition of tyrosine phosphorilation and calcium flux increase in CXCL8-activated human PMN.	A0211[2] (§ 6)
Inhibition by reparixin L-lysine salt of CXCL8 induced chemotaxis in whole blood-derived human PMN	Whole blood-derived human PMN	Reparixin L-lysine salt Single concentration 0.1-30 µg/mL	inhibition of CXCL8-mediated human PMN chemotaxis (IC_{50} 5 µg/mL corresponding to 30 ng/mL-100 nM as free concentration), when added directly to human whole blood,	A0314[2] (§ 7)
Effect of reparixin L-lysine salt metabolites on CXCL8-mediated human PMN chemotaxis	Human peripheral PMN	Reparixin metabolites Single concentration 0.1 nM-1 µM	DF 2243Y: significant inhibition of CXCL8-induced human PMN chemotaxis (IC_{50} 10 nM). Other metabolites (12 out of 13 tested): no influence on CXCL8 induced chemotaxis. DF 2188Y: no influence on reparixin inhibition of CXCL8 mediated PMN chemotaxis.	A0316[2] (§ 8)
Effect of reparixin on breast cancer cell growth: MTT and proliferation assays	Breast cancer cell line SUM159	Reparixin L-lysine salt 100 nM - 250 µM treatment for two time points (3 and 5 days).	Considerable decrease in ALDH activity after 3 days treatment at 500 µM. capability significant effect on cell viability after 3 and 5 days treatment at 100 and 500 µM . Reduction in cell number of 50% after 3 days treatment at 250 µM .	M1103[2] (§ 9)

Nonclinical Pharmacology Studies				
Study Type	**Species Number of animals Sex Cell type**	**Dose (mg/kg) Route Concentration Study design**	**Results**	**Reference**
Effect of reparixin and ladarixin on CSCs	Breast cancer cell line SUM159	Reparixin L-lysine salt and Ladarixin 10 µM	Treatment with both compounds leads to a general block of cell proliferation (about 50%, 1 day, 10µM treatment), not coupled with increase in cell death Both induce a decrease in CSC population (about 70%) Addition of chemotherapy does not exert synergistic or additive effect Induces a decrease in cell number and a decrease (about 80%) in the number of mammospheres formed; in addition the content of ALDH in formed mammosphere was drastically reduced No evidence of effect on cell signalling pathways and related genes	M1309 (§ 10)
The effect of Reparixin and Ladarixin on CSC signaling pathways	Breast cancer cell line SUM159	Reparixin and Ladarixin 100 nM	A significant decrease in IL8-induced phosphorylation of AKT and FAK due to Ladarixin and Reparixin treatment was observed in ALDH+/CXCR1+ cells, and not in the CD44+/CXCR1+ population.	M1409 (§11)
Computational Studies				
Selectivity of interaction between reparixin and cellular receptors of human CXCL8 and related agonists	Molecular models of human, rat, mouse, dog and rhesus CXCL8 counterparts receptors	- -	Reparixin is a weak inhibitor of dog and rhesus CXCL8 counterparts receptors according to the proposed binding model.	A0328[2] (§ 12)
Identification by site-directed mutagenesis of CXCR1 aminoacidic residues involved in reparixin L-lysine salt binding	L1.2 cell line	- Reparixin L-lysine salt Single concentration 1 nM-1 µM	Inhibition in a concentration dependent manner of CXCL8 chemotaxis of L1.2 transfectant expressing wild type CXCR1 (IC_{50} 1 nM). Completely resistance of L1.2 mutants expressing Y46A-CXCR1 or K99A-CXCR1 Partial resistance of E291A-CXCR1 .	A0332[2] (§ 13)
***In vivo* Studies**				
Effect of reparixin L-lysine salt in inhibiting auricular inflammation induced by cantharidin	Mouse 48 males	Reparixin L-lysine salt 15 mg/kg i.v 1h before cantharidin application plus 7.50 mg/kg/hour infusion s.c 24 h post-cantharidin application cantharidin (0.25%) topically applied to mice ears	Decrease of the amount of CXCL1 and TNF-α (50%), and of VEGF (14%) in the auricular tissue; inhibition of T lymphocyte (47%) and PMN (51%) infiltration.	A1025/E (§ 14)
Efficacy of reparixin L-lysine salt (15 mg/kg) in preventing renal damage in a rat model of DGF	Rat 13 males	Reparixin L-lysine salt 15 i.v., s.c. Three doses: • s.c. 24h before the transplant; • i.v. immediately before reperfusion; • s.c. 2h after reperfusion	• prevention of renal damage (assessed by plasma creatinine increase) in this rat model of DGF • reduction of creatinine increase by 80% at 24h after reperfusion.	A0108[2] (§ 15)
Efficacy of reparixin L-lysine salt at doses of 5, 15 and 30 mg/kg in preventing renal damage in a rat model of DGF	Rat 14 males	reparixin L-lysine salt 5, 15, 30 i.v., s.c. Three doses: • s.c. 24h before the transplant; • i.v. immediately before reperfusion; • s.c. 2h after reperfusion	• 5 mg/kg: reduction of plasma creatinine increase by 30% • 15 mg/kg: reduction of plasma creatinine increase by 77% and PMN infiltration into the reperfused kidney by 80-90% at 24h after reperfusion. • 30 mg/kg : reduction of plasma creatinine increase by 54% (at 30 mg/kg data were inconclusive)	A0132[2] (§ 16)
Efficacy of reparixin L-lysine salt in preventing renal damage in a rat model of DGF, when administering the first dose of reparixin L-lysine salt at 2h, 4h or 8h before transplant	Rat 14 males	reparixin L-lysine salt 15 i.v., s.c. Three doses: • s.c. 2h, 4h or 8h before the transplant; • i.v. immediately before reperfusion; • s.c. 2h after reperfusion	• first dose administered 2h or 4h before the transplant: inhibition of plasma creatinine increase is 30% and 10%, respectively. • first dose administered 8h before the transplant: inhibition of plasma creatinine increase is 77% after 24h of reperfusion.	A0133[2] (§ 17)

Nonclinical Pharmacology Studies				
Study Type	**Species Number of animals Sex Cell type**	**Dose (mg/kg) Route Concentration Study design**	**Results**	**Reference**
Efficacy of reparixin L-lysine salt in preventing renal damage in a rat model of DGF, when administering the first dose of reparixin L-lysine salt at 2h before transplant	Rat 16 males	reparixin L-lysine salt 15 i.v., s.c. Three doses: • s.c. 2h before the transplant; • i.v. immediately before reperfusion; • s.c. 2h after reperfusion	prevention of the increase of serum creatinine levels (47% of inhibition; n=8) 24h after kidney transplantation.	A0327[2] (§ 18)
Effect of reparixin L-lysine salt in preventing renal damage in a rat model of kidney allotransplantation	Rat 22 males Lewis rat:donor Brown Norway: recipient	reparixin L-lysine salt 10, 15, 20 i.v., s.c.Three doses: • s.c. 8h before the transplant; • i.v. immediately before reperfusion; • s.c. 2h after reperfusion	20mg/kg: prevention of renal function impairment with plasma creatinine concentration strongly reduced at 16h and 24h after reperfusion (76% and 78% of inhibition at 16h and 24h after reperfusion, respectively).	A0402[2] (§ 19)
Effect of reparixin L-lysine salt in preventing lung damage in a rat model of lung transplant.	Rat 28 males Fischer 344 rats served as donors and recipients	reparixin L-lysine salt 15, 30 i.v., s.c. Three doses • iv 15 min before reperfusion • sc 2 hours after reperfusion • sc 4 hours after reperfusion	15 and 30 mg/kg: improvement of lung graft oxygenation as assessed by PaO_2 (untreated = 188 mmHg; = 443 mmHg; 30 mg/kg = 424 mmHg; sham = 517 mmHg), block of lung oedema 6h after lung reperfusion 70% reduction in neutrophil infiltration.	A0420[2] (§ 20)
Efficacy of reparixin L-lysine salt in preventing liver damage and PMN recruitment in a rat model of liver ischaemia/ reperfusion injury	Rat 60 males	reparixin L-lysine salt 3, 15, 30 i.v., s.c. four doses • iv: 15 min before reperfusion • sc:2 hours after reperfusion; • sc:4 hours after reperfusion • sc: 6h after reperfusion	15 mg/kg: prevention of hepatocellular necrosis (assessed by ALT levels and morphological analysis of the tissue) and PMN infiltration induced by reperfusion of post-ischaemic rat liver. reduction of ALT increase and PMN infiltration by 80% at 12h and 24h after reperfusion.	A0229[2] (§21)
Efficacy of reparixin L-lysine salt in preventing liver damage and PMN infiltration in a rat model of steatotic liver ischaemia / reperfusion injury	Rat 30 males	reparixin L-lysine salt 15 i.v., s.c. Four doses • iv: 15 min before reperfusion • sc:2 hours after reperfusion; • sc:4 hours after reperfusion • sc: 6h after reperfusion	prevention of hepatocellular necrosis (assessed by plasma ALT activity and morphological analysis of the tissue) and PMN infiltration. reduction of ALT activity increase and PMN infiltration by 70%-80% at 24h after reperfusion.	A0323[2] (§ 22)
Effect of reparixin L-lysine salt infusion treatment on liver ischaemia/ reperfusion injury in the rat	Rat 22 males	reparixin L-lysine salt: 3 doses 4.5, 2.5, 0.4 i.v. (loading dose) + 2.4, 1.2, 0.2 infusion (48h) s.c. (maintenance dose) 15 min before reperfusion	infusion of 2.4 mg/h/kg and 1.2 mg/h/kg: block of ALT plasmatic increase (80% of inhibition) and PMN tissue infiltration (96% of inhibition) 24h after reperfusion. infusion of 0.2 mg/h/kg : partially reduction of ALT plasmatic increase (50% of inhibition) and PMN infiltration (33% of inhibition).	A0414[2] (§ 23)
Effect of DF 2243Y, a metabolite of reparixin L-lysine salt, on liver ischaemia/ reperfusion injury in the rat	Rat 20 males	reparixin L-lysine salt: 15 DF 2243Y: 1.5, 7.5,15 For both drugs: two doses • i.v. 15min. before reperfusion • s.c. 2h after reperfusion	1.5 mg/kg DF 2243Y: no contribution to reparixin L-lysine salt efficacy. 7.5 or 15 mg/kg DF 2243Y: no significant reduction of PMN infiltration and tissue damage	A0403[2] (§ 24)

Nonclinical Pharmacology Studies

Study Type	Species Number of animals Sex Cell type	Dose (mg/kg) Route Concentration Study design	Results	Reference
Efficacy of reparixin L-lysine salt in preventing brain damage and PMN infiltration in a rat model of transient cerebral ischaemia	Rat 65 males	reparixin L-lysine salt 15 i.v., s.c. Four doses • iv: 5 or 90 min before reperfusion • sc:2 hours after reperfusion; • sc:4 hours after reperfusion • sc: 6h after reperfusion	prevention of tissue damage (44%-52% inhibition of infarct volume) and PMN infiltration (42%-54% inhibition) 24h after middle cerebral artery reperfusion. The protective effect is obtained both when the first treatment was done at the time of ischaemia and just before reperfusion.	A0324[2] (§ 25)
Efficacy of reparixin L-lysine salt in preventing PMN infiltration and oedema formation in a rat model of intestine ischaemia / reperfusion injury	Rat 110 males	reparixin L-lysine salt mild injury 3, 10, 30 i.v. 10min before reperfusion severe injury: 30 i.v. 10min before reperfusion	mild injury prevention of intestine PMN infiltration (70%-80% of inhibition) and oedema formation (80%-90% of inhibition) 30min (mild injury) or 2h (severe injury) in dose dependent manner severe injury: significant inhibition (80%-90% of inhibition) of TNF-α increase (tissue and serum levels) and lethality (100% of prevention) after of the gut.	A0326[2] (§ 26)
Efficacy of reparixin D,L-lysine salt in preventing PMN infiltration and tissue damage in rat model of cardiac ischaemia /reperfusion injury	Rat 31 males	reparixin L-lysine salt 15 i.v., s.c. Two doses: • i.v. 5min before reperfusion; • s.c. 90min after reperfusion	prevention of PMN infiltration (77% of inhibition) and tissue damage (evaluated as tissue loss of creatine kinase activity; 47% of inhibition) 4h after reperfusion.	A0330[2] (§ 27)
Efficacy of reparixin L-lysine salt in preventing PMN infiltration, oligodendrocyte apoptosis and hind limb disability in a rat model of spinal cord injury (SCI)	Rat 48 males	reparixin L-lysine salt 15 i.v., s.c. Fourteen doses after SCI • i.v. within 30min; • s.c. 2h; • s.c. 4h; • s.c. 6h; • s.c. at 8 am and 5 pm until the 7thday	prevention of PMN infiltration (80% of inhibition) and oligodendrocyte apoptotic nuclei formation (62% of inhibition) 1 day and 7 days after SCI. improvement of recovery of hind limb function at 7, 11 and 14 days after SCI.	A0331[2] (§ 28)
Effect of reparixin D,L-lysine salt on carrageenan-induced oedema formation in the rat	Rat 79 females	reparixin D,L-lysine salt and ibuprofen racemate D,L-lysine salt 11.8, 39, 118, 393 μmoles/kg corresponding to 5, 16, 50, 168 for reparixin) p.o. 1h before carrageenan injection	• High dose reparixin D,L-lysine salt: marginally reduces carrageenan-induced paw oedema. • ibuprofen racemate D,L-lysine salt : reduces in a dose dependent manner carrageenan-induced oedema formation being the ID_{50} 32.4 μmoles/kg.	A0320[2] (§29)
Effect of reparixin on graft outcome in murine models of pancreatic islet transplantation	Mouse 174 males and females	reparixin L-lysine salt 8/mg/kg/h s.c. syngeneic model: continuous infusion for 7 or 14 days starting from day -1 of transplantation allogeneic model: continuous infusion for 7 days starting from day -1 of transplantation	According to preliminary data obtained in the syngeneic and allogeneic models, reparixin appears to improve islet engraftment. In the allogeneic model, reparixin was also able to delay the time to rejection.	M0903[2] (§ 30)
Efficacy of reparixin in a model of MLD-STZ diabete in mice.	Mouse 23 males	reparixin L-lysine salt 8/mg/kg/h s.c. Continuous infusion for 7 days starting from day -1 of STZ injection.	Preliminary data indicates that reparixin is able to deyed diabetes development. Even after development of diabetes, mice treated with reparixin had a better glycaemic control.	M0903[2] (§31)
Effect of reparixin L-lysine salt in an advanced breast cancer model in mice..	NOD/SCID Mouse 30 Females 30 Females for secondary re-implantation	reparixin L-lysine salt:15 mg/ kg twice daily s.c. x 28 days docetaxel:10 mg/kg weekly x 4 weeks paclitaxel : 10 mg/kg weekly x 4 week	• reparixin L-lysine salt: reduction of CSC population. • docetaxel and paclitaxel in association with reparixin: reduction of tumor size and CSC population.	UM040811-001 M1104[2] (§ 32)

Nonclinical Pharmacology Studies				
Study Type	**Species Number of animals Sex Cell type**	**Dose (mg/kg) Route Concentration Study design**	**Results**	**Reference**
Effect of reparixin L-lysine salt on cantharidin-induced auricular inflammation	Mouse 24 males	reparixin L-lysine salt: 65 mg/kg, s.c. repeated injections (at 0, 6, 12, 24, 30, 36 hrs) compared to 15 mg/kg i.v plus 7.50 mg/kg/hr/36hrs s.c.continuous infusion, Cantharidin (0.25%) was topically applied to mice ears immediately after the first treatment	Reduction in auricle weights and KC/CXCL1 levels into ear tissue (62%) in mice treated with reparixin s.c. by repeated injections. Inhibition of KC/CXCL1 levels into ear tissue (55%) in mice treated with reparixin administered by continous infusion.	A1210/E (§33)
Biodistribution of reparixin in an experimental model of breast cancer metastasis to brain	Nude mouse 62 females	Reparixin 15mg/Kg (one single administration) or 45 mg/Kg (one single administration or three administrations, every 8h) s.c. 21 days after tumor cells injection	In absence of tumor, the drug does not cross BBB. In tumor cells-injected animals, the dosage of 15 mg/Kg was not enough to be detected at cerebral level, while the dosage of 45 mg/Kg, was detected in a small but significant amount, at cerebral level.	A1304/E (§34)
Treatment with reparixin in an experimental model of breast cancer metastasis to brain	Nude mouse 114 females	Reparixin 45 mg/kg s.c. x2 (every 8h) for 14 days alone or in combination with paclitaxel 10mg/kg i.p.weekly. starting at the day of TC injection (T0-T14) or at 7 days after TC injection (T7-T21).	The different treatment options did not induce significant differences in the animals weight	A1432/E (§35)
Ex vivo Magnetic Resonance Imaging characterization of breast cancer metastasis to brain	Nude mouse 114 females, the same animals of A1432 study	Reparixin 45 mg/kg s.c. x2 (every 8h) for 14 days alone or in combination with paclitaxel 10mg/kg i.p.weekly. starting at the day of TC injection (T0-T14) or at 7 days after TC injection (T7-T21).	The treatment with reparixin alone, paclitaxel alone or reparixin+paclitaxel showed an inhibitory effect in reducing metastasis numbers and volume. The different treatment options result more effective when the treatment started at T0 rather than when the treatment started at T7. Nonetheless, when treatment started at T7, reparixin and paclitaxel alone were still able to inhibit the total metastasis volume and the effect on this parameter was higher when the drugs were given in combination. In addition the combination of reparixin and paclitaxel demonstrated a statistically significant inhibitory effect on metastasis number	A1433/E (§36)
Effect of reparixin in a rat model of neuropathic pain	Rats 30 males	reparixin L-lysine salt 8/mg/kg/h s.c. Continuous infusion for 14 days starting from day -3 of paclitaxel first injection.	Reparixin showed significant antyallodinic effects on mechanical and cold allodynia	A1482E (§37)
Interaction Studies				
Effect of reparixin L-lysine salt on cyclosporine A-induced inhibition of human lymphocyte proliferation	Human peripheral lymphocytes	1, 10, 100 µM reparixin L-lysine salt; 1-3000 ng/mL CsA Single concentration	at all tested concentrations no influence on cyclosporine-A-induced inhibition of human lymphocyte proliferation.	A0119[2] (§38)
Effect of cyclosporine A on the inhibitory activity of reparixin L-lysine salt on CXCL8-mediated PMN chemotaxis	Human peripheral PMN	1 µM reparixin L-lysine salt; 1-100 pM CsA Single concentration	no influence on inhibitory activity of reparixin-L-lysine salt on CXCL8-mediated PMN migration.	A0302[2] (§39)
Safety Pharmacology Studies				
Renal safety after intravenous administration	Sprague-Dawley Rat 40 Males	Reparixin L-lysine salt 4.5, 15, 45 i.v. Single dose reference drug: furosemide (7 mg/kg)	no influence on renal function: urinary volume, pH, protein, glucose, specific gravity, electrolytes and the glomerular filtration rate.	RTC 8480EXT A0112[1] (§40)

Nonclinical Pharmacology Studies

Study Type	Species Number of animals Sex Cell type	Dose (mg/kg) Route Concentration Study design	Results	Reference
DF 2243Y (metabolite of reparixin): Assessment of effects on renal function in rats	Wistar Rat 40M	DF 2243Y 20, 40, 80 i.v. Single dose reference drug: furosemide (20 mg/kg)	No effects on specific gravity, urine output, electrolyte, protein excretion, creatinine levels or numbers of samples containing blood. Dose-related reductions in urinary pH statistically significant at 40 and 80 mg/kg but not biologically relevant.	DOM 075/ 042659[1] (§41)
Cardiovascular safety after intravenous injection	Sprague-Dawley Rat 18 Males	Reparixin L-lysine salt 1.5, 4.5, 15, 45 i.v. Single dose	15 mg/kg: no effect on CV parameters; 45 mg/kg: transient decrease diastolic pressure (-20%) and frequency (-10%) (after the first minute disappeared) no modification of systolic and diastolic blood pressure no differences on the heart rate.	A0111[1] (§42)
Cardiovascular and respiratory safety in the anaesthetised dog	Beagle Dog 4 Males	Reparixin L-lysine salt 4.5, 15, 45 i.v. Single dose	no effect on all of the measured cardiorespiratory parameters (arterial blood pressure, heart rate, left ventricular systolic pressure, left ventricular dp/dt maximum, electrocardiogram intervals, femoral flow, femoral resistance, tidal volume, respiration rate and minute volume)	DOM 043/013041[1] (§43)
Effect on action potential duration (APD)	Rabbit Purkinje fibers	- Reparixin L-lysine salt 10,100 1000 µM Single concentration	10 µM: no effect (NOEL) 100, 1000 µM: significant decrease of the V_{max} of fibers stimulated at 1 Hz. 1000 µM : -Significant decrease of APD_{90} and APD_{50} of rabbit Purkinje fibers stimulated at 1 Hz. -Decrease of APD_{90} during bradycardia. -Significant decrease of APD_{50} during bradycardia. -Significant decrease of APA of fibers stimulated at 1 Hz and during bradycardia. no significant effect on RPM.	1495/ DOM/01[1] (§44)
Effect of reparixin L-lysine salt on □1-adrenergic receptors	Guinea-pig isolated atria	Reparixin L-lysine salt 10 µM, single concentration propranolol (β_1 adrenergic antagonist): 1 µM	Reparixin L-lysine salt: no significant effects on isoproterenol-stimulated cardiac force and cardiac rate, no β_1-adrenergic antagonism	A0130[2] (§45)
DF 2243Y (metabolite of reparixin): Telemetric evaluation of cardiovascular effects	Beagle Dog (conscious) 2M + 2F	DF 2243Y 8, 16, 24 i.v. Single dose	no evidence of a direct cardiovascular action or ECG effect, at the dose levels tested.	DOM 070/042643[1] (§46)
DF 2243Y (metabolite of reparixin): Evaluation of respiratory parameters using whole body bias flow plethysmo-graphy	Wistar Rat (conscious) 40M	DF 2243Y 20, 40, 80 i.v. Single dose	No marked or statistically significant effects on respiration rate, tidal volume or minute volume were seen at all doses tested.	DOM 074/042694[1] (§47)
Modified Irwin dose-range following intravenous administration	Wistar Rat 12 Males	Reparixin L-lysine salt 4.5, 15, 45 i.v. Single dose	no changes in the behavioural or physiological state of the rats. no marked or statistically significant effects on body temperature or spontaneous locomotor activity	DOM 042/013043[1] (§48)
Effects of reparixin L-lysine salt on histaminergic, cholinergic and serotoninergic receptors	Guinea-pig isolated smooth muscle preparations: - parenchymal - lung strips, -tracheal strips - ileum strips.	- 10 µM Single concentration	Reparixin L-lysine salt showed no significant effects on histaminergic, cholinergic and serotoninergic receptors. Furthermore, it did not modify the basal tone.	A0114[2] (§4949)
Evaluation of the haemolytic effect of reparixin in a preparation of rabbit blood	Blood from New Zealand rabbit	25, 250 mg/5mL Single concentration	25 mg/5mL: no effect 250 mg/5mL: haemolysis	A0121BPL[1] (§ 50)

[1] GLP; [2] non-GLP

5.1.2. *In vitro* assays

The in vitro assays included studies on mouse [1], rat [2] and human [3, 4, 5, 6, 7, 8] PMN (Polymorphonuclear neutrophils) and one ongoing study on SUM 159, a breast cancer cell line [9].

Reparixin L-lysine salt inhibited mouse PMN migration induced by the mouse counterpart of CXCL8 (mKC: IC_{50} 1 nM or mMIP-2 : IC_{50} 1 nM) and only slightly inhibited rabbit PMN chemotaxis stimulated by CXCL8 (IC_{50} 1 μM), suggesting that the rabbit is not an appropriate animal model for investigating reparixin L-lysine salt in the preclinical setting **[**1**]**.

Reparixin L-lysine salt inhibited rat PMN migration induced by the rat counterpart of CXCL8 (KC: IC_{50} 1 nM) or rat MIP-2chemokines, suggesting that the rat is an appropriate species to assess *in vivo* the inhibitory activity of reparixin on PNM and tissue damage associated with DGF [2].

Reparixin L-lysine salt inhibited chemotaxis of human PMN induced by an optimal concentration of CXCL8 (1 nM) in a concentration-dependent manner. IC50 was approximately 1 nM as assessed using freshly isolated PMN from blood of human normal donors. Chemotaxis of human PMN and monocytes, induced by other chemotactic and activating factors, including C5a, fMLP, TNF and MCP-1, was not affected by the same range of concentrations found efficacious in inhibiting CXCL8-induced chemotaxis. These data rule out the possibility that reparixin is a non specific inhibitor of cell motility, while it is a potent and specific inhibitor of human PMN chemotaxis induced by human CXCL8 **[**3**].**

Moreover, reparixin, in addition to CXCL8, resulted to be able to inhibit human PMN chemotaxis induced by the CXCL8-related chemokine GRO-α, indicating that reparixin acts on both CXCL8 II and I receptors. Indeed, human CXCL8 activates cells through two surface cellular receptors, CXCL8R I (CXCR1) and II (CXCR2), whereas the CXCL8-related chemokine GRO-α acts only through the CXCL8 II receptor **[**4**]**.

The study of mechanism of action involved in reparixin L-lysine salt-induced inhibition of CXCL8 chemotaxis was performed with receptor binding experiments. The results indicated that reparixin did not affect CXCL8 receptor expression on PMN surface and receptor binding of radiolabelled CXCL8 on PMN surface and, therefore, reparixin is not a receptor antagonist of CXCL8 **[**5**].**

Reparixin resulted to inhibit two key events, such as tyrosine phosphorilation and calcium flux increase, associated with the intracellular biochemical cascade triggered by activated CXCL8 receptors. These data are in keeping with the concept that the compound is an inhibitor of CXCL8-induced signal transduction in human PMN **[**6**].**

Reparixin was efficacious in inhibiting PMN chemotaxis induced by CXCL8 also when target cells were exposed to the drug in the natural context, representative of *in vivo* conditions, of whole blood. The IC_{50} was 5 μg/mL and corresponded to a free concentration of 30 ng/mL (100 nM) **[**7**]**.

The effect of reparixin L-lysine salt metabolites on CXCL8-mediated human PMN chemotaxis was evaluated. Only one metabolite (DF 2243Y) out of 13 significantly affected PMN migration.. DF 2243Y had an IC_{50} of 10 nM on CXCL8 chemotaxis of human PMN.

Moreover, the ability of DF 2188Y, one of the metabolite identified in volunteers plasma, to interfere with reparixin inhibitory effect on CXCL8 chemotaxis was also evaluated. To this aim, PMN were preincubated with reparixin L-lysine salt (10 nM) in the presence or absence of DF 2188Y (1 μM). The inactive metabolite DF 2188Y did not affect reparixin inhibition of CXCL8 mediated PMN chemotaxis **[**8**]**.

A study was performed to evaluate if reparixin (100 nM, 10 μM, 250 μM and 500 μM) is able to affect breast cancer cell growth. SUM159 cell line, a model for breast cancer, has been used to evaluate the relevance of CXCR1 blockade for targeting cancer stem cells.

SUM 159 was stained for CXCR1 and ALDH expression. Considering that CXCR1 positive population ranges from 2 to 4% and ALDH positive population is around 5% (percentage resembling that of CXCR1 positive cells), it could be speculate that CSCs subset in the SUM 159 cell line is identified by a CXCR1+/ALDH+ population.

SUM159 cell line was treated at different time points and at different concentration and tested for ALDH activity, viability and proliferation.

The assay showed a considerable decrease in the ALDH activity after 3 days of treatment at 500 µM, a significant effect on cell viability after 3 and 5 days of treatment at 100 and 500 µM and a 50% decrease in cell number at day 3 at 250 µM. This decrease was not associated with the accumulation of cells in any phase of the cell cycle and did not correspond to an increase of cell death mediated by FASL, as assessed by its dosage **[**9**].**

A study was performed to evaluate the effect of reparixin and ladarixin on cancer stem cells in the breast cancer cell line, SUM159 compared to classical chemotherapy (docetaxel and paclitaxel). Dose ranges between 0.1 and 100 µM and timeframes between 1 and 7 days were conducted depending on the assay.

Results showed that both compounds, reparixin and ladarixin, led to a general block of cell proliferation (about 50%, 1 day, 10 µM treatment), that is not coupled with an increase in cell death. Both compounds also induced a decrease in CSC population (about 70%). The addition of chemotherapy to reparixin and ladarixin did not exert a synergistic or additive effect.

The ability of reparixin and ladarixin to interfere in mammosphere formation was also evaluated. Exposure of the cells to all treatments (single and combo) induced a decrease in cell number. In the reparixin and ladarixin treatment groups, there was also a decrease (about 80%) in the number of formed mammospheres, and the content of ALDH in the formed mammosphere was dramatically reduced.

Lastly, there were no particular evidences seen with the compounds on cell signalling pathways and related genes in the whole cancer population [10].

A study to determine the percentage of Cancer Stem Cell in SUM159 according to conventional (CD44/CD24) and new markers (CXCR1/ALDH) was performed.

In this study the effect of reparixin on cell signalling of CSC was also investigated. To this Aim CSC were sorted and an IL-8 priming stimulus (100 ng/ml) was provided before reparixin (100 nM) treatment.

A significant decrease in IL-8 induced phosphorylation of AKT and FAK due to Reparixin treatment was observed in ALDH+/CXCR1+ cells, and not in the CD44+/CXCR1+ population.

This result enhanced the consistency of the association of CXCR1 and ALDH in defining cancer stem cell per se but also CSC function. These data confirm previous observation in the literature (Ginestier et al. 2010) of the key role in CSC of AKT and FAK in the signalling cascade triggered by IL-8 and silenced by reparixin [11].

5.1.3. Computational studies

Computer-aided technique was used to study the structural interactions of reparixin with human CXCL8 receptors CXCR1 and CXCR2 [12] and to identify the amino acids of CXCR1 involved in reparixin binding [13].

In the first study, the ability of computational modelling studies to correctly anticipate the sensitivity of cellular receptors of CXCL8 (or animal counterparts of) from different animal species to reparixin was evaluated. The results of the computational study ruled out that the evaluation of the hydrophobic interactions is useful to predict the affinity of reparixin towards the examined receptor subtypes. Reparixin showed a high affinity for the human CXCR1 due to a well structured hydrophobic region formed by Ile43, Val42 and Val58. The lack of potency of reparixin towards the human CXCR2 is caused by the replacement of Ile43 with Val51 that interacts less favourably with reparixin. The evaluation of the predicted hydrophobic pattern of interactions in highly homologous receptors seems adequate to predict the sensitivity of different animal species to the inhibitory activity of reparixin. The proposed model of interaction was in good agreement with the experimental data in rat, rabbit and mouse PMN chemotaxis assay and indicated a weak interaction between reparixin and the CXCL8 receptors of dog and rhesus. In the last two cases, no comparison with experimental data was possibe, since the activity of reparixin in the inhibition of rhesus and dog PMN chemotaxis was not available. The evaluation of the predicted hydrophobic pattern of interactions in highly homologous receptors seems adequate to predict the sensitivity of different animal species to the inhibitory activity of reparixin. These results were considered in making decisions as to animal species deemed relevant and appropriate for *in vivo* testing of reparixin, even if the reliability of the model is limited to highly homologous receptors such as those considered in this study where the polar interactions pattern appears strictly conserved [12].

In the second study, the amino acids of CXCR1 involved in reparixin binding were identified. Computational modeling of the reparixin L-lysine salt target molecule CXCR1 predicted a direct interaction of specific reparixin residues with three polar aminoacids (Y46, K99, E291) of CXCR1. To put into a test this model, each aminoacid of CXCR1 putatively involved in the interaction with reparixin, was evaluated by alanine-replacement mutagenesis experiments and tested for sensitivity to reparixin. Data showed that CXCL8-induced chemotaxis of L1.2 transfectants expressing wild type CXCR1 was concentration dependently inhibited by reparixin L-lysine salt (IC_{50} 1 nM), as expected, whereas Y46A-CXCR1 or K99A-CXCR1 mutants completely resisted the action of the compound. The E291A-CXCR1 mutant had a partial resistance to reparixin. These data support the proposed model of a hydrophobic channel defined by helices 1, 3, 6, and 7 in the transmembrane domain as the binding pocket for reparixin interaction with CXCR1 [13].

5.1.4. *In vivo* efficacy studies

In vivo studies included experiments evaluating the ability of reparixin to induce a decrease in local inflammation [14], to preserve renal function in syngeneic and allogeneic kidney transplantation [15, 16, 17, 18, 19], to ameliorate ischemia/reperfusion injury in lung transplant model [20], to prevent PMN infiltration and tissue damage in other animal models of ischemia/reperfusion injury of liver [21, 22, 23, 24], brain [25], intestine [26], heart [27] and spinal cord [28] and to inhibit paw oedema [29].

Two studies were performed in diabetic mice [30, 31] and one study assessed the efficacy in breast cancer model [32].

5.1.4.1. Efficacy in decrease local inflammation

One study was performed to assess the ability of reparixin L-lysine salt to induce a decrease in local inflammation. The compound was tested in a mouse model of auricular inflammation induced by cantharidin, a compound that induces aseptic inflammation. For the purpose, cantharidin (0.25%) was topically applied to mice ears and reparixin was injected as follow: an i.v. bolus of 15 mg/kg before cantharidin application and a 7.50 mg/kg/hour infusion s.c. in the 24 hours post-cantharidin application. At the end of treatment, there was a 50% reduction of CXCL1 and TNF-α and 14% reduction of VEGF in comparison to control mice (no cantharidin). Moreover, ears were tested for leukocyte infiltration, finding that there was a 47% and a 51% decrease in T lymphocytes and PMN respectively. All these results indicate that systemically administered reparixin is able to induce a decrease in local inflammation, due to a decrease of both pro-inflammatory cytokine and cells (T lymph and PMN) [14].

5.1.4.2. Efficacy in syngeneic and allogeneic kidney transplantation models

Four studies [15, 16, 17, 18] were performed in an experimental rat model of syngeneic kidney transplantation to evaluate the effect of reparixin on renal function preservation early after the ischaemia/reperfusion injury and to assess whether the compound prevents intragraft leukocyte infiltration that occurs after ischaemia/reperfusion injury.

To this purpose, in all the four studies, the left kidney of donor animals was exposed to cold ischaemia for 4h or 6h. Recipient animals were prepared by removal of the left kidney. An anastomosis was created between the recipient and the donor renal artery as well as between renal vein and an end-to-end anastomosis. Vascular clamps were released after 30min (warm ischaemia). The native right kidney of the recipient animals was then removed. Renal function was assessed by measuring plasma creatinine concentration.

In the first study [15] reparixin L-lysine salt was administered at 15 mg/kg and in the second study [16] it was given at 5, 15 or 30 mg/kg. In both studies, each dose of reparixin was administered s.c. 24h before the transplant, i.v. immediately before reperfusion and s.c. 2h after reperfusion.

The results from the first study indicated that in animals receiving 4h ischaemic kidneys, the treatment with 15 mg/kg of reparixin L-lysine salt protects animals from renal damage (24h after reperfusion) as assessed by plasma creatinine levels. Reparixin reduced creatinine increase by 80% at 24h after reperfusion. Plasma creatinine levels in vehicle and treated groups were 2.09±0.6 and 0.78±0.2 mg/dL, respectively. In addition, reparixin significantly reduced (50% of reduction) plasma creatinine levels also in animals receiving 6h ischaemic kidney, an experimental condition characterized by a more severe renal function impairment.

Creatinine levels in vehicle and reparixin treated groups were 3.27±0.4 and 1.83±0.1 mg/dL, respectively. Plasma creatinine levels in sham operated animals were in the range of 0.5-0.6 mg/dL. Prevention of renal damage induced in this animal model of DGF by reparixin was paralleled by a significant reduction of PMN infiltration in the interstitial (80% of inhibition) and intraglomerular (50% of inhibition) areas of the transplanted kidney [15].

The results from the second study confirmed those of the previous experiment, reparixin administered at a dose of 15 mg/kg reduced by 77% the plasma creatinine increase induced by reperfusion at 24h after organ reperfusion. Prevention of kidney damage was paralleled by a significant reduction of PMN infiltration in the graft (80-90% reduction of PMN recruitment in the graft). On the other hand, the dose of 5 and 30 mg/kg only partially reduced creatinine levels (30 and 54% respectively, inhibition of creatinine increase) without affecting PMN recruitment in the graft. However, results at the dose of 30 mg/kg were inconclusive since only few animals (3 out of 5) were administered and, due to technical problems, rats were misdosed and this caused a severe inflammation at the injection site (dorsal vein of penis) that conceivably interfered with the activity of the test compound. Thus, among the three doses tested (5, 15 and 30 mg/kg), 15 mg/kg was found efficacious in preventing both DGF and PMN infiltration into the transplanted kidney in a rat model of DGF [16].

The third study evaluated the efficacy of 15 mg/kg reparixin L-lysine salt in preventing DGF and PMN infiltration when the first dose was administered at different time intervals before the transplant, namely 2 or 4 or 8h prior to transplantation. After the first dose, all animal groups were then treated with a second dose and a third dose as in all previous experiments (15, 16).When the first dose was administered 2 or 4h before transplant, the treatment did not prevent DGF, whereas PMN infiltration was reduced when it was administered 4h before transplant. Finally, when the first dose was administered 8h before transplant, the treatment prevented both renal function impairment (77% inhibition of creatinine increase) and PMN infiltration, indicating that the interval of 8h was efficacious in preventing both DGF and PMN infiltration [17].

In the fourth study, reparixin L-lysine salt was given at 15 mg/kg with the following treatment schedule: 2h before transplantation, immediately before reperfusion and 2h after reperfusion. The results showed that reparixin significantly prevented the increase in serum creatinine levels (47% of inhibition; n=8) 24h after kidney transplantation and reduced PMN recruitment induced by reperfusion of the transplanted kidney in the interstitium and in the perivascular area **[**18**].**

One study evaluated the efficacy of reparixin in preventing ischaemia/reperfusion injury in an experimental model of allogeneic rat kidney transplantation.
Lewis rat taken as a donor in Brown Norway recipient was chosen as a highly incompatible strain combination. Donor left kidney was exposed to cold ischaemia for 6h until transplant. Recipients were prepared by removal of the left kidney. An anastomosis was created between the recipient and the donor renal artery as well as renal vein and end-to-end anastomosis. Vascular clamps were released after 30min (warm ischaemia). Donor and recipient ureteres were connected through end-to-end anastomosis. The native right kidney was then removed.
Recipient animals were treated with vehicle or different doses (10, 15, 20 mg/kg) of reparixin L-lysine salt 8h before transplantation (s.c.), immediately before reperfusion (i.v.) of the transplanted kidney and 2h after transplantation (s.c.). Renal function was assessed by plasma creatinine concentrations at 16h and 24h after reperfusion. Data showed that reparixin L-lysine salt (20 mg/kg) prevented renal function impairment since plasma creatinine concentration strongly reduced at 16 and 24h after reperfusion (76% and 78% of inhibition at 16 and 24h after reperfusion, respectively) [19].

5.1.4.3. Efficacy in lung transplant model

One study was conducted in a rodent orthotopic left lung transplant model between isogeneic rats to evaluate if reparixin ameliorates transplantation-associated ischaemia/reperfusion injury. Fischer 344 rats served as donors and recipients. Left lungs were harvested and transplanted using the modified cuff technique for rodent lung transplantation. Twelve hours of cold preservation was used to create ischaemia/reperfusion injury.

Recipients were divided into 4 groups: a sham group which underwent anaesthesia, intubation, and thoracotomy without transplantation, a control group which received saline, and two reparixin groups which received reparixin L-lysine salt (15 or 30 mg/kg) 15 min before transplantation (i.v.) and 2h and 4h after transplantation (s.c.). Recipients were sacrificed 6h after transplantation. Isolated graft oxygenation, pulmonary oedema and neutrophil infiltration were evaluated.
Reparixin significantly ameliorated ischaemia/reperfusion injury associated with 12h of cold preservation. Both reparixin doses produced nearly identical improvements in isolated lung graft oxygenation, as assessed by PaO2 values (untreated=188 mmHg; 15 mg/kg=443 mmHg; 30 mg/kg= 424 mmHg; sham=517 mmHg) with an associated reduction of pulmonary oedema. Significantly, treatment with reparixin produced a 70% reduction in neutrophil infiltration into lung tissues [20].

5.1.4.4. Efficacy in animal models of ischemia/reperfusion injury of liver, brain, intestine, heart and spinal cord]

One study was performed to evaluate the effect of reparixin L-lysine salt in a rat model of liver ischaemia/reperfusion injury with two sets of experiments [21].

The liver was subjected to a selective inflow occlusion by clamping the portal triad (hepatic artery, portal vein, and bile duct) to the left and median liver lobes by placing a bulldog clamp for 1h. Reperfusion was initiated by removing the clamp. Tissue samples from the median and left lateral lobes were taken at the end of the reperfusion phases (12h or 24h) for biochemical and morphological evaluation. Irreversible hepatic damage was evaluated by determination of alanine aminotransferase (ALT) levels in blood plasma.
In the first set of experiments, rats were treated with 3, 15 or 30 mg/kg (15 min before reperfusion i.v. and 2h after reperfusion s.c.). Treatment with reparixin induced a dose–related protection against PMN infiltration (85% inhibition of PMN infiltration with 15 mg/kg) and hepatocellular necrosis (80% inhibition of ALT increase with 15 mg/kg) 12 hours after reperfusion.
In the second set of experiments, the protective effect of reparixin L-lysine salt (15 mg/kg 15 min before reperfusion i.v. and 2h, 4h and 6h after reperfusion s.c.) was evaluated 24h after reperfusion. Results indicate that reparixin reduced hepatocellular necrosis (80% inhibition of ALT increase) and PMN infiltration (80% inhibition of intra-hepatic infiltration).

Another study was performed to evaluate the effect of reparixin L-lysine salt in a rat model of steatotic liver ischaemia/reperfusion injury.
Steatosis was induced by feeding the animals a diet deficient in choline for additional 4 days. Control rats consumed a semipurified diet containing adequate levels of choline.
Animals were treated with reparixin L-lysine salt (15 mg/kg) or vehicle 15 minutes before reperfusion (i.v.) and every two hours after reperfusion for three times (s.c.; 2h, 4h and 6h after reperfusion). The effect of reparixin was evaluated 24h after reperfusion.
Results indicate that reparixin strongly reduced liver PMN infiltration induced by reperfusion of steatotic liver with PMN recruitment similar to values observed in the sham group (80% inhibition). Similarly, reparixin dramatically reduced hepatocellular necrosis (70% inhibition of ALT activity increase) [22].

One study was performed to determine the schedule of treatment by s.c. infusion of reparixin L-lysine salt in the experimental model of I/R of the rat liver to obtain plasmatic concentrations of reparixin in the range of 3-15 µg/ml.
In addition, the efficacy of reparixin L-lysine salt administered by s.c. infusion against PMN infiltration and tissue damage induced by reperfusion of post-ischaemic rat liver was evaluated.
Plasma concentration-time profile of reparixin in the I/R model after administration of reparixin L-lysine salt by s.c. infusion at a dose of 8 mg/kg/h was determined. The results showed a plasma reparixin Css of 15 µg/ml during the infusion period (0-48h).
The efficacy of reparixin L-lysine salt in liver I/R injury after s.c. infusion was evaluated after administration of three different doses: loading 4.5, 2.5, 0.4 mg/kg i.v. and maintenance 2.4. 1.2, 0.2 mg/kg/h s.c.. Data showed that reparixin L-lysine salt at doses of 2.4 mg/kg/h (Css reparixin 7.2 µg/mL corresponding to 22.1 ng/mL of unbound reparixin) or 1.2 mg/h/kg (Css reparixin 3.6 µg/mL corresponding to 10.3 ng/mL of unbound reparixin) strongly prevented hepatocellular necrosis induced by reperfusion, as assessed by ALT level inhibition (80% of inhibition). Reduction of ALT levels was paralleled by 96% of inhibition of PMN tissue infiltration. On the contrary, reparixin L-lysine salt at 0.2 mg/h/kg (Css reparixin 0.44 µg/mL) only

partially reduced ALT level increase (50% of inhibition) as well as it partially inhibited PMN tissue infiltration (33%) [23].

The contribution and the efficacy in the prevention of PMN recruitment and tissue damage of DF 2243Y, a metabolite of reparixin L-lysine salt, was evaluated in a comparative I/R rat liver study.
Rats were treated with reparixin at 15 mg/kg. DF 2243Y was administered at 1.5 mg/kg to determine its contribution in reparixin efficacy and at doses superimposable to reparixin L-lysine salt effective doses (7.5 or 15 mg/kg) to assess its efficacy. The two compounds were given with a schedule of treatment (i.v. 5 minutes before reperfusion and s.c. 2h after reperfusion) known to prevent PMN infiltration and tissue damage 12h after liver reperfusion.
Data showed that 12h after reperfusion, DF 2243Y at none of the three doses reduced PMN infiltration and tissue damage, as assessed by plasma ALT activity, indicating that this metabolite did not contribute to reparixin L-lysine salt efficacy in rat liver I/R injury and did not significantly reduce PMN infiltration and tissue damage induced by reperfusion of post-ischaemic liver [24].

The efficacy of reparixin in preventing tissue damage and PMN infiltration was investigated in a rat model of transient cerebral ischaemia after the administration of 15 mg/kg or vehicle at the time of ischaemia or 5min before reperfusion (i.v.) and next every 2h after reperfusion (s.c.; 2, 4 and 6h after reperfusion) and evaluated 24h after reperfusion.
Transient cerebral ischaemia was induced in male rats by occlusion of the middle cerebral artery for 90min followed by 24h of reperfusion.
Reparixin, given at the time of ischaemia, significantly reduced brain PMN infiltration (54% of inhibition) and tissue damage (44% of inhibition of infarct volume) and, when given 5min before reperfusion, inhibited cerebral PMN recruitment (42%) and infarct volume (52%). In conclusion, in this model reparixin significantly inhibited cerebral PMN recruitment and infarct volume either when the first treatment was done at the time of ischaemia or just before reperfusion [25].

The efficacy of reparixin L-lysine salt in preventing PMN infiltration and oedema formation was assessed in a mild and severe rat model of intestine ischaemia/reperfusion injury.
In the model of mild injury (30min of ischaemia of the superior mesenteric artery followed by 30min of reperfusion), reparixin L-lysine salt given at 3, 10 or 30 mg/kg 10 min before reperfusion inhibited in a dose dependent manner both the increase in vascular permeability (80%-90% at 30 mg/kg) and PMN recruitment (70%-80% at 30 mg/kg) in the intestine and lung.
In the model of severe intestine I/R injury (2h of ischaemia followed by 2h of reperfusion), reparixin L-lysine salt given at 30 mg/kg 10 min before reperfusion virtually abolished the intestine and lung increase of vascular permeability (80%-90% of inhibition) and PMN recruitment (70%-80% of inhibition). In addition, reparixin significantly inhibited (80%-90%) TNF-α increase in tissue and serum after severe I/R injury. Since it was reported that intestine severe reperfusion injury is accompanied by significant TNF-α-dependent lethality (reaching 60% in most experiments), the efficacy of reparixin L-lysine salt (30 mg/kg) in preventing rat lethality was evaluated. The compound totally prevented lethality.
In conclusion, reparixin prevents local (intestine) and remote (lung) tissue injury and lethality that follow reperfusion of the ischaemic superior mesenteric artery in the rat [26].

The efficacy of reparixin L-lysine salt in preventing PMN infiltration and tissue injury was assessed in a rat model of cardiac ischaemia/reperfusion injury. Cardiac ischaemia was induced by ligation of left coronary artery (30min of ischaemia) and confirmed by the appearance of ventricular ectopies and discoloration of the heart surface.
Reperfusion was verified by reddening of previously discolored area. Animals were treated with reparixin D,L-lysine salt (DF 1681A; 15 mg/kg) 5min before reperfusion (i.v.) and 90min after reperfusion (s.c.) and the effect of reparixin was evaluated 4h after reperfusion.
Data showed that reparixin significantly inhibited cardiac PMN recruitment (evaluated as myeloperoxidase activity; 77%) and tissue damage (evaluated as tissue loss of creatine kinase activity; 47%) 4h after reperfusion [27].

The efficacy of reparixin L-lysine salt in preventing PMN infiltration, oligodendrocyte apoptotic nuclei formation and hind limb disability was investigated in a rat model of spinal cord injury (SCI).
SCI was performed in the rat using a contusion apparatus. The lesioning apparatus was computer controlled and free of the influence of gravity force. The force applied was 1N per 1 sec. Animals were treated with

vehicle or reparixin L-lysine salt (15 mg/kg) within 30 min after SCI (i.v.), then s.c. every 2h in the following 6h. In the following days, s.c. treatment was performed at 8 am and 5 pm until the 7th day.
Rats treated with saline began to show signs of recovery from hind limb disability between 4 and 7 days after SCI. The improvement of control animals was slow and reached 8.5 degrees (according to the "BBB scale" developed at the Ohio University) 14 days after SCI. Animals treated with reparixin had a better recovery of hind limb function with 5.5 degrees at 7 days and 13.2 degrees at 14 days after SCI. In addition, reparixin significantly reduced oligodendrocyte apoptotic nuclei (62%) and PMN infiltration (80%) in the injured cord [28].

5.1.4.5. Efficacy on carrageenan-induced oedema in the rat

The effect of reparixin on carrageenan-induced paw oedema in the rat was evaluated after the oral administration of reparixin D,L-lysine salt (DF 1681A) (5, 16, 50, 168 mg/kg corresponding to 11.8, 39, 118 or 393 μmoles/kg), ibuprofen racemate D,L,-lysine salt (DF 1663A; 11,8, 39, 118 or 393 μmoles/kg) or vehicle. Basal volume of the right paw was measured using a hydropletismometer and immediately after rats were treated with reparixin, ibuprofen or vehicle.
One hour after treatment, 0.1 mL of a 1% (w/v) of solution of carrageenan type IV in saline at 37 °C was given by subplantar injection into the right paw and evaluation of oedema formation was performed 3h after carrageenan injection.
The results showed that reparixin was able to inhibit paw oedema, although the maximal effect was observed at 118 μmoles/kg (corresponding to 50 mg/kg; 40% of inhibition). On the contrary, ibuprofen dose dependently reduced oedema formation with the ID_{50} 32.4 μmoles/kg. In conclusion, reparixin only marginally reduces carrageenan-induced paw oedema formation in the rat, suggesting that reparixin is not a classical anti-inflammatory (COX inhibitor) molecule [29].

5.1.4.6. Efficacy in syngeneic and allogeneic intrahepatic islet transplantation models

One study was performed to evaluate the effect of reparixin (lysine salt) in murine models of syngeneic and allogeneic intrahepatic islet transplantation. Islets from 12 week old C57 mice were transplanted in liver of diabetic C57 mice (alloxan induced, glycaemia >450 mg/dl) in the syngeneic model. Islets from 12 week old Balb/c mice were transplanted in liver of diabetic C57 mice (alloxan induced, glycaemia >450 mg/dl) in the allogeneic model. Reparixin (lysine salt) was administered by s.c. continuous infusion (through an osmotic pump) for a total of 7 (syngeneic and allogeneic models) or 14 (syngeneic model only) days starting from day -1 of islet transplantation at a dose of 8 mg/kg/h. Control animals received continuous s.c. vehicle.

In the syngeneic model, the group treated with reparixin for 14 days was limited to 19 mice since the incidence of surgical death (death within the first 7 days after transplantation) was higher (about 50%) than expected and higher than that observed in mice treated for 7 days (about 27%). This increase in surgical mortality was likely due to the size of the osmotic pump that prevented appropriate access to food and water in the post-operative period.

Experiments in the allogeneic model were also carried out in the presence or absence of rapamycin (daily i.p. injections starting with an induction dose of 0.3 mg/kg on day 0 followed by a maintenance dose of 0.15 mg/kg until day 14) and FK506 (0.1 mg/kg i.p daily starting on day 0).

In the syngeneic model, primary endpoint was the ability to reach a non-fasting blood glucose level less than 200 mg/dl for two consecutive measurements after islet transplantation. Preliminary results showed that the probability and median time to reach euglycaemia (<200 mg/dl) were higher in mice treated with reparixin as compared to vehicle (Log Rank $p<0.012$). An intravenous glucose tolerance test (IVGTT) and an oral glucose tolerance test (OGTT) were performed at 1 and 3 months after transplantation to evaluate the function of the grafted islets. Glucose elimination constant (KG, expressed as percent elimination of glucose per minute) was calculated between 1 and 15 min (KG_{1-15}) and 1 and 60 min ($KG_{1–60}$) after intravenous glucose (0.5 g/kg). KG_{1-15} and KG_{1-60} appeared higher in reparixin treated mice as compared to control animals. Similarly, the area under the curve (AUC) for glucose during OGTT in mice treated with reparixin remained lower than that in vehicle treated mice. Circulating levels of alanine aminotransferase (ALT) measured 24h and 48h after the transplant were not affected by reparixin.

According to preliminary results, reparixin appeared to improve islet engrafment also in the allogeneic model, as demonstrated by its ability to increase the likelihood of and to reduce the time to gain normo-

glycaemia. Moreover, in the allogeneic islet transplantation model, reparixin could delay the time to rejection (defined as two consecutive non-fasting blood glucose readings greater than 300 mg/dl), as demonstrated by its ability to prolong the normo-glycaemia. When associated to rapamycic, reparixin indefinitely prolonged graft function in about 20/30% of animals, suggesting a potential role in tolerance induction.

In parallel, single cell suspensions were prepared from two liver lobes of known weight and analysis of the intrahepatic leukocyte (IHL) population was perfomed by flow cytometry. Reparixin treatment decreased the total intraephatic number of PMNs, macrophages, T helper cells and dendritic cells. Circulating levels of alanine aminotransferase (ALT) measured 24h and 48h after the transplant were not affected by reparixin both in the presence or in the absence of rapamycin.

Additional experiments will be required to further confirm and characterize the initial results obtained in syngeneic and allogeneic models of islet transplantation [30].

5.1.4.7. Efficacy in multiple low dose streptozotocin induced diabetes model

Reparixin was tested in a model of MLD-STZ diabetes in mice. STZ was injected i.p. at a dose of 40 mg/kg/day for 5 consecutive days in male C57BL/6J mice. Reparixin was administered by s.c. continuous infusion (through an osmotic pump) for 7 days starting from day -1 of first STZ injection at a dose of 8 mg/kg/h.
Mice with glycemia >250 mg/dl on three consecutive daily measurements were considered diabetic. The presence of reparixin appears to delay diabetes development as assessed by glucose concentrations in venous blood. The median diabetes free time was 12 ± 1 days (n=12) and 6±2 days (n=11) respectively for reparixin and vehicle treated mice. More importantly, even after diabetes development, glycaemic levels remained constantly lower in reparixin treated than in vehicle treated group [31].

5.1.4.8. Efficacy in breast cancer model

A study was performed to evaluate if CXCR1 blockade could target CSCs in vivo. To this aim, the effects of the cytotoxic agents docetaxel and paclitaxel were compared with those of reparixin on the CSC compartment and on tumor growth in NOD/SCID mice. CSC population was assessed by the ALDEFLUOR assay and by serial transplantation in NOD/SCID mice. To test reparixin activity on tumor growth, 5 animals/group were injected with 50,000 cells from SUM159 cell line. When the tumor size reached approximately 4 mm, treatment was initiated: reparixin alone (15 mg/kg twice daily for 28 days), docetaxel alone (10 mg/kg once weekly for 4 weeks), paclitaxel alone (10 mg/kg once weekly for 4 weeks) or a combination of both drugs. For the second transplantation assay, serial cell diluition (100, 1,000, 10,000) from treated tumor was injected. Results showed that chemotherapy treatment (both docetaxel and paclitaxel) alone resulted in either no change or a relative increase in the CSC populations. In contrast, reparixin treatment alone or in combination with chemotherapy significantly reduced the CSC population. Despite the significant reduction in the tumor-initiating population, use of reparixin alone did not result in a significant reduction in tumor size. Nonetheless, the combination of reparixin plus chemotherapy resulted in significant reduction in tumor size and in the CSC population, which suggests that a stategy of combined therapy may maximize the efficacy of single agents. Docetaxel and paclitaxel target the differentiated tumor cells while reparixin is capable of dramatically reducing CSC re-growth thereby effectively targeting both the bulk of the tumor mass as well as the resident CSC population [32].

5.1.4.9. Efficacy on cantharidin-induced auricular inflammation

In a previous study we investigated the anti-inflammatory activity of reparixin L-lysine salt administered by continuous infusion in a mouse model of auricular inflammation induced by cantharidin [14]. Aim of the present study was to compare the efficacy of the compound administered either as multiple subcutaneous injections or via subcutaneous implanted osmotic pump.

Male Balb/c mice were used. Reparixin L-lysine salt was administered as the following treatment schedule:
16 mice received reparixin s.c. at the dose of 65 mg/kg at 0, 6, 12, 24, 30 and 36hrs. Eight mice were sacrificed after 37hrs starting from time 0, while other eight mice were sacrificed after 48hrs starting from time 0.
8 mice received an i.v. injection of reparixin 15 mg/kg an instantly an osmotic pump that delivered subcutaneously 7.5 mg/kg/h of compound for 36h. This group of mice were sacrificed at 37hrs.

Immediately after compounds/vehicles treatments (time 0), fifteen µl of 0.25% solution of cantharidin (in acetone) were applied onto the inner and upper surfaces of the right ear. After sacrifices, both ears were quickly excised at the hair line.
Auricle weights and KC/CXCL1 levels into ear tissue (ELISA method) were determined. One-way Anova followed by Dunnett's test was used for statistical analysis, significance was set at $p< 0.05$.
In the group of mice treated with reparixin s.c. by repeated injections and sacrificed 37hrs from time 0, a significant reduction in the weight of the right auricles was observed. In the same group, a marked, significant reduction of KC/CXCL1 (-61%) was seen. Also after 12 hrs starting from the end of the s.c. treatment reparixin was still able to significantly decrease (about 24%) the KC/CXCL1 levels in the cantharidin-exposed right auricles.
In the group of mice treated s.c by the osmotic pump, the reduction of KC/CXCL1 levels in the cantharidin-exposed right auricles was of about 55%.
On the whole, the present results confirm a remarkable activity of Reparixin, also following its s.c. administration at intervals of 6-12 hrs, in opposing inflammation processes by actions on KC/CXCL1 level [33].

5.1.4.10. Efficacy of reparixin in an experimental model of breast cancer metastasis to brain

Three studies [34, 35, 36] were performed in an experimental mouse model of breast cancer metastasis to brain. The first study [34] had the purpose to determine whether reparixin can cross the blood–brain barrier (BBB) in presence of brain metastasis. The other two studies [35, 36] had the aim to assess the possible effects of the compound in inhibiting brain metastasis formation.
To this purpose, in all three studies, brain metastasis were generated by inoculation of 10^6 MDA-MB-231 cells/100 µl (Tumor Cells; TC) in the internal carotid artery of nude female *BALB/c* of 4-5 weeks.
In the first study, 21 days after tumor cells injection, reparixin was administered subcutaneously (s.c.) at the dosage of 15mg/Kg (one single administration) or 45 mg/Kg (one single administration or three administrations, every 8h). Mice were sacrificed 2h or 5h after treatment and plasma and brain collected. After HPLC analysis of blood and brain samples at the different reparixin concentration and times, the ratio Cb/Cp (ratio between reparixin brain concentration and plasma concentration), which constitutes an important indicator of cerebral uptake, was calculated.
The results showed that in absence of brain metastases the drug does not cross BBB. In MDA-MB-231-injected animals, the dosage 15 mg/Kg was not enough to be detected at cerebral level. In MDA-MB-231-injected animals treated with reparixin 45 mg/Kg, mainly in those sacrificed after 5 h of treatment, a small but significant amount of drug at cerebral level was observed. In particular, evaluating the temporal kinetic it was possible to observe a decrease of drug in plasma from 5h to 2 h (0.298±0.09 µg/ml and 1,640±0,2430 respectively), and an increase at cerebral level from 5h to 2h (0.137±0.06 µg/g and 0,040±0,0110 respectively); as consequence, the Cb/Cp ratio increase about 20 folds from 2 to 5 h (0,0244±0,010 and 0,460±0,13, respectively).
The passage of reparixin across BBB does not seem affected by the increase of drug amount. A general decrease of reparixin both at cerebral and plasma level, with respect to animals treated with a single administration of reparixin 45mg/Kg and sacrificed at 5 h was observed [34].

On the basis of the obtained results, the potential effects of reparixin as single agent or in association with paclitaxel was characterized in the same mouse model. Two different experimental sessions that differed for the day of metastasis detection at day 14th and 21st respectively, has been set up, with the aim to study the temporal progression of metastasis in the brain and to identify a predictive experimental design for testing the potential effects of the drugs.
In the first experimental session, mice were injected with TC at T0; drugs treatment started at T7 and lasted for 14 days until T21. In the second experimental session, mice were injected with TC at T0; drugs treatment started at T0 and lasted for 14 days until T14. For each session, the experimental groups were: TC + vehicle (Group V); TC + reparixin 45 mg/kg s.c. twice daily (Group R), TC + paclitaxel (10 mg/kg i.p.) once weekly (Group T); TC + paclitaxel (10 mg/kg i.p.) once weekly + reparixin 45 mg/kg s.c. twice daily (Group TR).
After the different treatments, mice were anesthetized with Ketamine/xylazine (100mg/Kg and 10mg/Kg body weight) injected i.p. and intracardiacally perfused with physiological saline, followed by 10% formalin solution neutral buffered. Brains were dissected out and transferred in formalin for MRI imaging.

Of note, the different treatment options did not induce significant differences in the animals weight, both at in 14 and 21 days animals [35].

The brain metastasis in mice were characterized and quantified by means of *ex vivo* 2.35 Tesla Magnetic Resonance Imaging (MRI) that provided a comprehensive accounting of the number and size at the different time points selected.

In the experimental session in which the drugs treatment started at T0 and lasted for 14 days until T14, the different treatment options showed a similar inhibitory effects both on number and volume of brain metastasis. In fact the mean number of metastasis were significantly lower in the group R (1.2), group T (2.1) and group RT (1.4) when compared with the metastasis mean number of group V (4.0). A similar effect was found for the metastasis volume; in fact the mean for the different groups were 0.11 for R, 0.20 for T, 0.11 for RT and 0.41 for V.

In the subsequent session the mice were analyzed at day 21 post inoculation. The first observation was that the metastasis mean number and volume of the group V were significantly greater than at day 14 (16.2 vs 4.0 and 2.36 vs 0.41 respectively) showing a severe progression in the development of the metastasis. In this experimental session the drugs treatment started at T7 and lasted until T21. The results obtained showed a significant inhibitory effect on metastasis number only for the association of reparixin with paclitaxel (group RT 9.1 vs 16.2 for group V); on the other hand the statistically significant ability to inhibit the metastasis volume was shown by all the treatment options with a trend of increased inhibitory effect for the combination treatment vs the single treatments (1.67 for R, 1.347 for T, 0.99 for TR vs2.362 for V) [36].

5.1.4.11. Efficacy in a rat model of neuropathic pain

Aim of this study was to assess the antiallodynic effects of reparixin following continuous subcutaneous administration in a rat model of paclitaxel-induced neuropathy.

Neuropathic pain behaviour was induced in male Wistar rats (200-250g) by four once daily intraperitoneal injections of paclitaxel (2 mg/kg/day i.p.) administered on alternate day. Reparixin was administered by osmotic pumps implanted 3 days before paclitaxel first injection (-3 day). Pumps were filled with reparixin in order to obtain a rate of infusion of 8 mg/hr/kg for 14 days. Testing for the development of mechanical allodynia was performed with a dynamic plantar aesthesiometer. Cold sensitivity was measured as the number of foot withdrawal responses after application of acetone to the dorsal surface of the paw. Testing for cold and mechanical allodynia were performed on day -1 and then on 5 th, 7th, 10 th and 14th days after paclitaxel first administration.

The significance of difference between compound-treated group and vehicle group was determined by two-way ANOVA followed by Bonferroni post hoc tests for multiple comparisons. Significance was set at $P< 0.05$.

Following paclitaxel administration animals showed an evident mechanical and cold allodynia as compared to sham rats. Animals treated with reparixin showed a significant reduction of mechanical and cold allodynia at days 5 ($P<0.01$), 7 ($P<0.001$), and 10 ($P<0.001$), whereas no activity was determinated on day 14, three days after the interruption of reparixin administration. These data confirm that the antiallodynic activity was directly correlated with the delivery of the compound [37].

5.1.5. Interaction studies

Since the proposed clinical indication of reparixin L-lysine salt is the prevention of DGF in organ transplantation, and the immunosuppressant cyclosporine A is widely used in the clinical management of transplants, it was important to investigate whether reparixin L-lysine salt might interfere with the immunosuppressant activity of cyclosporine A.

Two studies were performed to investigate the interaction between reparixin L-lysine salt and cyclosporine A [38, 39].

The first study investigated whether *in vitro* inhibition of lymphocyte proliferation mediated by cyclosporine A might be affected by reparixin. *In vitro* proliferation of human lymphocytes was induced by the polyclonal mytogen phytohaemagglutinin A (PHA) at the optimal concentration of 21 µg/mL. The effect of three different concentrations of reparixin L-lysine salt (1, 10 and 100 µM) on different concentrations of

cyclosporine A (1, 3, 10, 30,100, 300, 1000, 3000 ng/mL) was evaluated in terms of cell viability (MTT assay) and proliferation (thymidine incorporation).
The results showed that reparixin, at all tested concentrations, does not affect cyclosporine A induced inhibition of human lymphocyte proliferation **[**3832**]**.

The second study investigated whether in vitro inhibition of CXCL8-induced PMN migration mediated by reparixin L-lysine salt might be affected by cyclosporine A.
The effect of cyclosporine A was evaluated in a range of concentrations (1, 3, 10, 30 and 100 pM) representative of free unbound cyclosporine A levels in transplanted patients, on an optimal inhibitory concentration of reparixin L-lysine salt (1 μM).
The results showed that cyclosporine A, at all tested concentrations, does not affect the activity of reparixin in inhibiting CXCL8-induced PMN migration **[**39].

5.1.6. Safety pharmacology studies

Safety pharmacology studies were conducted on renal, cardiovascular, respiratory and central nervous systems.

5.1.6.1. Renal Function

The effect of reparixin L-lysine salt and DF 2243Y (metabolite of reparixin) was assessed in two studies [40and 41].

The effect of reparixin L-lysine salt on renal function was evaluated on male Sprague-Dawley rats, lightly ether anaesthetised treated with the compound at 4.5, 15 and 45 mg/kg (2 mL/kg) by single i.v. injection (1 mL/min) in the tail vein. As reference drug, furosemide (7 mg/kg) was used. Immediately after the i.v. injection, the animals were submitted to a gavage with 25 mL/kg of saline solution and placed in individual metabolic cages for urine collection.
The evaluation of the renal function was performed by measuring the following parameters: urinary volume, pH, protein, glucose, specific gravity, electrolytes (sodium and potassium excretion) and the glomerular filtration rate (GFR). The method employed for assessment of urinary pH, protein, glucose and specific gravity was a semi-quantitative reagent strip test; GFR was evaluated measuring the creatinine clearance. Sodium and potassium concentration in the urine was determined by means of a flame-photometer. Urines collected during periods 0-4h and 4-8h were used to determine renal parameters except for creatinine clearance where urines were collected during 0-24h period.
Reparixin, at all tested doses, did not affect the urinary tested parameters at any collecting times.
Furosemide group, as expected, showed, 4h after dosing, a marked increase in urinary Na+ concentration and an increased urinary volume. On the contrary, furosemide was ineffective on glucose urinary excretion, K+ urine concentration and did not modify the creatinine clearance.
In conclusion, the data obtained suggest that reparixin has a safe profile for renal functionality in rats 40.

The renal function was also evaluated following administration of DF 2243Y (metabolite of reparixin). This compound was administered to male Wistar rats (8/group) at 20, 40 and 80 g/kg. The effects on urine volume and electrolyte excretion were evaluated. Two additional control groups received either an equivalent volume (2 ml/kg) of vehicle (0.08M phosphate buffer) or 20 mg/kg furosemide.
DF 2243Y at the dose levels tested produced no marked or statistically significant decrease in urine output between 1h and 24h post-dose and on specific gravity, electrolyte, protein excretion, creatinine levels or numbers of samples containing blood when compared to the control group. DF 2243Y produced dose-related reductions in urinary pH which were statistically significant at 40 and 80 mg/kg. However, these changes were not considered to be of biological significance in terms of actual pH changes [41].

5.1.6.2. Cardiovascular and Respiratory System

The effect of reparixin L-lysine salt and DF 2243Y (metabolite of reparixin) on cardiovascular and respiratory system was evaluated in four [42, 43, 44, 45] and two studies [46, 47] for reparixin and DF 2243Y, respectively.

The first study conducted with reparixin L-lysine salt was assessed on male Sprague-Dawley rats, urethane-anaesthetised, treated i.v. as single bolus with four increasing doses (1.5, 4.5, 15 and 45 mg/kg, 2 mL/kg) in a cumulative manner.
The evaluation of the cardiovascular activity included the following parameters: blood pressure, frequency and ECG. Blood pressure was assessed in the carotid artery by a pressure transducer, whereas frequency and ECG were evaluated through electrodes.
reparixin L-lysine salt, at the dose of 15 mg/kg, did not have any effect on CV parameters; only the highest dose tested (45 mg/kg) was active on pressure and frequency. In fact, a significant reduction of the diastolic pressure (-20%) and frequency (-10%) was observed. However, these effects were transient and disappeared after the first minute. Furthermore, the time course of the cardiovascular effect was measured using the effective dose (15 mg/kg/2 mL) injected as single bolus.
Reparixin did not modify the systolic and diastolic blood pressure during all the considered times (30 sec, 1, 5, 10, 20 min after injection) and no differences were also observed on the heart rate.
In conclusion, the results obtained suggest that reparixin has a safe profile on the cardiovascular system in rats [42].

The second study conducted with reparixin L-lysine salt was assessed on four anaesthetised male Beagle dogs treated by i.v. bolus with vehicle (saline) and increasing doses (4.5, 15 and 45 mg/kg) of reparixin L-lysine salt using a constant dose volume of 2 mL/kg administered by a Kds infusion pump at 30 min intervals.
The effects of reparixin on cardiovascular parameters (arterial blood pressure, heart rate, left ventricular systolic pressure, left ventricular dp/dt maximum, electrocardiogram intervals, femoral blood flow, femoral resistance, tidal volume, respiration rate and minute volume) were measured at 5 min intervals during the stabilisation period and then at pre-dose, 1, 2 and 5 min post-dose and then 5 min intervals thereafter.
All measured cardiorespiratory parameters were unaffected by i.v. administration of vehicle and reparixin. Slightly significant statistical differences ($p<0.05$) in absolute values for heart rate and QT values following 15 and 45 mg/kg and in QTc values following 45 mg/kg reparixin L-lysine salt were recorded at various time points when compared with vehicle. These were considered to be time related changes due to the effect of anaesthesia and therefore were not thought to be of any biological significance.
In conclusion, i.v. administration of reparixin L-lysine salt produces no significant effects on any of the measured cardiorespiratory parameters when compared to vehicle in dogs [43].

The third study evaluating the effects of reparixin L-lysine salt on action potential duration were performed in rabbit Purkinje fibers electrically paced at 1.0 and 0.2 Hz stimulation frequencies at concentration of 10, 100, 1000 μM.
The effects of reparixin were determined by measuring the resting membrane potential, the action potential amplitude (APA), the maximum upstroke velocity (Vmax) and the action duration at 50% and 90% repolarization (APD50 and APD90, respectively).
The highest concentration caused a significant decrease of APD90 and APD50 of rabbit Purkinje fibers stimulated at 1 Hz, a decrease of APD90 and a significant decrease of APD50 during bradycardia and a significant decrease of APA in fibers stimulated at 1 Hz and during bradycardia.
The two higher concentrations of reparixin L-lysine salt (100 and 1000 μM) decreased significantly the Vmax of fibers stimulated at 1 Hz, while at 10 μM no effect on APA, Vmax, APD50 and APD90 was seen.
In conclusion, reparixin L-lysine salt only at the concentration of 1000 μM is able to affect, by decreasing, the action potential duration of rabbit Purkinje fibers while at the concentration of 10 μM it does not show any effect. The No Observed Effect Level (NOEL) was 10 μM [44].

The fourth study evaluated the interaction between reparixin and β_1-adrenergic receptors on guinea-pig isolated atria stimulated with isoproterenol. Tissues were exposed to reparixin L-lysine salt (10 μM) or propranolol (β-adrenergic antagonist; 1 μM). The compounds were allowed to interact with the tissues for 15min prior to the addition of isoproterenol (β_1-adrenergic agonist; 30 nM). The atrial force was calculated graphically as mm of contraction before and after each single drug-addition. Similarly, the atrial rate, calculated as beats/minute, was evaluated before and after each single drug-addition. Spontaneous atrial contractions and atrial rate were recorded by isometric transducers connected to a polygraph.
The cardiac effects of reparixin were compared with those of propranolol. Isoproterenol caused a marked increase of atrial force and atrial rate. On the contrary, reparixin showed no significant effects on both the parameters evaluated, while propranolol inhibited in a significant manner the increase of both atrial force and

atrial rate induced by the agonist. Since reparixin has no significant effects on isoproterenol-stimulated cardiac force and cardiac rate in guinea-pig atria, a β1-adrenergic antagonism of the compound can be excluded [45].

The first study conducted with DF 2243Y investigated the cardiovascular effects in conscious dogs. Arterial blood pressure, heart rate and ECG (lead II) were measured in 2 male and 2 female non-naive Beagle dogs which were chronically implanted with telemetry transmitters. With the exception of the dosing period, feeding times and for behavioural observations, the animals were left undisturbed in the monitoring room during recording periods.
Prior to the first test session, a 12-hours telemetric recording was obtained to check the compliance of the transmitters and to obtain baseline data which were used to calculate individual regression lines for QT corrections. Each animal received a single i.v. dose (over a period of between 1-1.5 min) of DF 2243Y at 8, 16 or 24 mg/kg or vehicle control. The treatment design used was an escalating dose design. Animals were monitored for a period of 12 hours after each dosing.
DF 2243Y did not induce any adverse behavioural signs. There was no evidence of a direct cardiovascular action or electrocardiogram effect, at the dose levels tested, which would compromise the safety of the compound [46].

The second study conducted with DF 2243Y investigated the effects on respiratory system in conscious male Wistar rats (8/group), following i.v. administration of 20, 40 and 80 mg/kg. The effects on respiration rate, tidal volume and minute volume were registered. An additional control group received an equivalent volume (2 ml/kg) of vehicle (0.08M phosphate buffer). To ensure the validity of the test system, morphine sulphate at i.v. dose of 20 mg/kg was also administered. Prior to dosing, rats were randomised into study groups on the basis of their pre-dose respiration rates. At 0, 30, 60, 120 and 240 min post-dose, the respiratory parameters were recorded using whole body bias flow plethysmography.
DF2243Y at the dose levels tested did not produce any marked or statistically significant effects on respiration rate, tidal volume or minute volume when compared to the vehicle-treated control group [47].

5.1.6.3. Central Nervous System

The effects on general behavior, body temperature and locomotor activity, according to the modified Irwin test were evaluated in male Wistar rats treated with reparixin L-lysine salt at doses of 4.5, 15 and 45 mg/kg by single i.v. administration. These evaluations were performed at 15, 30, 60, 120 min and 24h after treatment. Further pre-dose and a 0-5 min subjective observations were also performed for behaviour evaluation.
Reparixin did not produce changes in the behaviour or physiological state of the rats at all doses. Furthermore, no marked or statistically significant effects on rectal body temperature or spontaneous locomotor activity were observed with reparixin when compared with the vehicle. The animals were also observed daily during a 7-day post-dose period to show delayed toxic effects of the treatment.
No mortalities or gross sign of toxicity were observed **[**48**]**.

5.1.6.4. Other organs and systems

Since reparixin is a potent and selective inhibitor of CXCL8 receptor activation, it was important to investigate whether it might inhibit the activation of diverse receptors belonging to 7-transmembrane domain receptor family.
To this aim, the effect of reparixin on histaminergic, cholinergic and serotoninergic receptors was evaluated.
The effect of reparixin L-lysine salt (10 μM) was evaluated on the basal and stimulated tone of three classical guinea-pig isolated tissues: parenchymal lung strips contracted with histamine, tracheal muscle preparations contracted with acetylcholine and ileum strips stimulated with 5-hydroxytryptamine. As reference compounds, we used selective histaminergic (mepyramine 30 nM), cholinergic (atropine 30 nM) and serotoninergic (methysergide 10 nM) receptor antagonists. Tissue responses were recorded by isometric transducers connected to a polygraph. The guinea-pig isolated organs were exposed to cumulative concentrations of the agonists; antagonists or vehicle were added 15 min prior to the addition of the agonist.

The results show that reparixin L-lysine salt has no significant effects on histaminergic lung parenchymal receptors, cholinergic tracheal receptors and serotoninergic ileum receptors when tested at 10 μM on guinea-

pig smooth muscle preparations. In addition, the compound does not modify the basal tone of all the considered organ preparations.
On the contrary, all the reference receptor antagonists tested caused a statistically significant rightward displacement of the concentration-response curve induced by the correspondent agonist [49].

5.1.6.5. Hemolysis

The haemolytic effect of reparixin L-lysine salt was evaluated in rabbits.
Blood was withdrawn from ether-anaesthetised New Zealand rabbits and incubated for 30 min at 37°C with reparixin L-lysine salt at two different concentrations (25 mg/5mL and 250 mg/5mL).
As reference compounds were used two commercial drugs: Artrosilene® ampoules (ketoprofen lysine salt) and Arfen® (ibuprofen lysine salt; 0.12M) tested at a concentration equimolar to the concentration of reparixin L-lysine salt 250 mg/5mL.
The assay was based on the spectrophotometric reading (at 562 nm) of the haemoglobin released from red blood cells lysed in presence of drugs or solutions able to affect the integrity of erythrocyte cellular membrane.
The results showed that reparixin had no haemolytic activity at 25 mg/5mL. On the contrary, the haemolytic effect was consistent at 250 mg/5mL with a corresponding IC50 ± SEM (percent of drug in the well-plate in which was observed 50% of haemolysis) of 40.7 ± 2.35.
Arfen® showed a haemolytic activity very similar (IC50 34.0 ± 1.03) to that of reparixin L-lysine salt at 250 mg/5mL. Artrosilene® ampoule showed a low haemolytic activity: 20.3% at the highest concentration tested (74.8% in the well plate).
In conclusion, the results indicate that the ability of reparixin and Arfen® to affect the integrity of erythrocyte cellular membrane is related to the chemical structure of the compounds. In addition, the haemolytic effect of reparixin is concentration-dependent and at 25 mg/5mL is completely safe [50].

5.1.7. References (section 5.1)

1. Effect of reparixin L-lysine salt on mKC- and mMIP-2-induced murine PMN chemotaxis and on CXCL8-induced rabbit PMN chemotaxis [A0321]
2. Inhibition by reparixin of rat PMN chemotaxis induced by the rat IL-8-related chemokines KC and MIP-2 [A0212]
3. Reparixin inhibits IL-8 induced chemotaxis of PMN (human polymorphonuclear cells) [A0142, A0212]
4. Inhibition by reparixin of human PMN chemotaxis mediated through both type I and type II IL-8 receptors [A0142]
5. IL-8 binding to human PMN surface IL-8 receptors in the presence of reparixin [A0142]
6. Inhibition by reparixin of IL-8-induced signal transduction in human PMN [A0211]
7. Inhibition by reparixin L-lysine salt of CXCL8-induced chemotaxis in whole blood-derived human PMN [A0314]
8. Effect of reparixin L-lysine salt metabolites on CXCL8-mediated human PMN chemotaxis [A0316]
9. In vitro feasibility study for evaluation of the relevance of CXCR1 blockage for targeting of human cancer stem cells [M1103]
10. Reparixin and Ladarixin characterization in a breast cancer model [M1309]
11. The effect of Ladarixin and Reparixin on SUM-159 [M1409]
12. A computational study for the evaluation of the selectivity of interaction between reparixin and cellular receptors of human CXCL8 and related agonists in different animal species [A0328]
13. Identification by site-directed mutagenesis of CXCR1 aminoacidic residues involved in reparixin binding [A0332]

14. Effects of reparixin L-lysine salt in inhibiting auricular inflammation induced by cantharidin [Dompé A1025/E]
15. Effects of interleukin-8 inhibitor DF 1681B on cell infiltrate and renal function during ischaemia/reperfusion injury in a rat model of syngeneic kidney transplantation [Dompé A0108]
16. Effects of the interleukin-8 inhibitor DF 1681B on cell infiltrate and renal function during ischaemia/reperfusion injury in a rat model of syngeneic kidney transplantation. Phase A1 [Dompé A0132]
17. Effects of the interleukin-8 inhibitor DF 1681B on cell infiltrate and renal function during ischaemia/reperfusion injury in a rat model of syngeneic kidney transplantation. Phase A2 [Dompé A0133]
18. Effect of the interleukin-8 inhibitor DF 1681B on renal function and leukocyte infiltrate during ischaemia/reperfusion injury in a rat model of syngeneic kidney transplantation. Extension of Phase A2 [Dompé A0327]
19. Efficacy of reparixin L-lysine salt in preventing renal damage in a rat model of kidney allotransplantation [Dompé A0402]
20. Reparixin, a novel CXCL8 receptor inhibitor, ameliorates ischaemia-reperfusion injury after experimental lung transplantation [A0420]
21. Role of DF 1681B (Reparixin) against neutrophil infiltration in a rat model of liver injury by ischaemia-reperfusion [Dompé A0229]
22. Efficacy of reparixin L-lysine in preventing liver damage and PMN infiltration in a rat model of steatotic liver ischaemia/reperfusion injury [Dompé A0323]
23. Effect of reparixin L-lysine salt infusion treatment on liver ischaemia/reperfusion injury in the rat [Dompé A0414]
24. Effect of DF 2243Y on liver ischaemia/reperfusion injury [Dompé A0403]
25. Efficacy of reparixin L-lysine salt in preventing tissue damage and PMN infiltration in a rat model of transient cerebral ischaemia [Dompé A0324]
26. Efficacy of reparixin L-lysine salt in preventing PMN infiltration and oedema formation in a mild and severe rat model of intestine ischaemia/reperfusion injury [Dompé A0326]
27. Efficacy of reparixin D,L-lysine salt in preventing PMN infiltration and tissue injury in a rat model of cardiac ischaemia/reperfusion injury [Dompé A0330]
28. Efficacy of reparixin L-lysine salt in preventing PMN infiltration, oligodendrocyte apoptotic nuclei formation and hind limb disability in a rat model of spinal cord injury [Dompé A0331]
29. Effect of oral administration of reparixin D,L-lysine salt (DF 1681A) on carrageenan-induced oedema in the rat [A0320]
30. Studies on reparixin in models of pancreatic islet transplantation in mice – protection of graft failure [M0903]
31. Studies on reparixin in a model of multiple low dose streptozotocin induced diabetes in mice [M0903]
32. Preclinical in vivo modelling of advanced breast cancer with CXCR1 inhibition in the presence of conventional chemotherapeutics [UM040811-001] [M1104,]
33. Effect of reparixin L-lysine salt subcutaneously administered in the cantharidin model in mice [A1210/E]
34. Biodistribution of reparixin in an experimental model of breast cancer metastasis to brain [A1304/E]
35. Treatment with reparixin in an experimental model of breast cancer metastasis to brain [A1432/E]

36. Ex vivo Magnetic Resonance Imaging characterization of breast cancer metastasis to brain [A1433/E]
37. Antiallodynic effect of DF1681B in a model of neuropathic pain by paclitaxel [A1482/E]
38. Effects of DF 1681B on cyclosporine-A inhibition of PHA-induced proliferation of human peripheral blood lymphocytes [A0119]
39. Cyclosporine A does not affect the inhibitory capacity of reparixin L-lysine salt on interleukin-8 mediated human PMN chemotaxis [A0302]
40. Evaluation of the renal safety of DF 1681B after intravenous administration in the male rat [RTC8480EXT, A0112]
41. DF 2243Y (metabolite of DF 1681B): Assessment of effects on renal function in rats (intravenous administration) [DOM 075/042659]
42. Evaluation of DF 1681B cardiovascular safety after intravenous injection in the anaesthetised rat [A0111]
43. DF 1681B. Cardiovascular and respiratory safety evaluation in the anaesthetised dog following intravenous administration [DOM043/013041]
44. Effects of DF 1681B on action potential duration in rabbit Purkinje fibers [1495/DOM/01]
45. Effects of DF 1681B on β_1-adrenergic receptors in isolated guinea-pig atria [A0130]
46. DF 2243Y (metabolite of DF 1681B): Telemetric evaluation of cardiovascular effects in the conscious dog (intravenous administration) [DOM 070/042643]
47. DF 2243Y (metabolite of DF 1681B): Evaluation of respiratory parameters in the conscious rat using whole body bias flow plethysmography (intravenous administration) [DOM 074/042694]
48. DF 1681B. Modified Irwin dose-range in rats following intravenous administration (including effects on locomotor activity and body temperature) [DOM 042/013043]
49. Effects of reparixin L-lysine salt on histaminergic, cholinergic and serotoninergic receptors in three isolated smooth muscle preparations derived from guinea-pigs. Comparison with mepyramine, atropine and methysergide [A0114]
50. Evaluation of the haemolytic effect of DF 1681B in a preparation of rabbit blood [A0121BPL]

5.2. PHARMACOKINETICS AND PRODUCT METABOLISM IN ANIMALS

5.2.1. Summary

The pharmacokinetics of reparixin L-lysine salt was studied following single dose by i.v. and p.o. route in rats and by i.v. route in dogs; following continuous infusion in rats and dogs and after 14 days repeated administration by oral route in rats and by i.v. route in dogs. The continuous infusion and the repeated studies were part of toxicity studies. The metabolic profile *in vivo* and *in vitro*, the potential inhibition on CYP450 isoenzymes, and the protein binding were also evaluated.

In all studies, the compound was administered as reparixin L-lysine salt (DF 1681B) except for the oral toxicokinetic studies where the compound was administrered as reparixin (DF 1681Y); in both cases the concentrations of the drug are expressed in terms of reparixin (DF 1681Y).

The results indicated a different pharmacokinetic profile between rats and dogs. Reparixin was more rapidly eliminated in rats than in dogs ($t_{1/2}$ 0.5-3 h vs 12-28 h, respectively) both after single IV and continuous infusion.

Absolute bioavailability of reparixin in rats after single oral administration accounted for more than 80%.

In both species, the compound showed a low volume of distribution indicating a limited distribution into tissues. Indeed, the distribution of total radioactivity into tissues was generally much smaller than those in plasma. Total radioactivity crossed the placenta in pregnant rats.

[^{14}C] reparixin was highly bound to plasma proteins (> 98.9%), mainly to albumin and more distributed in plasma than in blood.

The pharmacokinetics of reparixin was linear in rats after i.v. infusion in the range of 100-900 mg/kg/day for 28 days. In dogs the AUCs were linear in the range 0.4-10.8 mg/kg/day with 14 days i.v. infusion.

In dogs, after 14 days continuous infusion, the concentrations in plasma of reparixin declined after 48 h after administration of 100, 200 or 400 mg/kg/day indicating a possible induction of its metabolism, while the plasma levels remained constant up to day 14-15 after administration of 15, 30, 50, or 60 mg/kg/day.

After 14 days or 13 weeks of repeated oral administration (bis in die) of reparixin in rats, exposure in females was slightly higher than in males. The duration of treatment did not induce accumulation of the parent compound DF1681Y or its metabolite DF1674Y in both sexes. Apparent half-life was not influenced by the doses.

No definitive conclusions regarding gender difference in dogs were available since no difference was observed after single i.v. route, but after 2 weeks continuous infusion, male dogs showed lower concentration at 15, 30 and 60 mg/kg/day, similar concentrations after 50 mg/kg/day and higher concentrations after 100 and 200 mg/kg/day in comparison to females.

The pharmacokinetic profile of reparixin in dogs after single i.v. bolus administration suggests the presence of an enterohepatic recirculation.

Plasma clearance in rats and dogs was low. Reparixin was poorly excreted in rat urine (< 2% up to 48h), while in dogs it was not detectable in urine (up to 96h).

Reparixin was extensively metabolised in rat, lesser in human, and slightly in dog hepatocytes. Three main metabolic pathways were identified: hydroxylation, carboxylation and hydrolysis. Hydroxylation and carboxylation were the biotransformation routes observed in rats, hydrolysis, hydroxylation and carboxylation in humans and just hydrolysis in dogs.

In human liver microsomes, metabolism was catalysed by CYP2C9 and, to a lesser extent, by CYP2C19 to give two hydroxylated metabolites named DF 2188Y and DF 2260Y.

Qualitative identification of reparixin L-lysine salt metabolism indicated the presence of 8 and 10 metabolites in rats and dogs plasma, respectively. Eight metabolites were detected in rat urine and some of these such as ibuprofen, DF 2260Y, DF 2239Y and DF 2243Y, were the same found in the plasma.

In both rats and dogs, the majority of drug related radioactivity was excreted in urine (about 80%).

Reparixin L-lysine salt *in vitro* resulted as a minor inhibitor of the isoenzymes CYP3A4 (IC_{50} = 8 µM), CYP2C9 (IC_{50} = 79 µM) and of CYP2C19 (IC_{50} = 868 µM).

Reparixin L-lysine salt indicates some potential for the uncompetitive inhibition of the human hepatic enzyme CYP3A4 involved in the phase 1 metabolism of both cyclosporine A and sirolimus. Since the free plasma concentration of reparixin at steady state in human is approximately 0.1 µM, then the C_{max}/K_i ratio << 0.1 and thus it is predicted that the clinical relevance of any inhibition of cyclosporine A or sirolimus metabolism by DF 1681B is remote.

Reparixin L-lysine salt and its major metabolite, DF 2243Y did not affect P-gp activity. Reparixin and DF 2243Y did not interfere with the P-gp also in presence of cyclosporine A, sirolimus and tacrolimus. Besides, reparixin is not a substrate for P-gp.

The methodology and the results are summarised in the below table and detailed in the subsequent paragraphs.

<table>
<tr><th colspan="4">Nonclinical Pharmacokinetic and Metabolism Studies</th></tr>
<tr><th>Type of Study (Species / No. of animals / sex)</th><th>Dose (mg/kg/day)*/ Route/Frequency</th><th>Comments</th><th>Reference</th></tr>
<tr><td>Methods of Analysis</td><td></td><td></td><td></td></tr>
<tr><td>MET in vivo (R to S DF 1681 interconversion)
Rat plasma samples from: RBM 12110 study</td><td>5, 15, 45 / i.v./ single dose</td><td>15 mg/kg: low concentrations of S-isomer (2.1µg/mL) at 5min (1F),
45 mg/kg : 3.6±0.5 µg/mL (mean±SD) (M+2F,)</td><td>Chiman R032/01[1]
(§ 1)</td></tr>
<tr><td>MET in vivo (R to S DF 1681 interconversion)
Dog plasma samples from: RBM 14700 study</td><td>1, 10, 50 / i.v. / single dose</td><td>DF 1672Y (S-isomer) was not detected in dog plasma.</td><td>Chiman R033/01[1]
(§ 2)</td></tr>
<tr><td colspan="4">Pharmacokinetics after single dose</td></tr>
<tr><td>PK (plasma)
rat/4 M per dose</td><td>15, 30 / p.o. / single dose
15 / i.v. / single dose</td><td>Cmax (p.o.) = 16.86 ± 7.02 and 63.94 ± 25.78·µg/mL (rapid absorbtion)
Tmax (p.o.) = 10.00 ± 0.00 and 10.00 ± 0.00 min
t½ elim. (p.o.) = 107.07 ± 87.34 and 51.36 ± 10.95 min (rapid elimination)
AUCtot (p.o.) = 1034 ± 121 and 2945 ± 614 ·µg·min /mL
t½ elim. (i.v.) = 49.45 ± 12.73 min
AUCtot (i.v.) = 1224 ± 326 ·µg·min /mL
Vz (i.v.) = 620 ± 246 mL/kg (low distribution)
Fabs = 84% and 120% after oral administration of 15 and 30 mg/kg/day.</td><td>A0109[2]
(§ 3)</td></tr>
<tr><td>PK (plasma, urine)
rat/56 per dose: 2M+2F each time point</td><td>5, 15, 45 / i.v. / single dose</td><td>t½ elim. = 0.6, 1.1 and 1.0 h (rapid elimination)
AUCtot = 13, 47 and 144 µg·h /mL
CL = 260, 213, and 206 mL/h/kg
Vz = 233, 342, 297 mL/kg (poor distribution)
Ae from 0 to 48 h = 1.3, 0.4 and 0.3% respectively for 5, 15 and 45 mg/kg. (poor excretion)</td><td>RBMR12110[1]
(§ 3)</td></tr>
<tr><td>PK (plasma, urine)
dog/3M+3FM</td><td>1, 10, 50 / i.v. / single dose</td><td>
<table>
<tr><td>dose (mg/kg)</td><td>1</td><td>10</td><td>50</td></tr>
<tr><td>AUCtot (µg·h /mL)</td><td>119.7</td><td>801.4</td><td>2048.1</td></tr>
<tr><td>CL (mL/h/kg)</td><td>6.0,</td><td>8.5</td><td>16.6</td></tr>
<tr><td>t½ (h)</td><td>26.8</td><td>12.6</td><td>28.2</td></tr>
<tr><td>Vz (mL/kg)</td><td>193.3</td><td>149.3</td><td>663.2</td></tr>
</table>
increase less than proportional)
not detection in urine</td><td>RBMR14700[1]
(§5)</td></tr>
<tr><td colspan="4">Pharmacokinetics after repeated dose</td></tr>
</table>

Nonclinical Pharmacokinetic and Metabolism Studies

Type of Study (Species / No. of animals / sex)	Dose (mg/kg/day)*/ Route/Frequency	Comments	Reference
TK (plasma) rat/12 M (6 per group): 5 sampling times after the second daily administration of each dose. wash-out period of 2 daysbetween doses	100-400/ oral (gavage)/ bid after administration of DF 1681B and DF 1681Y PK after second daily administration DF 1681B:_100, 200, 300 and 400 mg/kg b.i.d (corresponding to 66, 132, 168 and 198 mg/kg b.i.d as DF 1681Y) DF 1681Y: 100, 200, 300 and 400 mg/kg b.i.d	DF 1681B dose 100 300 200 400 Cmax (µg/mL) 70 150 115 192 tmax (h) 0.50 0.25 0.25 0.83 AUClast (µg·h/mL) 101 221 225 299 DF 1681Y dose 100 300 200 400 Cmax (µg/mL) 96 305 301 277 tmax (h) 0.25 0.25 0.25 0.25 AUClast (µg·h/mL) 116 442 453 586 rapidly absorbtion dose dependent PK AUC DF 1681Y > DF 1681B	C99443[2] (§ 5.3.3.1) A1019[1] (Analytical report) M1102[2] (PK report) (§ 6)
TK (plasma) rat/30 (15M + 15F): 5 per sex and dose group; 5 sampling time on day 1 and on day 14 after each dose The samples were taken after the first dose on D1 and after the last dose on D14	DF 1681Y 100, 200, 400 oral (gavage) bid (8h apart) for 14 days	**DF 1681Y** / **Male** / **Female** **Days** 1 14 1 14 **100 mg/kg** Cmax (µg/mL) 139 220 211 277 tmax (h) range 0.25 0.25-1.00 0.25 0.25 AUClast (µg·h/mL) 221 301 398 413 t½ (h) 1.6 1.2 2.8 2.1 **200 mg/kg** Cmax (µg/mL) 240 337 351 369 tmax (h) 0.25-1.00 0.25-1.00 0.25 0.25 AUClast (µg·h/mL) 610 657 1020 877 t½ (h) 2.2 1.3 3.0 2.1 **400 mg/kg** Cmax (µg/mL) 293 331 561 400 tmax (h) 0.25-1.00 0.25-1.00 0.25-1.00 0.25-2.00 AUClast (µg·h/mL) 977 892 1990 1270 t½ (h) 2.2 1.9 -- 1.9 Day1: AUC increase more than in direct proportion Day14: AUC increase less than in direct proportion No accumulation . gender difference: AUC females > males.	D01208[1] (§ 5.3.3.1) A1126BPL[1] (Analytical report) (§7)

Nonclinical Pharmacokinetic and Metabolism Studies

<table>
<tr><th>Type of Study (Species / No. of animals / sex)</th><th>Dose (mg/kg/day)*/ Route/Frequency</th><th>Comments</th><th>Reference</th></tr>
<tr><td>TK (plasma)
Rat: 6M+ 6F for each treated groups; 3M + 3F for the control group. Total 42 TK animals.

The blood samples for treated animals were taken on day 1 and on day 93 after the first administration in the day at the following time points: 0.25, 0.5, 1, 2, 4, 8 hrs</td><td>DF1681Y:

100, 200, 400 oral (gavage) bid (8h apart) for 13 weeks</td><td>A summary of the toxicokinetic parameters of DF1681Y is given in the following table:
<table>
<tr><th></th><th>Parameter</th><th>Units</th><th>100 mg/kg/day bid
Male</th><th>Female</th><th>200 mg/kg/day bid
Male</th><th>Female</th><th>400 mg/kg/day bid
Male</th><th>Female</th></tr>
<tr><td rowspan="4">Day 1</td><td>Cmax</td><td>µg/mL</td><td>198.53</td><td>366.80</td><td>367.90</td><td>504.76</td><td>503.41</td><td>562.90</td></tr>
<tr><td>tmax</td><td>h</td><td>0.3</td><td>0.3</td><td>0.3</td><td>0.5</td><td>0.3</td><td>0.5</td></tr>
<tr><td>AUC0-t</td><td>µg·h/mL</td><td>371.15</td><td>519.18</td><td>869.06</td><td>1093.75</td><td>2008.94</td><td>2309.74</td></tr>
<tr><td>t1/2 app</td><td>h</td><td>-</td><td>1.8</td><td>1.5</td><td>2.8</td><td>-</td><td>-</td></tr>
<tr><td rowspan="4">Day 93</td><td>Cmax</td><td>µg/mL</td><td>220.38</td><td>256.68</td><td>335.25</td><td>441.25</td><td>471.16</td><td>699.79</td></tr>
<tr><td>tmax</td><td>h</td><td>0.3</td><td>0.5</td><td>0.5</td><td>0.5</td><td>0.5</td><td>0.5</td></tr>
<tr><td>AUC0-t</td><td>µg·h/mL</td><td>275.98</td><td>499.93</td><td>536.86</td><td>771.45</td><td>1472.06</td><td>2610.04</td></tr>
<tr><td>t1/2 app</td><td>h</td><td>1.8</td><td>-</td><td>1.9</td><td>-</td><td>3.4</td><td>2.8</td></tr>
</table>
Time to peak plasma level ranged from 0.3 to 0.5 h for the parent compound and from 0.5 to 8 h post administration for the metabolite DF1674Y at all dose levels and for both sexes.
The duration of treatment did not induce accumulation of the parent compound DF1681Y or its metabolite DF1674Y in both sexes</td><td>Harlan Laboratories study D55388; Sponsor reference no. A1232 BPL/E (§65)</td></tr>
<tr><td>TK (plasma)
dog/2M+2F</td><td>30 / i.v./ bolus every 24h for 14 days</td><td>Mean reparixin plasma concentration (µg/mL)
5min 4h 24h (after administration)
Day 1: 175.03 108.53 18.94
Day 14: 170.06 111.99 22.87

No differences between day 1 and day 14</td><td>RBMR14500[1] (§ 5.3.3.2)
A0113[1] (Analytical report) (§ 9)</td></tr>
<tr><td colspan="4">Pharmacokinetics after continuous infusion</td></tr>
<tr><td>TK (plasma)
rat/6 per dose: 1 M + 1 F each sampling time</td><td>4, 8, 16, 32, 64.1, 128.1, 256.2, 512.6, 1025 / i.v. / continuous infusion for 8h</td><td>Plasma concentrations at 8h: 0.59, 0.99, 1.77, 7.74, 24.16, 1.18, 71.85, 263.15, 222.78 µg/mL.

linear PK up to 256.2 mg/kg/day.</td><td>RTC7780[1] (§ 5.3.2.2)

A0009[1] (Analytical report) (§ 10)</td></tr>
</table>

Nonclinical Pharmacokinetic and Metabolism Studies			
Type of Study (Species / No. of animals / sex)	**Dose (mg/kg/day)*/ Route/Frequency**	**Comments**	**Reference**
TK (plasma). rat 3M+3F	DF 1681B: 100, 300 and 800 mg/kg continuous infusion for 14 days /	At the end of administration period plasma levels of reparixin ranged between 79.99 and 175.95 µg/mL. Since in some animals (1 rat at 100 mg/kg/day and 4 rats at 300 mg/kg/day) plasma concentrations of reparixin were found at predose, pharmacokinetic analysis was not performed	RTC8226[1] (§5.3.3.1) A0147[2] (§ 11)
TK (plasma) Sprague Dawley rat/12 per dose: 3M+3F each sampling time per 7 time points	100, 300, 900 / i.v./ continuous infusion for 28 days	Cmax= 12.27, 59.34, 202.94 µg/mL at 24 h after infusion t½ elim. = 1.14, 3.07, 1.33 h AUCtot = 7067, 34917, 116178 µg·h /mL CL = 396, 241, 217 mL/h/kg Cmax slightly higher than C672h. PK dose independent ibuprofen detectable only in some plasma samples after 100 mg/kg/day detectable up to 3h - 5h after end of infusion of 300 and 900 mg/kg/day.	RTC8594[1] (§ 5.3.3.1) A0124BPL[1] (Analytical and Addendum 2 PK report) A0207[2] (Analytical and PK report ibuprofen) (§ 12)
TK (plasma) CD rat/12 per dose: 3M+3F each sampling time per 6 time points.	300, 500, 1000 / i.v./ continuous infusion for 28 days	Male / Female C24h: 6.58, 13.95, 56.6 / 23.02, 27.41, 59.43 µg/mL C672h 4.87, 33.37, 85.37 / 34.10, 43.98, 96.54 µg/mL AUCtot 3892, 15834, 47419 / 19112, 24047, 52500 µg·h/mL t½ elim. 1.28, 1.47, 1.15 / 1.64, 1.45, 1.27 h CL 2567, 623, 488 / 473, 367, 432 mL/h/kg Vz 4727 , 1318, 808 / 867, 987, 793 mL/kg Cmax slightly higher than C672h. PK time and dose independent	DOM056[1] (§ 5.3.3.1) A0304BPL[1] (Analytical and PK report) (§ 13)
TK (plasma) dog/1M+1F	0.4, 1.2, 3.6, 10.8, 32.4, 97.2, 194.4, 388.8 / i.v. / continuous infusion for 8h	t½ elim. = 27, 24, 28, 21, 20, 9, 8, 5 h AUCtot = 39, 81, 377, 981, 1703, 2232, 2529, 2631 h µg/mL PK dose independent up to 10.8 mg/kg/day t½ decrease with the dose	RTC7781[1] (§ 5.3.2.3) A0010[1] (Analytical report) (§ 14)
TK (plasma) DOM/046: dog/2M+2F DOM/048: dog/1M+1F	DOM046: continuous i.v. infusion for 72h Phase 1: 15 mg/kg/day Phase 2.: 30 mg/kg/day Phase 3: 10 mg/kg/day DOM048: continuous i.v. infusion for 72h 10 mg/kg/day continuous	Reparixin concentrations in plasma are consistent with those obtained in the study RTC7781. The presence of ibuprofen (reparixin metabolite) is confirmed also in this study.	DOM046[1] DOM048[1] (§ 5.3.3.2) A0149BPL[1] (Analytical and PK report) A0207[2] (Analytical report ibuprofen) (§ 15)
TK (plasma) dog/3M+3F per dose	15, 30, 60 / i.v. / continuous infusion: 14 days for males 15 days for females	Male (14 days) / Female (15 days) Cmax= 59, 93, 106 / 74, 92, 119 µg/mL t½ = 24, 23, 19 / 20, 22, 24 h AUCtot = 18925, 26794, 32936 / 23601, 30563, 40255µg·h /mL CL = 11.24, 15.69, 25.58 / 9.56, 14.81, 22.59 mL/h/kg Vz = 389, 511, 714 / 279, 481, 765 mL/kg slowly cleared from plasma after the end of infusion AUC: increase less than in direct proportion with dose AUC female slight > male Steady state reached after 48 h of infusion Ibuprofen t½ ranging from 4.78 to 11.61h. AUCtot ibuprofen/reparixin: 3-5% of reparixin. (results confirm those found in the previous study RTC8850).	DOM047[1] (§ 5.3.3.2) A0206BPL[1] (Analytical and PK report) A0207[2] (Analytical report ibuprofen) (§ 16)

Nonclinical Pharmacokinetic and Metabolism Studies

Type of Study (Species / No. of animals / sex)	Dose (mg/kg/day)*/ Route/Frequency	Comments	Reference
TK (plasma) dog/3M+3F	50, 100, 200, 400 / i.v. / continuous infusion for 14 days	Cmax= 92.16, 134.80, 175.64, 256.66# µg/mL Tmax = 148.1, 40.0, 24.0, 24.0# h t½ elim. = 12.75, 11.11, 20.93, 9.30# h AUCtot = 27707, 38379, 40241, 40289# µg·h /mL CL = 25.38, 36.78, 70.96, 139.00# mL/h/kg Vz = 460, 598, 2143, 1866# mL/kg (# = only 1 dog) steady state after 50 mg/kg/day: reached after 48h of infusion from 100 onwards: plasma levels decreased from day 1 to day 14. Ibuprofen t½ ranged 5.49-15.66 h AUCtot ibuprofen/reparixin: 4-5% of reparixin. In dog 25F after 400 mg/kg/day the ibuprofen AUCtot ratio accounted for 7.75%.	RTC8850[1] (§ 5.3.3.2) A0125BPL[1] (Analytical and Addendum 2 PK report) A0207[2] (Analytical and - PK report Ibuprofen) (§ 17)
Distribution			
PK *In vitro* protein binding	[^{14}C]reparixin L-lysine salt concentrations: 1, 50, 250, 500, 1000 µg/mL (0.66, 32.98, 164.92, 329.85, 659.69 µg/mL as reparixin)	Protein binding [^{14}C]reparixin L-lysine salt 1 - 1000 (µg/mL) Rat plasma 98.9% - 88.2% Dog plasma 99.2% - 89.0% Rabbit plasma 99.3% - 94.7% Cynomolgus plasma 99.1% - 93.6% Human plasma 99.4% - 93.3% Human serum albumin 99.2% - 85.2% Human α_1-acid glycoprotein 3.9% - 4.0%	DOM/058[1] (§ 19)
PK (distribution, metabolism and excretion – [14C] DF 1681B PK: Sprague-Dawley rats : 60M +60F (3M+3F) for each time point) between 5min and 168h after dosing Distribution: Albino 5M + 5F (1M+1F each time point) Pregnant Sprague-Dawley rats: 3F Lister Hooded strain (partially pigmented: 6M (1 each time point) Recovery: 3M+3F Excretion balance: 3M+3F Biliary excretion: 3M+3F HPLC of urine and bile samples (before and after treatment with a β-glucuronidase/sulphatase mixed enzyme preparation) and extracts of faeces samples	45 / i.v. / single dose	**Radioactivity plasma:** / **Male** / **Female** C_0 (µg eq/mL) 229 332 AUCtot (µg·h /mL) 163 187 t½ elim. (h) 22.5 37.8 **DF 1681Y plasma:** C_0 (µg eq/mL) 219 285 AUCtot (µg·h /mL) 77 131 t½ elim. h 0.4 0.5 Ae168 h (%) 82.46 81.72 Faeces 168 h (%) 11.21 12.55 Biliary excretion (%) 22.50 23.79 concentration of radioactivity in all tissues < plasma greatest concentrations (other than those in plasma) in liver smallest in CNS. radioactivity detectable up to 24h (few tissues) pigmented rats: no evidence of persistence in melanin containing tissues. pregnant rats: radioactivity rapidly crossed the placenta, distribution in all foetal tissues (no foetal brain)< maternal tissue; in foetuses no radioactivity detectable 24h after dosing. Cp>Cb estensive metabolism: oxidation of the isobutyl sidechain to hydroxylation products (DF 2188Y and DF 2260Y) and the carboxylic acid (DF 2243Y) of DF 2260Y plus further metabolism of each of these to form glucuronide or sulphate conjugates.	DOM057[1] (§ 20)

<table>
<tr><th colspan="4">Nonclinical Pharmacokinetic and Metabolism Studies</th></tr>
<tr><th>Type of Study (Species / No. of animals / sex)</th><th>Dose (mg/kg/day)*/ Route/Frequency</th><th>Comments</th><th>Reference</th></tr>
<tr><td colspan="4">Metabolism</td></tr>
<tr><td>MET in vitro
rat, dog, human hepatocytes</td><td>incubastion with reparixin L-lysine Tested concentrations 10, 100 µM after 4h incubation</td><td>10 µM: disappearance of 100% in rats, 84% in humans, and less than 5% in dogs (after 4h incubation)
100 µM: disappearance of 72% in rats, 37% in humans, and 17% in dogs (after 4h incubation)

rat metabolism seems to be more similar to the human, considering the type of metabolites detected, but the rate of metabolites formation is much higher in the rat.
Qualitative analysis : ibuprofen (human and dogs, only after incubation of 100 µM), OH-reparixin and carboxy-reparixin (human and rat).</td><td>MDS PS 003271[2]
(§ 21)</td></tr>
<tr><td>MET in vitro
human microsomial metabolism CYP1A1/2, CYP2C9, CYP2C19, CYP2DP6, CYP2E1, CYP3A4.</td><td>Tested concentrations 25, 250, 500, 1000, 5000 µM</td><td>Michaelis-Menten kinetic parameters for the metabolism of [14C]reparixin L-lysine salt:
Km of 27.0 µM and
Vmax of 2024 pmoles/min/mg protein for the consumption of [14C]reparixin L-lysine salt.
Phase 1 metabolism is catalysed by CYP2C9 and to a lesser extent by CYP2C19.
Two major metabolite fractions, DF 2188Y and DF 2260Y were produced.
DF 2188Y formation appears to be principally catalysed by CYP2C9, with a lesser contribution by CYP2C19.
DF 2260Y formation appears to be essentially catalysed solely by CYP2C9.</td><td>DOM062/ 034034[1]
(§ 22)</td></tr>
<tr><td>PK (metabolism and excretion – [^{14}C] labelled compound) dog (3M+3F)

HPLC of urine samples (before and after treatment with a β-glucuronidase/sulphatase mixed enzyme preparation) and extracts of faeces samples</td><td>45 / i.v. / single dose</td><td>
<table>
<tr><th>Radioactivity plasma:</th><th>Male</th><th>Female</th></tr>
<tr><td>C_0 (µg eq/mL)</td><td>251</td><td>266</td></tr>
<tr><td>AUCtot (µg·h /mL)</td><td>2963</td><td>3337</td></tr>
<tr><td>t½ elim. # (h)</td><td>61.5</td><td>36.5</td></tr>
<tr><td>Reparixin plasma:</td><td></td><td></td></tr>
<tr><td>C_0 (µg eq/mL)</td><td>255</td><td>270</td></tr>
<tr><td>AUCtot (µg·h /mL)</td><td>2610</td><td>2910</td></tr>
<tr><td>t½ elim. (h)</td><td>9.9</td><td>12.3</td></tr>
<tr><td>Ae168 h (%)</td><td>80.11</td><td>81.61</td></tr>
<tr><td>Faeces 168 h (%)</td><td>9</td><td>11</td></tr>
<tr><td>DF 2243Y plasma:</td><td></td><td></td></tr>
<tr><td>C_{max} (ng eq/mL)</td><td>197</td><td>281</td></tr>
<tr><td>t_{max} (h)</td><td>3</td><td>2</td></tr>
<tr><td>AUCtot (ng·h /mL)</td><td>1360</td><td>1860</td></tr>
<tr><td>t½ elim. (h)</td><td>5.7</td><td>5.0</td></tr>
<tr><td>DF 2188Y plasma:</td><td></td><td></td></tr>
<tr><td>C_{max} (ng eq/mL)</td><td>554</td><td>739</td></tr>
<tr><td>t_{max} (h)</td><td>3</td><td>3</td></tr>
<tr><td>AUCtot (ng·h /mL)</td><td>4840</td><td>6570</td></tr>
<tr><td>t½ elim. (h)</td><td>4.4</td><td>4.3</td></tr>
</table>
extensive metabolism prior to excretion:
oxidation of the isobutyl sidechain, DF 2260Y (formed by hydroxylation of a terminal carbon of the isobutyl sidechain). This was, however, a minor metabolite found only in urine. The greatest amounts of radioactivity (accounting for ca 50% of the dose) eluted in the void volume of the column, a portion of which (in urine), was confirmed as methanesulfonamide by TLC.
data to be interpreted with caution</td><td>DOM 067/034028[1]
(§ 23)</td></tr>
<tr><td>MET in vivo
plasma samples from: RTC7780, RTC7781 studies</td><td>Plasma samples at 8 h (end of i.v. infusion):
D OG: 388.8 mg/kg/day
RAT: 256.2 mg/kg/day

structural identification of dog and rat metabolites was performed by HPLC/MS analysis</td><td>In dog plasma, 10 metabolites were found: I, II, III=OH-DF 1681; IV=carboxy-DF 1681; V=ibuprofen; VI=OH-ibuprofen; VII=carboxy-ibuprofen; VIII=ibuprofen glucuronate; X=alanylamide-ibuprofen;
XI= methanesulfonamide (theoretical metabolite)
In rat plasma, 7 different metabolites were found: I, II, III=OH-DF 1681; V=ibuprofen; VI=OH-ibuprofen; IX=dehydrated of II and/or III;
XI= methanesulfonamide (theoretical metabolite)</td><td>A0123[2]
(§ 24)</td></tr>
</table>

Nonclinical Pharmacokinetic and Metabolism Studies			
Type of Study (Species / No. of animals / sex)	**Dose (mg/kg/day)*/ Route/Frequency**	**Comments**	**Reference**
MET *in vivo* human urine samples from: Phase 1 clinical study REP0101 and rat urine samples from RTC7780 study	Analysis of metabolites was performed by reverse phase HPLC/MS analysis on urine samples after β-glucuronidase digestion	13 metabolites in human urine 8 metabolites in rat urine (all metabolites detected in rats, except for DF 2239Y, also detected in humans). 6 out of 13 metabolites detected in humans not detected in rats, (DF 2151Y, DF 2196Y, DF 2233Y, dehydro-hydroxy-reparixin, di-hydroxy-ibuprofen and ibuprofen. DF 2188Y and DF 2243Y: major metabolites found in rat and human urine. Di-hydroxy-reparixin and DF 2260Y third and fourth most important metabolites in human urine, In rats, di-hydroxy-reparixin higher than in human DF 2260Y very low levels.	A0303, REP0101 (ME0706) (§ 25)
TK (plasma) rat/10 per dose: 5M+5F.	Dose of DF 2243Y 0, 600, 900 / i.v./ continuous infusion for 14 days	Plasma samples were collected during the toxicity study at 1, 8, and 14 days. Mean plasma levels for 600 and 900 mg/kg/day were: Male / Female Day 1 36.6, 54.9 / 38.2, 58.2 µgh/mL Day 8 39.0, 63.0 / 39.9, 56.0 µgh/mL Day 14 42.4, 51.8 / 51.7, 62.2 µgh/mL AUC0-day 14 12656, 18828 / 13627, 18804 µg·h/mL	MDS AA17669[1] (§ 5.3.3.1) DOM073/ 042577[1] (Analytical report) (§ 26)
Drug-drug interaction			
MET *in vitro* CYP1A1/2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4	Tested concentrations 25, 250, 500, 1000, 5000 µM	no inhibition on CYP1A1/2 (IC_{50} = >5000 µM). Slight inhibition on CYP2D6 (IC_{50} = 2697µM) and CYP2E1 (IC_{50} = 1523 µM) unlikely to cause clinically relevant drug interactions. inhibition of CYP3A4 (IC_{50} = 8 µM), CYP2C9 (IC_{50} = 79 µM) and to a minor extent of CYP2C19 (IC_{50} =868 µM).	IPAS-MET-014-01[1] (§ 27)
INT *in vitro* microsomial metabolism	human liver microsomal proteins (2 mg/mL) and reparixin at 0.08, 0.8, 2.4, 8, 24, 80, 800 µM incubated in presence of cyclosporine A or sirolimus at 1, 3, 10, 30 and 100 µM.	un-competitive inhibition for cyclosporin A and sirolimus (.results not unequivocably because of a relatively large variability) K_i : cyclosporin A= 97,024 µM; sirolimus= 426 µM no effect on tacrolimus metabolism. Since the free plasma concentration of reparixin at steady state in human is approximately 0.1 µM, then the C_{max}/K_i ratio << 0.1 and thus it is predicted that the clinical relevance of any inhibition of cyclosporin A or sirolimus metabolism by reparixin is remote. However, given the variability in the data obtained, these conclusions must be regarded as being tentative.	DOM071/ 040089[2] (§ 28)
in vitro P-glycoprotein inhibition TC7 (a sub-clone of the Caco-2 cell line)	reparixin L-lysine salt and DF 2243Y at 0.1, 5, 25, 50µM tested in the absence and presence of Cyclosporine A and rapamycin: 10 µM Single concentration	Cerep 8089: reparixin and DF 2243Y: no influence on P-gp activity, reduction of P-gp inhibition exerted by cyclosporine A and rapamycin (not concentration related) (error in experimental procedure) Cerep 8543 (repeated in triplicate): reparixin and DF 2243Y: no influence on the ability of cyclosporine A or rapamycin to inhibit P-gp.(results are reliable and correct)	Cerep 8089[2] Cerep 8543[2] (§ 29)
P-glycoprotein inhibition TC7 (a sub-clone of the Caco-2 cell line)	reparixin L-lysine salt and DF 2243Y at 0.1, 5, 25 µM tested in the absence and presence of tacrolimus (FK506) 10 µM cyclosporine A Single concentration	• cyclosporine A and tacrolimus: strong P-gp inhibitors. • reparixin L-lysine salt and DF 2243Y: no influence on P-gp activity, no interference with the P-gp also in presence of tacrolimus.	Cerep 8366[1] (§ 30)

Nonclinical Pharmacokinetic and Metabolism Studies			
Type of Study (Species / No. of animals / sex)	**Dose (mg/kg/day)*/ Route/Frequency**	**Comments**	**Reference**
P-glycoprotein inhibition TC7 (a sub-clone of the Caco-2 cell line)	reparixin L-lysine salt 0.1, 5, 25 µM tested in the absence and presence of Cyclosporine A (2 and 0.2 µM) FK506 (Tacrolimus) (10 and 0.03 µM) Single concentration	• tacrolimus : strong P-gp inhibitors at 10µM • reparixin L-lysine salt no influence on P-gp activity, no synergic interaction in presence of cyclosporine A or tacrolimus • Cyclosporine A lower inhibition of the P-pg as per tacrolimus at 0.03 µM.	Cerep 9030[1] (§ 31)
P-glycoprotein Apical to basal (A-B) permeability and Basal to apical (B-A) permeability TC7 (a sub-clone of the Caco-2 cell line)	reparixin L-lysine salt 0.1, 1, and 10 µM Cyclosporine A 10 µM Single concentration	At the concentrations of 0.1 and 1 µM in presence or absence of cyclosporine, reparixin was not detected. The data at 10 µM indicate that the addition of CSA did not affect both the A-B and B-A permeability values. reparixin 10µM A-B permeability (10^{-6} cm/s): 31.9 B-A permeability (10^{-6} cm/s): 19.8 reparixin 10µM with cyclosporine A 10µM A-B permeability (10^{-6} cm/s): 28.5 B-A permeability (10^{-6} cm/s): 19.8 reparixin not effluxed (Efflux ratio = 0.62),, therefore no substrate for P-gp.	Cerep 8973[1] (§ 32)
Effect of reparixin L-lysine salt and metabolites DF 2243Y and DF 2188Y on P-glycoprotein activity *in vitro*	reparixin L-lysine salt, DF 2243Y and DF 2188Y 10 nM-10 □M Single concentration Membrane vesicles preparation (commercial assay kit) vinblastine, verapamil and progesterone positive control	reparixin, DF 2243Y and DF 2188Y: no influence on basal ATPase activity of P-glycoprotein. vinblastine, verapamil and progesterone:influenced P-gp	A0334[2] (§ 33)

i.v. = intravenous**, p.o.** = per oral, **PK** = pharmacokinetics, **TK** = toxicokinetics, **MET** = metabolism, **INT** = interaction **M** = male, **F** = female; [1] = GLP; [2] = non-GPL; * = dose as reparixin L-lysine salt (DF 1681B), **MTD** = maximum tolerated dose; **Cp**: concentration in plasma; Cb: concentration in blood

5.2.2. Methods of Analysis

Two HPLC analytical methods were applied to determine reparixin and DF 1672Y (enantiomeric form of reparixin) in rat [1] and dog [2] plasma samples. The two methods were linear, accurate and precise to assay concentrations between approximately the LOQ and 360 µg/mL; the quantitation limit of both the enantiomers was 1.2 µg/mL.

The concentrations of one of reparixin metabolites, ibuprofen, were determined by HPLC method with UV detection. DF 2243Y was determined using a validated LC-MS/MS method [26].

5.2.3. Pharmacokinetics after single dose

The pharmacokinetics of reparixin L-lysine salt was investigated after i.v. or oral administration in rats [3, 4], and just after i.v. route in dogs [5].

5.2.3.1. RAT

The bioavailability of reparixin, evaluated in one study where the compound was given at 15 and 30 mg/kg by oral route and 15 mg/kg by i.v. route, ranged from 84% to 120% [3].

After oral administration reparixin was rapidly absorbed, since the maximal concentration reached 10 min after administration, and then rapidly eliminated from plasma with a terminal $t_{1/2}$ lower than 2 h. After i.v. administration, the compound was rapidly eliminated from plasma ($t_{½}$ about 1 h) and poorly distributed [4]. The clearance was very low. The relationship of the administered dose to the plasma AUCs was found to be dose proportional. The pharmacokinetic parameters obtained in the two studies, after oral and i.v. administration [4] are reported in Table 1.

Table 1: Reparixin main pharmacokinetic parameters in rats after single oral and i.v. administration of 5, 15, and 45 mg/kg of reparixn L-lysine salt.

Study (ref)		A0109 (§ 3)[1]			RBMR12110 (§4) [2]		
Route		oral		iv	iv		
Dose	**mg/kg**	15	30	15	**5**	**15**	**45**
Parameter							
Cmax	µg/mL	16.86 ± 7.02	63.94 ± 25.78				
Tmax	min	10.00 ± 0.00	10.00 ± 0.00				
C_{5min}	µg/mL						
AUCtot	µg·min /mL	1034 ± 121	2945 ± 614	1224 ± 326	11	43	134
AUCtot	h µg/mL				13	47	144
t1/2	min	107.07 ± 87.34	51.36 ± 10.95	49.45 ± 12.73			
t1/2	h				0.6	1.1	1.0
CL	mL/h/kg				260	213	206
MRT	h				0.7	0.8	0.9
Vz	mL/kg			620 ± 246	233	342	297
Vss	mL/kg				188	180	188
Ae (0-48h)	% dose				1.3	0.4	0.3

[1]: mean ±SD are reported [2]: mean of males and females

5.2.3.2. DOG

The pharmacokinetic parameters obtained after i.v. dose in male and female dogs (mean values) [5] are reported in Table 2. Reparixin is slowly eliminated from plasma. The dose normalised AUCs decreased with increasing dose in both gender. There was no overall statistically significant gender difference. The non-linear cyclic natures of these elimination profiles suggested an enterohepatic recirculation.

Table 2: Reparixin pharmacokinetic parameters after single i.v. administration of 1, 10, 50 mg/kg of reparixin L-lysine salt in male and female dogs.

		1 mg/kg		10 mg/kg		50 mg/kg	
PK parameters		***mean***	***SD***	***mean***	***SD***	***mean***	***SD***
t_{last}	(hours)	**27***	-	**48***	-	**72***	-
Clast	(µg/mL)	**1.3**	0.3	**2.4**	1.2	**3.2**	1.5
C_{5min}	(µg/mL)	**8.1**	0.8	**66.8**	4.1	**181.8**	20.8
AUClast	(h µg/mL)	**71.3**	6.1	**754.9**	122.0	**1907.8**	325.3
AUCtot	(h µg/mL)	**119.7**	39.6	**801.4**	150.4	**2048.1**	403.4
T1/2	(hours)	**26.8**	18.1	**12.6**	2.4	**28.2**	5.6
CL	(mL/h/kg)	**6.0**	1.9	**8.5**	1.6	**16.6**	3.3
Vz	(mL/kg)	**193.3**	81.4	**149.3**	10.0	**663.2**	107.0
Vss	(mL/kg)	**164.2**	62.1	**131.3**	5.3	**316.7**	39.6
* Median values; C_{5min} = first sampling time							

5.2.4. Pharmacokinetics after repeated administration (toxicokinetics)

The pharmacokinetics of reparixin were investigated in rats after oral bid administration of DF 1681B (reparixin L-lysine salt) and DF 1681Y (reparixin) for a single day [6] and after DF 1681Y for 14 days [7]. The pharmacokinetics of reparixin was investigated in dogs after i.v. administration of DF 1681B given once a day for 14 days [9].

5.2.4.1. RAT

Pharmacokinetics of DF 1681B and DF 1681Y were evaluated in rats after oral bid administration for one day at 100, 200, 300, and 400 mg/kg [6].

Reparixin is rapidly absorbed over the range of doses studied both after administration of DF 1681B or DF 1681Y. The systemic exposure expressed as AUClast after administration of DF 1681B and DF 1681Y appeared not to be dose-proportional. DF 1681Y showed a higher relative bioavailability (AUClast with Frel ranging from 132% to 129%) at all tested doses except for the 100 mg/kg dose where the DF 1681B seems to have a higher bioavailability in comparison to DF 1681Y (Frel: 61%). The main PK parameters are reported in Table 3.

Table 3: Reparixin mean pharmacokinetics parameters in rats after oral gavage administration of DF 1681B (reparixin L-lysine salt correspond to 66, 132, 168 and 198 mg/kg b.i.d. of DF 1681Y) and DF 1681Y.

DF 1681B Group 1	**Rat 1-3**		**Rat 4-6**	
	D1	**D7**	**D4**	**D10**
	100	**300**	**200**	**400**
Cmax (µg/mL)	70	150	115	192
tmax (h)	0.50	0.25	0.25	0.83
AUClast (µg ·h/mL)	101	221	225	299
DF 1681Y Group 2	**Rat 7-9**		**Rat 10-12**	
	D1	**D7**	**D4**	**D10**
	100	**300**	**200**	**400**
Cmax (µg/mL)	96	305	301	277
tmax (h)	0.25	0.25	0.25	0.25
AUClast (µg ·h/mL)	116	442	453	586

In the 14 days study, DF 1681B was administered bid by oral gavage (8 hrs apart) at 100, 200 and 400 mg/kg [7]. Pharmacokinetic results are reported in Table 4. At day 1, reparixin increased more than in direct proportion with the dose, whereas on day 14, the exposure increase was slightly less than dose-proportional. After 14-day repeated administration, exposure to DF1681Y tended to be stable compared to exposure on day 1, and no accumulation was observed under these dosage regimens.
A slight gender difference, especially on day 1, was observed, since DF1681Y exposure in females was higher than the corresponding exposures in males.

Table 4: Reparixin mean pharmacokinetics parameters in rats after oral gavage administration of DF 1681Y

DF 1681Y PK PARAMETERS (mean±SD)			Day 1			Day 14		
	Dose	mg/kg bi.d.	100	200	400	100	200	400
Male	Cmax	(µg/mL)	139±50	240±55	293±62	220±51	337±39	331±18
	tmax (range)	h	0.25	0.25-1.00	0.25-1.00	0.25-1.00	0.25-1.00	0.25-1.00
	AUC_{0-8h}	h µg/mL	221±73	610±124	977±219	301±28	657±56	892±149
	t½	h	1.6±0.32	2.2±0.74	2.2	1.2±0.36	1.3±0.35	1.9±0.66
Female	Cmax	µg/mL	211±41	351±25	561±192	277±58	369±35	400±71
	tmax (range)	h	0.25	0.25	0.25-1.00	0.25	0.25	0.25-2.00
	AUC_{0-8h}	(h µg/mL)	398±54	1020±72	1990±240	413±64	877±133	1270±263
	t½	h	2.8±0.3	3.0±1.5	--	2.1±0.5	2.1±0.4	1.9±0.6

In the 13 weeks study, DF 1681Y was also administered bid by oral gavage (8 hrs apart) at 100, 200 and 400 mg/kg [8].Pharmacokinetic results are reported in 5. Similar to the 14 days oral administration, DF1681Y exposure in females was higher than in males with no apparent accumulation between day 1 and day 93. Time to Tmax was higher in females than in males (0.5 versus 0.3 hr).

Table 5: Reparixin mean pharmacokinetics parameters in rats after oral gavage administration of DF 1681Y for 13 weeks

			100 mg/kg/day bid		200 mg/kg/day bid		400 mg/kg/day bid	
	Parameter	***Units***	Male	Female	Male	Female	Male	Female
Day 1	C_{max}	*µg/mL*	198.53	366.80	367.90	504.76	503.41	562.90
	t_{max}	*h*	0.3	0.3	0.3	0.5	0.3	0.5
	AUC_{0-t}	*µg·h/mL*	371.15	519.18	869.06	1093.75	2008.94	2309.74
	$t_{1/2\ app}$	*h*	-	1.8	1.5	2.8	-	-
Day 93	C_{max}	*µg/mL*	220.38	256.68	335.25	441.25	471.16	699.79
	t_{max}	*h*	0.3	0.5	0.5	0.5	0.5	0.5
	AUC_{0-t}	*µg·h/mL*	275.98	499.93	536.86	771.45	1472.06	2610.04
	$t_{1/2\ app}$	*h*	1.8	-	1.9	-	3.4	2.8

5.2.4.2. DOG

Pharmacokinetics of DF 1681B were investigated in dogs after repeated bolus i.v. administration of reparixin L-lysine salt at 30 mg/kg/day, once a day for 14 days [9].

In Table 6 the individual and mean plasma levels of reparixin are reported.

Table 6: Reparixin plasma levels (µg/mL) (mean±SD, n=4) in dogs after repeated i.v. administration of reparixin L-lysine salt 30 mg/kg/day.

Time (h)	DAY 1		DAY 14	
	Mean	SD	Mean	SD
0.083	175.03	14.64	170.06	14.43
4	108.83	15.12	111.99	15.34
24	18.94	3.35	22.87	9.16

The results suggest that no accumulation or induction phenomena occurred in dogs after i.v. repeated bolus administration.

5.2.5. Pharmacokinetics after intravenous infusion (toxicokinetics)

Pharmacokinetics of DF 1681B were investigated in rats after 8 h [10] 14 days [11] and 28 days of infusion [12, 13] and in dogs after 8h [14], 72 h [15] and 14 days [16, 16].

5.2.5.1. RAT

During a preliminary infusion toxicity study, DF 1681B was administered at doses between 4-1025 mg/kg/day for 8 h infusion [**10**]. Plasma samples, collected at 140, 480, 680 min after starting 8h i.v. infusion suggested that the increase of reparixin concentration is linear up to 256.2 mg/kg/day (Table).

Table 7: Reparixin plasma levels in rats during i.v. administration of reparixin L-lysine salt 4-1025 mg/kg/day (8 h infusion; n =2; 1M+1F each time point).

	Mean plasma concentration (µg/mL)								
Dose (mg/kg/day)	**4**	**8**	**16**	**32.4**	**64.1**	**128.1**	**256.2**	**512.6**	**1025**
Time (min)									
140	0.38	1.63	3.88	3.90	8.33	81.26	24.71	94.16	219.43
480	0.59	0.99	1.77	7.74	24.16	71.85	1.18	263.15	222.78
660	0.00	0.20	0.00	0.32	0.34	3.43	0.99	56.46	83.74

During a toxicity study [11], plasma samples were collected from 3M and 3F after continuous infusion for 14 days of 100, 300 and 800 mg/kg of reparixin L-lysine salt. At the end of administration period plasma levels of reparixin ranged between 79.99 and 175.95 µg/mL.

DF 1681B was administered to Sprague Dawley (SD) for 28 days continuous infusion at the dose of 100, 300 and 900 mg/kg/day [12] and to CD rats at 300, 500 and 1000 mg/kg/day [13], during two toxicity studies.

Pharmacokinetic parameters obtained in the two studies are reported in Table [12] and Table 9

Table 9 [13], respectively.

After 100, 300 and 900 mg/kg/day, the concentrations of reparixin after 24h of infusion were slightly higher than those found at 672h.

In both studies, after the end of the infusion, reparixin was rapidly eliminated from plasma. After 100, 300 and 900 mg/kg/day, the AUCtot increased largely in direct proportion with the dose.

After 300, 500 and 1000 mg/kg/day, plasma disposition kinetics of reparixin was dose-linear in female rats over the whole range of tested doses (300-1000 mg/kg/day) and in male rats at the doses of 500-1000 mg/kg/day. In males, the drug plasma levels at the dose of 300 mg/kg/day were much lower than those observed in females. From the plasma level profiles at the first and last day of observation, it appears that there were no major time dependent changes in drug disposition, which could have suggested accumulation or induction phenomena. Finally, in both studies there was a tendency to higher plasma levels (probably lower drug clearance) in female rats compared to males.

Table 8: Mean pharmacokinetic parameters of reparixin after i.v. continuous 24 h infusion for 28 days of reparixin L-lysine salt in male and female SD rats. [12]

PARAMETERS		100 mg/kg/day (4.17 mg/kg/h)	300 mg/kg/day (12.51 mg/kg/h)	900 mg/kg/day (37.53 mg/kg/h)
Cmax	(µg/mL)	12.27	59.34	202.94
Tmax	h	24	24	24
C_{672h}	(µg/mL)	8.65	44.23	143.07
AUClast	(h µg/mL)	7065	34873	116163
AUCtot	(h µg/mL)	7067	34917	116178
t½	h	1.14	3.07	1.33
CL	(mL/h/kg)	396.229	240.569	216.909
Vz	(mL/kg)	653.25	1063.72	415.28

Table 9: Mean pharmacokinetic parameters of reparixin after i.v. continuous infusion for 28 days of 300, 500 and 1000 mg/kg/day of reparixin L-lysine salt in CD rats. [13].

PARAMETERS		300 mg/kg/day (12.5 mg/kg/h)		500 mg/kg/day (20.8 mg/kg/h)		1000 mg/kg/day (41.7 mg/kg/h)	
		M	F	M	F	M	F
C_{24h}	(µg/mL)	6.58	23.02	13.95	27.41	56.61	59.43
C_{672h}	(µg/mL)	4.87	34.10	33.37	43.98	85.37	96.54
AUClast	(h µg/mL)	3889	19107	15832	24043	47416	52495
AUCtot	(h µg/mL)	3892	19112	15834	24047	47419	52500
t½	h	1.28	1.64	1.47	1.45	1.15	1.27
CL	(mL/h/kg)	2567	367	623	473	488	432
Vz	(mL/kg)	4727	867	1318	987	808	793

The concentrations of one of reparixin metabolites, ibuprofen, were very low and detectable up to 3h/5h post infusion after 300 and 900 mg/kg/day [12] (Table 10).

Table 10: Mean plasma levels at Cmax, C_{24h}, C_{672h} and Clast of ibuprofen after continuous i.v. infusion of 100, 300 or 900 mg/kg/day reparixin L-lysine salt in male or female rats for 28 days.

PARAMETERS		100 mg/kg/day for 28 days	300 mg/kg/day for 28 days	900 mg/kg/day for 28 days
Cmax	(µg/mL)	0.38	0.44	2.56
Tmax	h	2	8	24
C_{24h}	(µg/mL)	0.04	0.38	2.56
C_{672h}	(µg/mL)	0.00	0.14	1.46
Clast	(µg/mL)	0.04	0.16	0.03
Tlast	h	24	673	678

5.2.5.2. DOG

After the administration of DF 1681B at doses ranging between 0.4-388.8 mg/kg/day for 8 h [14], AUClast and AUCtot appeared to be linear with the administered doses up to 10.8 mg/kg/day; afterwads AUC increased less than in direct proportion with the dose. Moreover, reparixin seems to be eliminated from plasma faster at the higher doses (97.2-388.8 mg/kg/day) than at the lower doses (up to 32.4 mg/kg/day) (Table 5).

Table 5: Reparixin pharmacokinetic parameters after 8h i.v. infusion of reparixin L-lysine salt in 1 male and 1 female dogs.

Dose mg/kg/day	n= 2	Cmax µg/mL	Tmax* h	AUClast h•µg/mL	AUCtot h•µg/mL	t½ h
0.4	**Mean**	1.00	0	14	39	26.99
1.2	**Mean**	3.82	0**	46	81	24.03**
3.6	**Mean**	11.05	0	117	377	28.18
10.8	**Mean**	35.20	0	373	981	21.30
32.4	**Mean**	68.06	0	708	1703	20.35
97.2	**Mean**	178.18	0	1540	2232	9.42
194.4	**Mean**	240.90	0	1899	2529	7.95
388.8	**Mean**	328.23	0	2316	2631	5.42
* = 0 corresponding to the end of infusion; ** = only dog 2M						

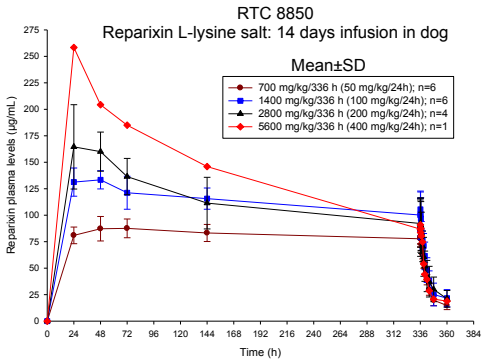
Reparixin concentrations in plasma are consistent with those obtained after 72h continuous infusion at the dose of 10, 15 and 30 mg/kg/day [15].

DF 1681B was administered to male and female dogs for 2 weeks continuous infusion at the dose of 15, 30 and 60 mg/kg/day [16] and 50, 100, 200 and 400 mg/kg/day [17] during two toxicity studies, respectively.

In the first study, reparixin Css were reached after 48h of infusion with the plasma levels remaining constant up to day 14-15, indicating no accumulation or induction phenomena up to 60 mg/kg/day.

After the administration of reparixin at 50, 100, 200 and 400 mg/kg/day, from the dose of 100 mg/kg/day onwards, plasma levels decreased from day 1 to day 14. An example of plasma profile is given in Figure 1.

Figure 1: Reparixin plasma levels in dogs after 24h infusion for 14 days of reparixin L-lysine salt at 50, 100, 200 and 400 mg/kg (3M+3F, at 400 mg only one animal survived). [17]



Small differences were observed between males and females, since plasma concentrations in females higher than in males up to 60 mg/kg/day (Table 6).

After 100 and 200 mg/kg/day plasma levels in male were slight higher than in female dogs (AUC0-360h ratios = 1.12 and 1.35 for the two dose regimens, respectively), while at 50 mg/kg/day the profiles were similar (AUC0-360h ratio = 0.98).No information on gender difference was available at 400 mg/kg/day since only females were treated at this dose level [16].

In both studies, it was apparent that reparixin AUCs increase less than in direct proportion with the dose.

Pharmacokinetic profiles obtained in the two studies are reported in Table 6.

Table 6: Pharmacokinetic parameters of reparixin (Mean ±SD) after i.v. continuous infusion of 15, 30 and 60 mg/kg/day of reparixin L-lysine salt for 14 days (male dogs) or 15 days (female dogs).

PK parameters after i.v. continuous infusion		Cmax (µg/mL)	Tmax (h)	AUClast (µg ·h/mL)	AUCtot (µg ·h/mL)	t½ (h)	CL (mL/ h/kg)	Vz (mL/kg)
Study A0206BPL [16] [1]								
15 mg/kg/day (0.625 mg/kg/h)	**Male (14 days)**	**58.79±** 4.47	**208±** 111	**18244±** 2361	**18925±** 2594	**24.21±** 4.70	**11.24±** 1.56	**388.93±** 64.93
15 mg/kg/day (0.625 mg/kg/h)	**Female (15 days)**	**74.30±** 6.90	**152±** 160	**22958±** 1527	**23601±** 1408	**20.27±** 3.70	**9.56±** 0.58	**279.27±** 51.22
30 mg/kg/day (1.25 mg/kg/h)	**Male (14 days)**	**92.60±** 5.15	**248±** 152	**25893±** 1044	**26794±** 940	**22.57±** 0.72	**15.69±** 0.55	**510.50±** 5.14
30 mg/kg/day (1.25 mg/kg/h) [2]	**Female (15 days)**	**91.58±** 12.18	**336±** 0.00	**29760±** 3397	**30563±** 3361	**22.37±** 2.23	**14.81±** 1.63	**480.66±** 100.17
30 mg/kg/day (1.25 mg/kg/h)	**Female (14 days)**	**106.34**	**336**	**31934**	**33199**	**25.75**	**12.65**	**469.99**
60 mg/kg/day (2.5 mg/kg/h)	**Male (14 days)**	**106.12±** 12.05	**336±** 0.05	**32186±** 2122	**32936±** 2224	**19.39±** 1.31	**25.58±** 1.70	**714.30±** 45.79
60 mg/kg/day (2.5 mg/kg/h)	**Female (15 days)**	**118.59±** 14.94	**152±** 160	**39118±** 4730	**40255±** 5116	**23.55±** 9.85	**22.59±** 2.75	**764.78±** 337.90
Study A0125BPL [16] [3]								
50mg/kg/day (2.08 mg/kg/h)	**Male+ Female**	**92.16±** 5.97	**148.10±** 146.07	**27415.9±** 2016.2	**27706.7±** 2100.2	**12.75±** 8.69	**25.38±** 1.89	**459.90±** 288.36
100mg/kg/day (4.17 mg/kg/h)	**Male+ Female**	**134.80±** 8.23	**40.00±** 12.39	**37989.8±** 3962.8	**38378.8±** 3904.1	**11.11±** 6.33	**36.78±** 3.56	**598.09±** 379.85
200mg/kg/day [4] (8.33 mg/kg/h)	**Male+ Female**	**175.64±** 21.21	**24.00±** 0.00	**39568.8±** 6051.9	**40241.3±** 6196.7	**20.93±** 2.98	**70.96±** 11.98	**2142.66±** 489.50
400 mg/kg/day (16.67 mg/kg/h)	**Female**	**256.66**	**24.00**	**40035.1**	**40288.7**	**9.30**	**139.00**	**1865.91**

[1]: n=3; [2]: mean of 2 dogs ; [3]: n=6; [4]: n=4

As already seen in rats, one of reparixin metabolites, ibuprofen was detected in dogs in the two studies. Ibuprofen after the end of infusion was eliminated from plasma with mean t½ ranging from 4.78 to 15.66 h. In terms of AUC, ibuprofen accounted for 3-5% of reparixin for all doses except for the 400 mg/kg/day dose, where the ratio is higher (just one animal).

Pharmacokinetic parameters of ibuprofen are shown in Table 7.

Table 7: Pharmacokinetic parameters of ibuprofen (Mean ±SD) after i.v. continuous infusion of 15, 30, 60 mg/kg/day [16] and 50, 100, 200 or 400 mg/kg/day reparixin L-lysine salt in male or female dogs [16]

PK parameters after i.v. continuous infusion		Cmax (µg/mL)	Tmax (h)	AUClast (µg ·h/mL)	AUCtot (µg ·h/mL)	t½ (h)	Ratio AUCtot Ibuprofen/Reparixin
Study A0206BPL [16] [1]							
15 mg/kg/day (0.625 mg/kg/h)	**Male (14 days)**	**2.60±** 0.70	**48.00±** 0.003	**680.64±** 173.89	**687.75±** 175.42	**9.39±** 2.12	**3.73% ±** 1.21
15 mg/kg/day (0.625 mg/kg/h)	**Female (15 days)**	**3.00±** 0.77	**48.00±** 0.00	**786.20±** 205.39	**795.00±** 209.70	**9.02±** 1.25	**3.41% ±** 1.05
30 mg/kg/day (1.25 mg/kg/h)	**Male (14 days)**	**3.64±** 0.14	**152.00±** 159.80	**952.89±** 241.20	**968.84±** 239.32	**11.61±** 5.35	**3.61% ±** 0.83
30 mg/kg/day (1.25 mg/kg/h)	**Female (15 days)**	**4.21±** 1.15	**240.00±** 135.76	**1045.04±** 108.04	**1047.85±** 108.23	**4.78±** 0.64	**3.43% ±** 0.02
30 mg/kg/day (1.25 mg/kg/h)	**Female (14 days)**	**3.73**	**144.00**	**1029.57**	**1038.76**	**8.27**	**3.13%**
60 mg/kg/day (2.5 mg/kg/h)	**Male (14 days)**	**5.00±** 1.54	**176.00±** 146.64	**1401.38±** 283.91	**1407.85±** 284.84	**7.73±** 1.70	**4.30% ±** 1.06
60 mg/kg/day (2.5 mg/kg/h)	**Female (15 days)**	**7.13±** 1.66	**56.00±** 13.86	**1946.85±** 524.00	**1952.41±** 524.82	**6.33±** 0.26	**4.93% ±** 1.57
Study A0125BPL [16] [2]							
50mg/kg/day (2.08 mg/kg/h)	**Male+ Female**	**4.29±** 0.98	**209.67±** 147.09	**1141.40±** 221.05	**1144.96±** 220.90	**5.49±** 1.10	**4.16%±** (0.89)
100mg/kg/day (4.17 mg/kg/h)	**Male+ Female**	**6.59±** 1.19	**48.00±** 21.47	**1558.08±** 165.70	**1563.81±** 166.20	**6.57±** 2.03	**4.11%±** (0.61)
200mg/kg/day [3] (8.33 mg/kg/h)	**Male+ Female**	**8.92±** 2.38	**33.60±** 13.15	**1793.33±** 294.36	**1798.15±** 296.11	**8.52±** 3.33 [4]	**4.84%±** (1.64)
400 mg/kg/day (16.67 mg/kg/h)	**Female**	**14.18**	**24.00**	**3048.86**	**3120.98**	**15.66**	**7.75%** -

[1]: n =3 [2]: n=6 [3] :n=5; [4] :n=4

5.2.6. Distribution

After single i.v. administration the volume of distribution in rats and dogs was small since it was lower than the total body water (see Table 1 and Table 2 for rats and dogs, respectively).

The protein binding of [^{14}C]reparixin *in vitro* was investigated in male rat, male dog, female rabbit, male cynomolgus monkey and human plasma, human serum albumin and human α_1-acid glycoprotein at concentrations near 1, 50, 250, 500 and 1000 µg [^{14}C]reparixin L-lysine salt/mL by ultrafiltration method [19].

The extent of binding was high for all species investigated, but slightly greater in dog, rabbit, monkey and man than in rat. There was very little protein binding to human α_1-acid glycoprotein but a high degree of binding to human albumin. Therefore, albumin is likely to be the major protein in the plasma protein binding in all species. There was a decrease in plasma protein and human serum albumin protein binding as the concentration increased (most notably between 250 – 1000 µg/mL) possibly due to saturation of binding sites by reparixin above 50 µg/mL. At 1000 µg/mL, the extent of binding to human albumin was smaller (85.2%) than in whole human plasma (93.3%). This could reflect the involvement of other binding proteins but, in view of the small extent of binding to α_1-acid glycoprotein, it is more likely that the difference is attributable to the difference in albumin concentrations (44 g/L in whole plasma; 40 g/L in human albumin solutions). The results are tabulated in Table 8.

Table 8: Protein binding of [^{14}C]reparixin L-lysine salt following ultrafiltration in rat, dog, rabbit, cynomolgus monkey and human plasma, human serum albumin and human α_1-acid glycoprotein.

Mean % protein bound							
Nominal concentration in plasma (µg/mL)	**Rat plasma**	**Dog plasma**	**Rabbit plasma**	**Cynomolgus monkey plasma**	**Human Plasma**	**Human serum albumin**	**Human alpha1-acid glycoprotein**
1	98.9	99.2	99.3	99.1	99.4	99.2	3.9
50	98.8	98.8	99.2	99.1	99.3	99.2	4.6
250	97.9	96.3	98.6	98.4	97.9	98.3	5.0
500	96.1	94.0	97.3	96.8	96.4	94.8	3.1
1000	88.2	89.0	94.7	93.6	93.3	85.2	4.0

After the i.v. administration of 45 mg/kg of [^{14}C]-DF 1681B to male and female rats [20], radioactivity was distributed in all analysed tissues, although at concentrations generally much smaller than those in plasma. Greatest concentrations (other than those in plasma) were in liver and smallest in the CNS tissues. Radioactivity was not persistent, being detectable in a small number of tissues at 24h and in none at 168h. In partially pigmented rats, there was no evidence of persistence in melanin containing tissues. In pregnant rats on *ca* day 18 of gestation, radioactivity rapidly crossed the placenta, and was measurable in all foetal analysed tissues at concentrations (with the exception of brain) much smaller than those in the corresponding maternal tissue. The radioactivity in the foetuses was transient, with no radioactivity detectable 24h after dosing.

Total radioactivity in plasma was higher and lasted longer than in blood. Moreover, radioactivity plasma and whole-blood concentration were generally greater in female rats than in males. The same gender difference was evident also when reparixin concentrations was considered. Comparison of AUC showed that reparixin accounted for 48% and 71% of total plasma radioactivity (male and female, respectively). The pharmacokinetic parameters obtained in plasma and whole blood are reported in Table 9.

The same blood to plasma distribution was observed in dogs. After the administration of 45 mg/kg of [14C]-DF 1681B in male and female dogs [23], most of the radioactivity was in the plasma fraction (only small amounts were associated with blood cells).

Table 9: Main pharmacokinetic parameters radioactivity and reparixin in plasma and whole blood after single intravenous administration of 45 mg/kg of reparixin L-lysine salt in rats (male and female).

Matrix	Analyte	Sex	C_0 µg /mL	AUC_t µg.h/mL	AUC_{168} µg.h/mL	AUC µg.h/mL	$t_{1/2}$ h	CL mL/h/kg	V_{ss} mL/kg
Plasma	Reparixin	female male	284.68 218.93	131.43 77.49	132.65 78.94	133.24 78.85	0.5 0.4	223 375	147 167
	radioactivity	female male	331.55* 229.02*	186.94* 162.82*	187.08* 163.13*	187.60* 163.26*	37.8 22.5		
Whole-blood	radioactivity	female male	167.37* 143.33*	112.96* 104.69*	113.18* 104.69*				

* = µg equivalents. h/mL for radioactivity

5.2.7. Elimination

After single i.v. administration, the plasma clearance in rats and dogs was low, accounting for less than 10 and 0.1% of hepatic blood flow (Hbf) in rats and dogs (Hbf: 3.3 and 1.9 L/h/kg for rats and dogs, respectively [21]. The compound was eliminated faster in rats than in dogs (mean about 1 h and 22h in rats and dogs, respectively), as reported in Table 1 and Table 2.

5.2.7.1. Metabolism

The metabolism of DF 1681B was studied in two *in vitro* studies [21, 22]. The *in vivo* metabolism was investigated in two studies after the administration of [^{14}C]-DF 1681B to rats [20] and dogs [23]. The metabolic profile was evaluated in dog and rat plasma [24] and in human and rat urine [25]. Moreover, the pharmacokinetic of one of the main metabolites, DF 2243Y was assessed during 2-week continuous intravenous infusion study in the rat [26].

5.2.7.1.1. *In vitro* metabolism

In vitro metabolism was evaluated using cryopreserved (dog and human) or freshly isolated rat hepatocytes in suspension incubated with 10 or 100 µM reparixin L-lysine salt in a 4-hour time-course study **[**21**].**

The disappearance of 10 µM of the compound after 4h incubation is approximately 100% in rats, 84% in humans, and less than 5% in dogs, while at 100 µM it was approximately 72% in rats, 37% in humans, and 17% in dogs.

The rat metabolism seems to be more similar to the human, considering the type of metabolites detected (which involved the same enzymes), but the rate of metabolite formation is much higher in the rat. In dogs, the drug appears to be metabolised at a very slow rate when compared to rats and humans. The enzymes responsible for the biotransformation could be low in the dog or be not present, hence the presence of other metabolites is not detectable even at a higher concentration (100 µM). These experiments also suggest that the dog might not be an appropriate model for human metabolism prediction of this compound.

Sample analysis by LC/MS resulted in the detection of a total of five metabolites after incubations with hepatocytes: three hydroxylated metabolites, one carboxylated metabolite and one hydrolysed metabolite identified as ibuprofen were detected. Reparixin was extensively metabolized by rat, to a lesser extent by human, and slightly metabolized by dog hepatocytes.

There were some species differences in the relative contributions of the different metabolic pathways. Hydroxylation appeared to be the major biotransformation route in rat, followed by carboxylation. In human, hydrolysis appeared to be the major biotransformation route followed by hydroxylation and carboxylation. In dog, the only biotransformation route observed was hydrolysis.

In human liver microsomes [22], phase 1 metabolism of [14C]-reparixin L-lysine salt was catalysed by CYP2C9 (*ca* 99% decrease in parent total chromatogram radioactivity) and to a lesser extent by CYP2C19 (*ca* 20%) with the production of two major metabolite fractions, DF 2188Y and DF 2260Y. CYP3A4 metabolised reparixin to a very limited extent (ca 4%) and CYP 1A2, CYP2D6 and CYP2E1 showed negligible capacity to metabolise reparixin (<2%).

DF 2188Y formation appears to be principally catalysed by CYP2C9, with a lesser contribution by CYP2C19 and DF 2260Y formation appears to be essentially catalysed solely by CYP2C9. The principal human in vivo metabolite, DF 2243Y was not detected in either the 10 µM nor 1000 µM by LC-MS(/MS) (see 6.1.2). It may be speculated that the formation in vitro of DF 2243Y from reparixin via DF 2260Y may require a longer incubation time than that used in this study.

Michaelis-Menten kinetic parameters for the consumption of [^{14}C]reparixin L-lysine salt were estimated to be: K_m of 27.0 ±2.7 µM (estimate ± standard error, CV=10.1%) and V_{max} of 2024 ± 54 pmoles/min/mg protein (estimate ± standard error, CV=2.7%).

5.2.7.1.2. *In vivo* metabolism

After the administration of 45 mg/kg of [^{14}C]-reparixin L-lysine salt in male and female rats, the compound was completely metabolised. Metabolites was analysed with HPLC using radioactivity detection and reference standards in urine and bile samples (before and after treatment with β-glucuronidase/arylsulfatase mixed enzyme preparation) and extracts of faeces samples. Metabolism was mediated almost exclusively by oxidation of the isobutyl side chain of reparixin to hydroxylation products (DF 2188Y and DF 2260Y) and the carboxylic acid (DF 2243Y) of DF 2260Y. There was further metabolism of each of these to form glucuronide or sulphate conjugates [20].

After the administration of 45 mg/kg of [14C]-DF 1681B in male and female dogs, 90% of the plasma radioactivity (in terms of AUC) was associated with reparixin whereas <0.1% and <1%, were associated with DF 2243Y and DF 2188Y, respectively and the remaining percentage with ibuprofen. HPLC of urine (before and after treatment with a β-glucuronidase/sulphatase mixed enzyme preparation) and extracts of faeces samples showed that [14C]-reparixin was almost completely metabolised prior to excretion. Metabolism included oxidation of the isobutyl sidechain to give DF 2260Y (formed by hydroxylation of a terminal carbon of the isobutyl sidechain). This was, however, a minor metabolite found only in urine. The greatest amount of radioactivity (accounting for ca 50% of the dose), were found corresponding to methanesulfonamide by TLC [23]. The pharmacokinetic parameters obtained are reported in Table 10.

Table 10: Main pharmacokinetic parameters of radioactivity and reparixin in plasma after single i.v. administration of 45 mg/kg of [^{14}C]-reparixin L-lysine salt in dogs (male and female).

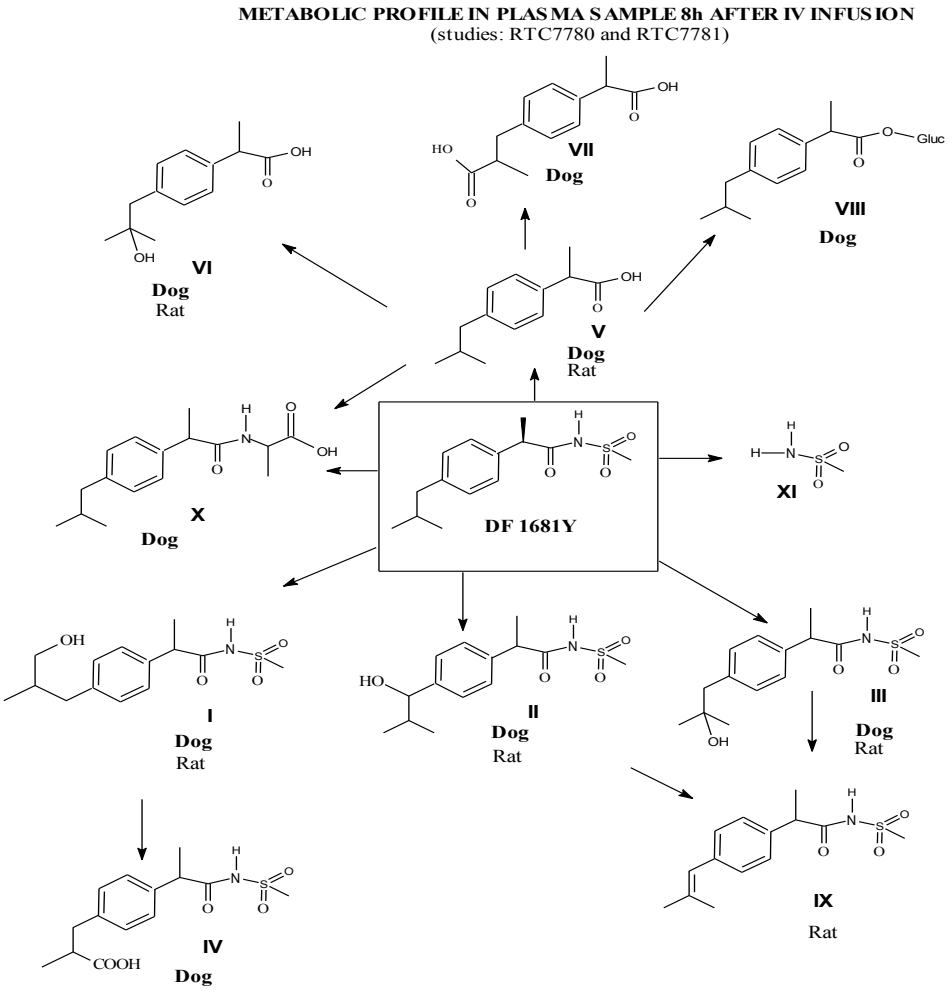
Analyte	Sex	C_0 (µg/mL)	C_{max} (ng/mL)	T_{max} (hours)	AUC_t (µg.h/mL)	AUC_{168} (µg.h/mL)	$t_{1/2}$ (hours)	CL (mL/h/kg)	V_{ss} (mL/kg)
Reparixin	Male	255			2610	2640	9.9	11.5	154
	Female	270			2910	2940	12.3	10.9	170
Radioactivity	Male	251			2963	2963	61.5[a]	-	-
	Female	266			3337	3337	36.5[a]	-	-
DF 2243Y	Male		197	3	1360	1520	5.7	-	-
	Female		281	2	1860	2010	5.0	-	-
DF 2188Y	Male		554	3	4840	5160	4.4	-	-
	Female		739	3	6570	6890	4.3	-	-

[a] Did not meet acceptance criteria, these values should be interpreted with caution; Note: Units for radioactivity are in µg equivalents

The structural identification of dog and rat metabolites performed on plasma samples from two studies [10 and 14] is shown in Figure 2 [24].

In dog plasma 10 metabolites were found: I, II, III=OH-DF 1681Y; IV=carboxy-DF 1681Y; V=ibuprofen; VI=OH-ibuprofen; VII=carboxy-ibuprofen; VIII=ibuprofen glucuronate; X=alanylamide-ibuprofen; XI= methanesulfonamide (theoretical metabolite).
In rat plasma 8 different metabolites were found: I, II, III=OH-DF 1681Y; V=ibuprofen; VI=OH-ibuprofen; IX=dehydrated of II and/or III; XI= methanesulfonamide (theoretical metabolite).

Figure 2: Qualitative metabolic profiling in plasma at 8h after infusion of reparixin L-lysine salt in rats (256.8 mg/kg/day) and dogs (388.8 mg/kg/day)

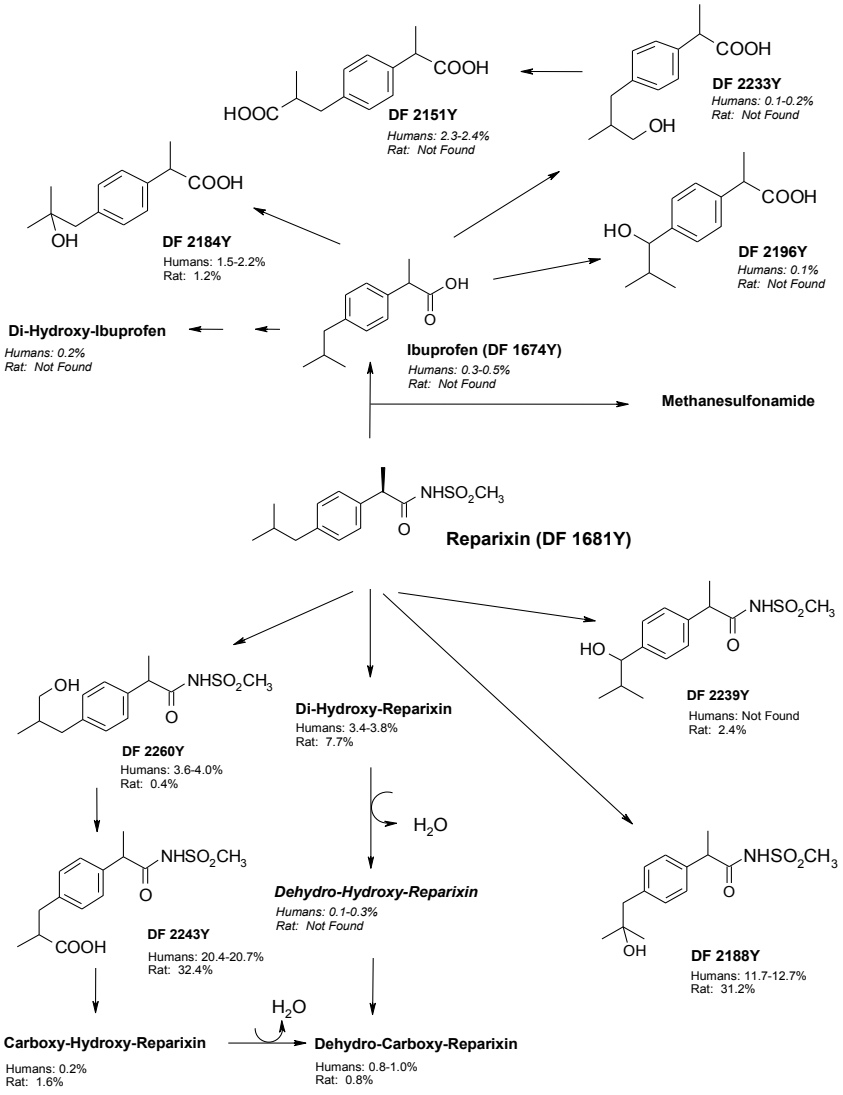


The qualitative and quantitative evaluation of metabolic profile of reparixin L-lysine salt was also evaluated in human and rat urine samples [25] collected during the Phase 1 clinical study REP0101 (§ 6.1.2) and the toxicological study RTC7780 [10], respectively. Analysis of metabolites was performed by reverse phase HPLC/MS analysis on urine samples after β-glucuronidase digestion.

Thirteen different metabolites were detected in human urine, whereas eight metabolites were detected in rat urine (Figure 3). All metabolites detected in rats, except DF 2239Y, were also detected in humans. Six out of thirteen metabolites detected in humans were not detected in rats, i.e. DF 2151Y, DF 2196Y, DF 2233Y, dehydro-hydroxy-reparixin, di-hydroxy-ibuprofen and ibuprofen.

DF 2188Y (on average 12% and 31% of reparixin dose in humans and rats, respectively) and DF 2243Y (on average 21% and 32% of reparixin dose in humans and rats, respectively) can be considered as the only two major metabolites excreted in urine in both rat and human species.
Di-hydroxy-reparixin and DF 2260Y resulted to be the third and fourth most important metabolites in human urine (on average 4% of reparixin dose for both). In rats, di-hydroxy-reparixin was seen at higher levels than in human (on average 8% of reparixin dose), whereas DF 2260Y was excreted at very low levels (on average 0.4%. of reparixin dose).

Figure 3: Reparixin - Comparative metabolic pathway scheme in urine for humans and rat after single i.v. doses.



DF 2243Y was administered to male and female rats as 14 days continuous infusion at 600 and 900 mg/kg/day [26] and its pharmacokinetic profile was evaluated using a validated LC-MS/MS method. Mean plasma concentrations at 600 mg/kg/day at day 1, 8 and 14 in male and female rats, were 36.6±3.6, 39.0±6.1 and 42.4±1.4 µg/mL, and 38.2±3.6, 39.9±5.2 and 51.7±8.1 µg/mL, respectively. Pharmacokinetic profile of DF 2243Y in male and female rats was similar. Mean AUCs from 0 to day 14 at 600 and 900 mg/kg/day and

female rats were 12656±1191 and 18828±1134 µg•h/mL in males and 13627±1016 and 18804±1561 µg•h/mL in females. The corresponding AUCs at steady state were 939 and 1343 µg•h/mL (this value corresponds to the average $AUC_{0\text{-day}14}$ divided by 14) after 600 and 900 mg/kg/day, respectively. Pharmacokinetics seems to be dose proportional.

5.2.8. Excretion

After i.v. administration of reparixin to male and female rats, urinary excretion (0-48 hours) was very low (1.3, 0.4 and 0.3% of administered dose) and decreased with increasing dose level from 15 to 45 mg/kg [4] (see Table 1). No reparixin was detected in the urine of any male or female dogs [5].

In the rat, after i.v. administration of [14C]reparixin, the excretion of radioactivity was relatively rapid and, within 168h after dosing, effectively complete. Most radioactivity was excreted in urine (about 82% dose) with about 12% dose in faeces. In bile duct-cannulated rats, about 23% was excreted in bile within 48h after dosing [20].

In dogs, after i.v. administration of [14C]reparixin, the major route of excretion of radioactivity (80.11 and 81.61% dose in 168h after dosing; male and female, respectively) was in the urine. About 9 – 11% of the dose was excreted in the faeces. Overall, >76% dose (male) and 82% dose (female) was excreted within 48h of dosing, so that excretion was relatively rapid and complete since <0.1% was recovered in the final excreta collection [23].

5.2.9. Drug-Drug interaction

Six studies were performed to assess the possible interaction between the test compound and other drugs. Two studied evaluated the potential for enzyme inhibition [27, 28]. Three studies investigated whether reparixin L-lysine salt and DF 2243Y act like a P-gp inhibitor of a known P-gp substrate digoxin with and without strong P-gp inhibitors such as cyclosporine A or rapamycin (sirolimus) [29] or cyclosporine A and tacrolimus [30] [31]. One study investigated if reparixin L-lysine salt is a substrate for P-gp [32] and another if reparixin L-lysine salt affects the basal ATPase activity of P-glycoprotein [33].

The first study investigated the potential inhi bitory effects of reparixin L-lysine salt on several human CYP isoenzymes (CYP1A12, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4). Reparixin did not cause any inhibitory effect on CYP1A12 and resulted as slight inhibitor of CYP3A4, CYP2C9, CYP2C19, CYP2D6 and CYP2E1 (Table 11). These slight inhibitions are unlikely to cause any clinically relevant drug interactions [27].

Table 11: Reparixin L-lysine salt inhibitory potential for each CYP isoform expressed as IC_{50}.

CYP isoform	IC_{50}DF 1681B (µM)	IC_{50} Classic Inhibitor (µM)
CYP1A12	> 5000	0.54 (furafylline)
CYP2C9	79	1.7 (sulfaphenazole)
CYP2C19	868	47.6 (tranylcypromine)
CYP2D6	2697	5.7 (quinidine)
CYP2E1	1523	6.07 (diethyldithio-carbamate)
CYP3A4	8	0.05 (ketoconazole)

Since reparixin L-lysine salt was found to slightly inhibit human CYP3A4 with an IC_{50} of 8 µM**,** a further study was performed to assess if reparixin inhibited the human hepatic microsomal metabolism of cyclosporin A (CsA), sirolimus and tacrolimus that are all substrates of this enzyme [28]. To this purpose, human liver microsomal proteins (2 mg/mL) and reparixin L-lysine salt (0.08, 0.8, 2.4, 8, 24, 80 or 800 µM) were incubated with the addition of either CsA, sirolimus or tacrolimus (1, 3, 10, 30 or 100 µM) and were stopped after either 15min (CsA), 3min (sirolimus) or 30min (tacrolimus) by the addition of acetonitrile (CsA and sirolimus) or 4% orthophosphoric acid (tacrolimus). The resulting supernatants were analysed for parent compound by LC-MS. Reparixin indicates some potential for the un-competitive inhibition of the human hepatic phase 1 metabolism of both CsA and sirolimus (K_i were 97,024 µM for CsA and 426 µM for sirolimus). Since the unbound plasma concentration of reparixin at steady state in human is approximately 0.1 µM, then the C_{max}/K_i ratio << 0.1 and thus it is predicted that the clinical relevance of any inhibition of

CsA or sirolimus metabolism by reparixin is remote. However, given the variability in the data obtained, these conclusions must be regarded as tentative. Furthermore, there was no evidence for the inhibition of the phase 1 metabolism of tacrolimus by reparixin.

Reparixin L-lysine salt and DF 2243Y, tested at various concentrations in the absence and presence of cyclosporine A (10 µM) or rapamycin (10 µM), did not show any effect on the ability of cyclosporine A or rapamycin to inhibit P-gp [29] (Table 12).

Table 12: Summary results on P-gp inhibition of reparixin L-lysine salt and DF 2243Y in presence or absence of cyclosporine A or rapamycin (sirolimus) [28].

Cerep 8543 **Compound**	**Concentration** **(µM)**	**P-gp Inhibition** (% inhibition)			**with 10 µM cyclosporine A** **P-gp Inhibition** (% inhibition)			**with 10 µM rapamycin** **P-gp Inhibition** (% inhibition)		
		Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
Reparixin L-lysine salt	0.1	29	26	40	104	104	98	99	106	100
	5	-11	16	35	101	106	97	102	105	101
	25	29	18	26	102	103	98	103	106	102
DF 2243Y	0.1	35	36	26	105	106	100	109	104	103
	5	36	24	40	111	107	101	104	119	105
	25	17	18	22	103	102	100	104	104	104
Cyclosporine A	10	101	104	98						
Rapamycin	10	99	102	101						

Moreover, the two compounds did not affect P-gp activity, nor they interfere with the P-gp also in presence of tacrolimus, a strong P-gp inhibitors [30].

Reparixin salt did not affect P-gp activity and did not show any synergic interaction as P-gp inhibitor in presence of cyclosporine A or tacrolimus [31].

Reparixin was not likely effluxed and therefore was not a substrate for P-gp, as resulted by the ratio of the B-A over the A-B permeability (efflux ratio: 0.62). Indeed, if a compound is a P-gp substrate, one would expect that the B-A permeability of the compound be significantly higher than the A-B permeability. As shown in Table , the addition of CsA, a strong P-gp inhibitor, did not affect both the A-B and B-A permeability values [32].

Table 19: Summary results of A-B and B-A permeability using TC7 cells

Cerep 89/73	**Reparixin L-lysine salt 10µM**	**Reparixin L-lysine salt 10µM with cyclosporine A 10µM**
A-B permeability (10^{-6} cm/s)	31.9	28.5
B-A permeability (10^{-6} cm/s)	19.8	19.8

Reparixin L-lysine salt, DF 2243Y and DF 2188Y did not affect ATPase basal activity of P-gp, when tested in vitro in the range of 10 nM to 10 µM [33].

5.2.10. References (section 5.2)

1. Validation Report Of A Stereoselective HPLC Method To Assay DF 1681Y And DF 1672Y In Rat Plasma. Analytical Determination In Plasma Samples From LCG-RBM Study No. R12110 [Chiman R032/01]
2. Validation report of a stereoselective HPLC method to assay DF 1681Y and DF 1672Y in dog plasma. Analytical determination in plasma samples from LCG-RBM study No. R14700 [Chiman R033/01]
3. Preliminary pharmacokinetics of DF 1681B after single intravenous (15 mg/kg) and oral (15 mg/kg and 30 mg/kg) administration in the rat [A0109]
4. DF 1681B. Pharmacokinetic study in the rat after single intravenous administration of three doses of DF 1681B [RBM R12110]
5. DF 1681B. Pharmacokinetic study in the dog after single intravenous administration of three doses of DF 1681B [RBMR14700]
6. DF 1681B & DF 1681Y: Ascending Oral Dose Pharmacokinetic Study in Male Wistar Rats [Harlan Laboratories Ltd. C99443, A1019, M1102]

7. DF1681Y: 14-Day Oral Toxicity (Gavage) Study in the Wistar Rat [Harlan Laboratories D01208; A1026BPL]
8. DF1681Y: 13-Week Oral Toxicity (Gavage) Study in the Wistar Rat with a 4-Week Recovery Period [Harlan Laboratories D55388; A1232BPL/E]
9. DF 1681B. Dose range-finding study in dogs by intravenous route [RBMR14500, A0113]
10. DF 1681B. Preliminary intravenous infusion toxicity study in rats [RTC7780, A0009]
11. DF 1681B. Preliminary intravenous infusion toxicity study in rats [RTC8226, A0147]
12. DF 1681B. 4-Week intravenous infusion toxicity study in rats followed by a 2-week recovery period [RTC8594, A0124BPL, A0124BPL Addendum 2, A0207]
13. DF 1681B. 4-Week intravenous infusion toxicity study in rats followed by a 2-week recovery period [DOM056, A0304BPL]
14. DF 1681B. Preliminary intravenous infusion toxicity in dogs [RTC7781, A0010]
15. DF 1681B: preliminary toxicity study by continuous intravenous infusion to Beagle dogs [DOM046, DOM048, A0149BPL, A0207]
16. DF 1681B. Toxicity study by continuous intravenous infusion to Beagle dogs for 2 weeks [DOM047, A0206BPL]
17. DF 1681B: 2-Week intravenous infusion toxicity study in dogs [RTC 8850, A0125BPL, A0125BPL Addendum 2, A0207]
18. B. Davies and T. Morris. Physiological Parameters in Laboratory Animal and Humans. Pharm. Res, 1993, 10:1093-1095.
19. ^{14}C-DF 1681B – Investigation of the binding of ^{14}C-DF 1681Y (the free acid of ^{14}C-DF 1681B) to plasma proteins of rat, dog, rabbit, cynomolgus monkey and man *in vitro* [DOM 058]
20. [^{14}C]-DF 1681B. Pharmacokinetic, distribution, metabolism, and excretion in rats following single intravenous doses [DOM057]
21. Species comparison of the metabolic stability and metabolite profile using rat, dog and human primary hepatocytes. [MDS-PS 003271]
22. To Determine if CYP1A2, 2C9, 2C19, 2D6, 2E1 and/or 3A4 are involved in the *in vitro* microsomial metabolism of DF 1681B [DOM 062/034034]
23. [^{14}C]-DF 1681B – Pharmacokinetics, metabolism and excretion in dogs following intravenous doses at target dose level of 50 mg/kg – Actual dose 45 mg/kg. [DOM067/034028]
24. Preliminary *in vivo* metabolic profiling of DF 1681B using dog and rat plasma samples. [A0123]
25. Preliminary *in vivo* metabolic profiling of reparixin L-lysine salt in human and rat urine [A0303, REP0101 (ME0706)]
26. DF 2243Y - 2-week continuous intravenous infusion study in the rat [MDS AA17669, DOM 073/042577]
27. Evaluation of DF 1681B inhibitory potential on different CYP450 isoenzymes [IPAS-MET-014-01]
28. Study of potential metabolic interactions with cyclosporine A and sirolimus [DOM 071/040089]
29. ADME: P-glycoprotein Inhibition – Study of DF 1681B, DF 2243Y, Rapamycin and Cyclosporine A [Studies CEREP 8089 and CEREP 8543]
30. ADME: P-glycoprotein Inhibition – Study of several Compounds –(tacrolimus) [CEREP 8366]
31. ADME: P-glycoprotein Inhibition - Study of Cyclosporine A, FK506 (tacrolimus) and DF 1681B [CEREP 9030]
32. ADME: A-B and B-A Permeability – Study of DF 1681B [CEREP 8973]
33. Effect of reparixin L-lysine salt, DF 2243Y and DF 2188Y on P-glycoprotein activity in vitro [A0334]

5.3. TOXICOLOGY

5.3.1. Summary

Reparixin under the form of the L-lysine salt (DF1681B) or as free acid (DF1681Y) was tested for toxicity in rodent and non-rodent animal species after single and repeated dose administrations either by i.v. or oral, according to the human foreseen administration route.

The general toxicological profile of reparixin L-lysine salt, as for the studies conducted to date, is characterised by a low toxicity after single administration by i.v. or oral route to mice (LD_{50} = i.v. 609 mg/kg; LD_{50} = p.o.>3 g/kg) and to rats (LD_{50} = i.v. 348 mg/kg; LD_{50} = p.o. 1303 mg/kg).

Single ascending doses of reparixin L-lysine salt (DF1681B) and reparixin acid (DF1681Y) were administered orally (by gavage) to rats up to the dose of 1000 mg/kg bis in die. The daily doses up to 2000 mg/kg were very well tolerated and no mortality, clinical signs or body weight changes were observed.

The repeated dose administration to rats by continuous infusion for 28 days resulted in a determination of a safe dose of 1000 mg/kg/day (NOAEL), while the continuous infusion administration to dogs for 2 weeks resulted in a safe dose of 60 mg/kg/day, even if after 2 weeks infusion at the dosage of 50 mg/kg/day one male animal showed a mucosal ulceration in the fundic area of the stomach.

The repeated oral administration by oral gavage in rats for a period of 14 days up to the doses of 400 mg/kg bis in die resulted to be well tolerated, and only minor adaptive liver changes of metabolic nature were observed in females at the dose of 400 mg/kg/bid. They consisted of an increase of liver weight and hepatocellur hypertrophy. The NOEL could be determined at 400 mg/kg/bid in males. In females 400 mg/kg/bid represents the NOAEL.

A 13-week oral administration by oral gavage in rats, followed by a 4-week recovery period, up to the doses of 400 mg/kg bis in die also resulted to be well tolerated. Adaptive liver and kidney changes of metabolic nature were observed in animals at 400 mg/kg, and consisted of an increased weight of these organs and hypertrophy of the hepatocytes and of the proximal tubules of the kidneys. Hyaline droplets accumulation observed in the proximal convoluted tubules of kidneys of males at 400 mg/kg, are well know of being species and sex-specific with no safety relevance for humans. These effects were completely reversible after the 4 weeks recovery phase; therefore, none of the effects was considered to be adverse.

The local tolerability of reparixin L-lysine salt was assayed in the rabbit lateral ear vein and the compound resulted well tolerated at 7.5 mg/mL.

An immunotoxicity study in rats by continuous infusion for 28 days at dose levels of up to 1000 mg/kg/day resulted in no treatment effect on splenic lymphocyte numbers or the Natural Killer cell activity of the splenocytes. The lymphocyte subsets in the spleen were analysed using flow cytometry.

Continuous i.v. infusion of reparixin L-lysine salt to the male and female rat at dose levels of up to 1000 mg/kg/day did not have any significant adverse effects on mating performance and fertility.

At high and cytotoxic concentrations after metabolic activation with S9, reparixin L-lysine salt had a clastogenic activity in human lymphocytes chromosome aberration assay. However, considering the high concentrations, this activity is unlikely to be reproduced *in vivo*.

All other tests performed (Ames test, micronucleus in rat, DNA repair in rat liver, UDS test) were devoid of any genotoxic activity. Therefore, reparixin L-lysine salt poses no genotoxic hazard for humans.

DF 2243Y (metabolite of reparixin L-lysine salt) was found to be active *in vitro* in inhibiting the chemotaxis and was produced in high amount both in rats and men. Because of that, it was investigated for any potential toxicologic adverse effect. A 2 week-study by continuous i.v. infusion was carried out in rat at the doses of 600 and 900 mg/kg. At these dose levels DF 2243Y was devoid of any toxicologic effect.

No carcinogenicity studies have been carried out.

Toxicological studies are summarised in the below table and detailed in the subsequent paragraphs.

Nonclinical Toxicology Studies

Study Type	Species Cell type N° of animals Sex	Dose regimen (mg/kg) Concentration Route	Results	Reference
Single dose and short infusion				
Acute toxicity in mice by intravenous route	Mouse 20 Males 20 Females	340-587.5 i.v. bolus	LD_{50}: 563 mg/kg (95% CI 501-632 mg/kg) in females; >587.5 mg/kg in males (not reached during the study); 609 mg/kg (95% CI 552-672 mg/kg) extrapolated by combining the genders. 408 mg/kg: reasonably tolerated resulting in no mortality.	RTC8245[1] (§ 1)
Acute toxicity in mice by oral route	Mouse 25 Males 25 Females	400-3266 p.o.	The mortality pattern allowed only an estimation of the median lethal dose: LD_{50} >3266 mg/kg (95% CI: not calculable) for males, females and combined.	RTC9083[1] (§ 2)
Acute toxicity in rats by intravenous route	Rat 20 Males 20 Females	210-461 i.v. bolus	LD_{50} 348 mg/kg (95% CI 304-397 mg/kg) both in males and in females. A combined LD_{50} value of 348 mg/kg (95% CI 316-382 mg/kg) was calculated. The dose of 210 mg/kg was reasonably tolerated, resulting in no mortality (max. non lethal dose).	RTC8244[1] (§ 3)
Acute toxicity in rats by oral route	Rat 20 Males 20 Females	781-3200 p.o.	The mortality pattern obtained allowed calculation of the median lethal point (LD_{50}), as follows: Males 1581 mg/kg ; Females 1223 mg/kg ; Combined 1303 mg/kg NB: 95% CI were not calculable.	RTC9084[1] (§ 4)
Infusional Maximum Tolerated Dose study in rats with TK determination	Rat 9 Males 9 Females	ascending doses separated by a 2 day wash-out period 4.0, 8.0, 16.0, 32.0, 64.1, 128.1, 256.2, 512.6, 1025 8 h continuous infusion	No clinical signs were observed at dosages up to 512.6 mg/kg, while hypoactivity and piloerection were observed in females receiving 1025 mg/kg. Reduced body weight gain or weight loss occurred at dosage of 1025 mg/kg. Reduction of food consumption was recorded in females following dosage of 256.2, 512.6 mg/kg and at 1025 mg/kg/8h in both sexes. Reparixin appeared to be well tolerated up to 1025 mg/kg/8h.	RTC7780[1] (§ 5) A0009[1] (Analytical report) (§5.2.4.1)
Infusional Maximum Tolerated Dose study in dogs with TK determination	Dog 1 Male 1 Female	0.4 up to 777.6 8 h continuous infusion increments with free periods of 16h	MTD is about 388.8 mg/kg. Alanine and aspartate aminotransferase were increased in animals receiving 777.6 mg/kg. These changes were more evident in the male. Urea concentration was also increased in animals of both sexes.	RTC7781[1] (§ 6) A0010[1] (Analytical report) (§5.2.5.2)
Repeated dose				
Dose Range-Finding (DRF) study after continuous i.v. infusion with TK determination	Rat 33 Males (M) 33 Females (F) divided in three groups of 5 animals/ gender	100, 300, 800 daily by i.v. 24h infusion for 14 days	No treatment-related death or relevant clinical signs Inflammatory lesions at the injection sites Dose: 800 — Key observation: RBC, HTC,Hb decrease; urea, AST increase; absolute kidney weight increase; absolute spleen and adrenal weights increase (F) Reparixin appeared to be well tolerated up to the dosage of 300 mg/kg/day for 14 days .	RTC8226[1] (§ 7) A0147[2] (Analytical report) (§5.2.5.1)
4 week intravenous infusion toxicity study in rats with TK determination	Sprague Dawley (SD) Rat 91 Males (M) 91 Females (F) divided in 3 groups of 14 animals/gender A fourth group received the vehicle. Control and high-dose groups included 7 additional animals per sex to be killed after 2 weeks of recovery	0, 100, 300, 900 daily by i.v 4 weeks continuous infusion .	No treatment-related death or relevant clinical signs Dose: 300 — AUCtot (h µg/mL): 34917 — Key observation: no systemic adverse or toxic effects; spleen weights decrease (F); changes in the caudal vena cava recovery: changes in the caudal vena Dose: 900 — AUCtot (h µg/mL): 116178 — Key observation: changes in the caudal vena cava; liver and kidney weights increase (M); spleen weights decrease (F); haematocrit, RBC, red blood cell count, haemoglobin and mean corpuscular haemoglobin decreases (M); AST increase; AP increase (F); recovery: changes in the caudal vena	RTC8594[1] (§ 8) A0124BPL[1] (Analytical and PK report) A0207[2] (Analytical and PK report ibuprofen) (§ 5.2.5.1)

Nonclinical Toxicology Studies

Study Type	Species Cell type N° of animals Sex	Dose regimen (mg/kg) Concentration Route	Results	Reference
Toxicity study by continuous intravenous infusion to CD rats for 4 weeks followed by a 2 week recovery period with immuno-toxicity and toxicokinetic investigations	CD Rat 78 Males 78 Females divided in 4 groups of 12 animals/gender Control and high-dose groups including 6 animals per sex, were assigned to the recovery phase (2 weeks without treatment	0, 300, 500, 1000 4 weeks continuous infusion daily by i.v.	Dose: 1000 AUCtot (h µg/mL): M:47419; F:52500 Key observation: NOAEL liver weight slightly high (F) thickening of the proximal vena cava chronic vascular changes in the vena cava perivascular chronic inflammation of the vena cava Centrilobular hepatocytes hypertrophy No effect upon splenic lymphocyte numbers or the Natural Killer cell activity of the splenocytes. **recovery:**, thickening of the proximal vena cava (1M)	DOM/056[1] (§ 9) A0304BPL[1] (Analytical and PK report) (§5.2.5.1)
DF1681B & DF1681Y: Ascending Dose Pharmacokinetic Study in Male Wistar Rats	Wistar Rat 12 Males	100-1000 oral (gavage) bid 6 hrs apart followed by a two days washout phase Single Ascending Dose initial dose: 100 2 days washout 200 For each dose: increase by 100 up to 1000	No treatment-related effects at all doses tested. MTD:1000 mg/kg/bid	Harlan Laboratories C99443 (§10)
DF1681Y: 14-Day Oral Toxicity (Gavage) Study in the Wistar Ra	SPF-bred Wistar Rat 38 Males 38 Females control group: vehicle, (Na-CMC) in purified water, 5 rats/sex/group sacrificed after 14 days of treatment	DF1681Y: 100, 200, 400 oral (gavage) bid Repeated Dose bis in die for 2 weeks	Dose: 400 mg/kg Key observation: increase in liver and adrenal (F) decrease in thymus weights (F). changes in adrenal and thymus weight not considered toxicologically relevant. hypertrophy in centrilobular hepatocellular (2 F) regarded as adaptive response of the liver. NOEL in males NOAEL in females	Harlan Laboratories D01208, (§ 10) A1026BPL[1] (Analytical and PK report) (§5.2.4.1)
DF1681Y: 13-Week Oral Toxicity (Gavage) Study in the Wistar Rat with a 4-Week Recovry Period	SPF-bred Wistar Rat	DF1681Y: 100, 200, 400 oral (gavage) bid Repeated Dose bis in die for 13 weeks	Dose / Key observation: No mortality No effects on body weight, food consumption, ophthalmic examination, hematology and urinalysis at any dose. 200 mg/kg: Increased liver weight in males 400 mg/kg: Decreased globulin concentration in males and protein/albumin in females; Increased sodium, potassium, phosphorus and chloride concentrations in females. Increased liver and kidneys weight in males and females. Centrilobular hepatocellular hypertrophy in males and females Tubular hypertrophy of kidneys of males and females Hyaline droplets in kidneys of males All changes considered not adverse and reversible after 4 weeks of recovery period. NOAEL: 400 mg/kg bid in males and females	Harlan Laboratories D55388; Sponsor Reference No. A1232BPL/E §5.2.4.1

Nonclinical Toxicology Studies

Study Type	Species Cell type N° of animals Sex	Dose regimen (mg/kg) Concentration Route	Results	Reference
DF 2243Y 2 weeks continuous intravenous infusion toxicity study in the rat	Sprague Dawley (SD) Rat 15 Males 15 Females	0, 600, 900 2 weeks continuous infusion	No treatment-related effects at all doses tested. NOAEL = 900 mg/kg	MDS AA17669[1] (§ 13) DOM 073/042577[1] (Analytical report) (§5.2.7.1.2)
Dose Range-Finding (DRF) study in dogs by consecutive intravenous injections with TK determination	Beagle dog 2 Males 3 Females	30, 50, 100 daily by i.v 50 mg/kg/day for 5 days and thereafter 100 mg/kg/day for 3 days After a 25-day washout period, 30 mg/kg/day for 14 days .	Dose / Key observation 30 — No general clinical signs 50 — no general clinical signs 100 — hepatic enzymes transient increase (1 dog) At all doses moderate-severe local changes, in general already after the first/second injection. reparixin was not well tolerated, inducing chronic vascular damage.	RBMR14500[1] (§ 14) A0113[1] (Analytical report) (§5.2.5.2)
Preliminary toxicity studies by continuous intravenous infusion	Beagle dog male female	10, 15, 30 daily 72h continuous infusion: with a washout period between each dose	Dosages of up to and including 30 mg/kg/day appropriate for a longer term administration	Preliminary studies DOM 046 – DOM 048[1] (§ 15) A0149BPL[1], A0207[2] (Analytical report) (§ 5.2.5.2)
2 week intravenous infusion toxicity in dogs with TK determination	Beagle dog 15 Males 15 Females divided in five groups of 3 dogs/gender	2 weeks continuous infusion 0, 50, 100, 200, 400 daily.	Dose / AUCtot (h µg/mL) / Key observation 50 — 27706.7[3] 1144.96[4] — mucosal ulceration in the stomach (1M). thymus: mild lymphoid depletion (2M, 3F) 100 — 38378.8[3] 1563.81[4] — ↓HCT, Hb, RBC(F) (terminal kill) 200 — 40241.3[3] 1798.15[4] — ↓body weight, food consumption (F) ↓HCT, Hb, RBC (terminal kill) (F) death (1F) 400 — 40288.7[3] 3120.98[4] — ↓ body weight, food consumption ↑liver, spleen and kidney weights ↓HCT, Hb, RBC (terminal kill) (F) humanely killed (2F, 3M) NOEL not reached, side effects attributable to ibuprofen [3] reparixin, [4]ibuprofen;	RTC8850[1] (§20) A0125BPL[1] (Analytical and PK report) A0207[2] (Analytical and PK report Ibuprofen) (§5.2.5.2)
Toxicity study by continuous intravenous infusion to Beagle dogs for 2 weeks with TK determination.	Beagle dog 12 Males 12 Females divided in 4groups of 3dogs/gender	0, 15, 30, 60 daily by i.v .2 weeks continuous infusion	No effects on any measured parameter up to 60 mg/kg/day. Findings attributable to the dosing procedure within normal limits: macro and/or micropathology lesions in the heart, jugular vein, subcutaneous port, superior vena cava and the l ungs	DOM047[1] (§21) A0206BPL[1] (Analytical and PK report) A0207[2] (Analytical report ibuprofen) (§5.2.5.2)

Nonclinical Toxicology Studies

Study Type	Species Cell type N° of animals Sex	Dose regimen (mg/kg) Concentration Route	Results	Reference
Special Studies				
Local tolerability by intravenous and paravenous routes	Sprague Dawley Rat 12 Males 3 rat/group sacrificed 24h and 7 days after treatment	100 Single dose i.v. bolus p.v. bolus	no mortality i.v route: no clinical abnormalities .. p.v route: moderate degenerative and/or inflammatory changes at different sites (muscle, blood vessels).	RBMR14660[1] (§ 22)
Effect on venous tolerability left lateral vein of the ear	New Zealand Rabbit 21 Males 21 Females	repeated: 2 doses i.v. bolus • 5, 6.5, 15, 25, 50 (1ml/kg; 1mL/min) • 7.5 (1mL/kg, 1mL/min) • 15, 25, 50 (1mL/kg, 2ml/kg; 1mL/min) • 100 (2ml/kg; 1mL/min) • 25 (0.2, 0.5, 1 mL/min)	safe doses: 7.5 mg/kg in 1 mL/kg and 15 mg/kg in 2 mL/kg. reduction of the vein injury with constant flux and at delivery rates near to physiological perfusion of the vein (200-500 µL/min)	A0126[2] (§ 23)
Evaluation of the venous tolerability	New Zealand Rabbit16 Females	Single dose i.v. bolus 5, 7.5, 15, 50 mg/mL (1 mL/kg)	Safe concentration: 7.5 mg/mL. Acute damage: Haemorrhage and infiltration of inflammatory cells (2/3 of animals 15 mg/mL, all animals at 50 mg/mL) chronic damage: vascular obliteration and focal moderate chronic inflammation (2/3 of the animals at 50 mg/mL)	A0127BPL[1]RTC9086[1] (§ 24)
Reproductive toxicity				
Fertility (Segment I) with toxicokinetic determinations.	Rat 80 Males 80 Females	continuous infusion Male: 28 days from pairing up to the day before necropsy Female:14 days from mating, up to day 7 of gestation (inclusive) 0, 300, 500, 1000 daily	Males and females: No adverse effects on mating performance and fertility at all doses tested.	MDS 35/004-D[1] (males); (§ 25) MDS 35/005-D[1] (females) (§ 26) A0311BPL[1] (Analytical and PK report)
Genotoxicity				
Evaluation of mutagenic capacity of reparixin	Salmonella typhimurium, Escherichia coli -	Single concentration 0-5000 µg/plate In absence or presence of metabolic activation	No significant increase in the number of mutations, both in absence or presence of metabolic activation.	RBMR05500[1] (§ 27)
Effect of reparixin on induction of chromosome aberration	Human lymphocytes -	Single concentration 39.06-2500 µg/mL in absence or presence of metabolic activation (S9 mix).	In presence of S9 mix, at 2500 µg/mL:induction of statistically significant increases in the proportion of cells with chromosomal aberrations.	DOM 041/012621[1] (§ 28)
Effect on micronucleus induction	Rat 35 Males 35 Females	Single dose 62.5, 125, 250 i.v. bolus	No significant increase in the frequency of micronucleated cells in the bone marrow.	RBMR14520[1] (§ 29) A0141BPL[1] (Analytical report)
Effect on DNA repair	Rat 32 Males	Single dose 108, 325 i.v. bolus	No significant increase in the nuclear grain count at any dose level at either sampling time (2h and 14 h) was induced.	DOM 045/014076[1] (§ 30)

Abbreviation: M=male; F=female; AP= alkaline phosphatase; AST= aspartate amino-transferase; Hb: haemoglobin; HCT= haematocri; RBC= Red blood cell; Na-CMC= Sodium Carboxymethyl Cellulose [1] GLP; [2] non-GLP

5.3.2. Single dose and short infusion (8h)

Single-dose toxicity of reparixin was evaluated in mouse by i.v. and oral route [1, 2] in rats by i.v., oral and infusion route [3, 4, 5] and in dogs by infusion [6].

5.3.2.1. Mouse

After the i.v. administration of reparixin L-lysine salt to male and female mice at doses ranging between 340-587.5 mg/kg, in females LD_{50} was 563 mg/kg (95% CI of 501-632 mg/kg) whereas in males, the LD_{50} was not reached during the study (DL_{50} >587.5 mg/kg). By combining the genders, an LD_{50} value of 609 mg/kg (95% CI 552-672 mg/kg) was extrapolated. In both genders mortality occurred immediately after dosing. Increased breathing and reduced activity were commonly observed immediately after dosing in all animals. Individual animals showed piloerection and/or lethargy. Convulsions were observed in animals dosed at 587.5 mg/kg and in one female dosed at 408 and 489.6 mg/kg. Recovery from these signs had occurred by day 2. A number of animals had damaged tails towards the end of the observation period. Changes in body weight observed in surviving animals during the period of the study appeared slightly reduced. Necropsy examination of both surviving and decedent animals revealed no abnormalities. The tail (injection site) was damaged and the tip was missing in a number of animals [1].

After the oral administration of reparixin L-lysine salt to male and female mice at doses ranging between 400-3266 mg/kg, mortality occurred at the two higher dose levels investigated (2333-3266 mg/kg). The mortality pattern allowed only an estimation of the median lethal dose: LD_{50} >3266 mg/kg (95% CI: not calculable) for males, females and combined. No clinical signs were observed in animals dosed at 400 mg/kg. Reduced activity and hunched posture were commonly observed in animals dosed at 720, 1296, 2333 and 3266 mg/kg. Swollen abdomen and a moribund appearance were seen in animals dosed at 2333 and 3266 mg/kg. Individual animals dosed at 2333 mg/kg showed difficulty in breathing, lethargy, part-closed eyes, rales, pallor and piloerection. No abnormalities were found on necropsy of animals. The results of this study indicate that reparixin L-lysine salt has little toxic effect in the mouse following oral administration of a single dose [2].

5.3.2.2. Rat

After the i.v. administration of reparixin L-lysine salt to male and female mice at doses ranging between 210-461 mg/kg, the LD_{50} was 348 mg/kg (95% CI 304-397 mg/kg) both in males and in females. A combined DL_{50} value of 348 mg/kg (95% CI 316-382 mg/kg) was calculated. Mortality occurred immediately after dosing. The dose of 210 mg/kg was reasonably tolerated, resulting in no mortality (max. non lethal dose). Piloerection was commonly observed at all dose-levels. Reduced activity, pronation, difficulty in breathing and/or convulsion were observed in animals from the three higher dose groups (286, 355, and 461 mg/kg). A number of animals had damaged tails (injection site) and the tip of the tail missing towards the end of the study [3].

After the oral administration of reparixin L-lysine salt to male and female mice at doses ranging between 781-3200 mg/kg, main study mortality occurred at the 3 higher dose levels (1250, 2000, and 3200 mg/kg). The mortality pattern obtained allowed calculation of the LD_{50}, as follow: males 1581 mg/kg, females 1223 mg/kg and combined 1303 mg/kg (95% CIs not calculable). Piloerection, hunched posture and moribund appearance were observed at all dose levels. Reduced activity and part-closed eyes were observed at all dose levels, with exception of the highest dose (3200 mg/kg). Ataxia, lachrymation and lethargy, were seen in animals dosed at 1250, 2000 and 3200 mg/kg. A number of animals showed difficulty in breathing. Individual animals showed pronation, pallor, swollen muzzle/abdomen, scabs on the eyelids, a dirty appearance around the urogenital region, brown staining around the muzzle, damaged eye, red discharge from the vagina, red staining in the litter tray and/or were thin or cold to touch. In particular, significant toxicity was observed also at the lower dose level investigated, 781 mg/kg, even though no mortality occurred [4].

After 8h continuous infusion of reparixin L-lysine salt to male and female mice at doses ranging between 4-1025 mg/kg, no animals died. No clinical signs were observed at dosages up to 512.6 mg/kg, while hypoactivity and piloerection were observed in females receiving 1025 mg/kg. Body weight loss occurred

following 1025 mg/kg. Reduction in food consumption was recorded at 256.2, 512.6 and 1025 mg/kg. Decreases in haematocrit, red cell count, haemoglobin, and mean corpuscular haemoglobin concentration were detected at the end of the treatment period. No macroscopic changes were observed in the examined organs/tissues of animal sacrificed at the end of the study attributable to the treatment. Reparixin appeared to be well tolerated up to 512.6 mg/kg, when a reduction in body weight gain and food consumption and changes in some clinical chemistry parameters were noted. Haematological changes detected in the surviving animals at the end of the study had no clear toxicological significance, as they could be attributable to a slight anaemia caused by the number of bleedings performed in the animals during the study and the inflammatory reaction at the infusion implant site. These modifications were noted only when reparixin plasma levels exceeded 200 µg/mL **[**5**]**. For pharmacokinetic details refer to § 5.2.4.1.

5.3.2.3. Dog

Male and female dogs were treated for 8h continuous infusion with reparixin L-lysine salt at doses ranging between 0.4 - 777.6 mg/kg, with free period of 16h. Both male and female treated with 777.6 mg/kg were killed for humanitarian reasons after 6h from the start of infusion. A marked poor health condition with loss of reaction to stimuli and hypothermia was described for these animals prior to their sacrifice. No toxicologically significant clinical signs were observed in both animals up to the dose of 388.8 mg/kg. Body weight and food consumption were not affected by the treatment. No changes of toxicological significance were observed at the haematological analyses performed during the study before each ascending dosage in any treated animal. Alanine and aspartate aminotransferase were increased in animals receiving 777.6 mg/kg at the analysis performed before their sacrifice. These changes were more evident in the male. Urea concentration was also increased in both animals at this time. Increase in kidney and spleen weights were observed in the male after sacrifice. Moreover, in the same animal distension of the urinary bladder, increased size of kidneys and a single pale area in the liver were observed at necroscopy. In conclusion, reparixin appeared to be well tolerated up to the dosages of 388.8 mg/kg at the Cmax of 328.23 µg/mL [6]. For pharmacokinetic details refer to § 5.2.5.2.

5.3.3. Repeated dose

Repeated -dose toxicity of reparixin L-lysine salt was evaluated rats and dogs. In rats, the toxicity of the test compound was assessed following either i.v. route in Sprague Dawley (14 days [7] and 4 weeks [8] infusion) and in CD rats (4 weeks infusion [9]) or oral route in Wistar rats (bid for one day and 2 weeks [10, 11**]**). In one study, the toxicity of DF 2243Y (metabolite of reparixin) was evaluated in rats [13]. In dogs, the toxicity of reparixin L-lysine salt was investigated after 5 and 3 days [14], 72h [15] and 2 weeks continuous infusion [20, 21].

5.3.3.1. Rat

In a dose range finding study reparixin L-lysine salt was administered for continuous 24h infusion to Sprague Dawley SD rats (5M+5F each) at 100, 300 and 800 mg/kg/day for 14 consecutive days. No treatment-related death occurred during the study. No relevant clinical signs were observed. Body weight and food intake were unaffected by the treatment. Statistically significant decrease in red blood cells, haemoglobin, and haematocrit were seen in both sex animals of high-dose group. Red blood cell count was decreased also in males of mid-dose group. Increase in urea and aspartate amino-transferase levels were detected in individual animals in high-dose group. Males and females of high-dose group showed an increase in the absolute kidney weight, while relative kidney weight was increased only in males. In addition, females receiving the high dose showed an increase in the absolute spleen and adrenal weights. No other changes of toxicological significance were observed. The main macroscopic observations in animals sacrificed during the course or at the end of the study were inflammatory lesions at the injection sites (femoral vein and caudal vena cava). The lesions reported at the histopathological evaluation were considered to be an expression of spontaneous pathology under the experimental condition. In conclusion, reparixin appeared to be well tolerated up to the dosage of 300 mg/kg/day for 14 days [7]. The range of reparixin plasma levels for the three groups was between 79.99 and 175.95 µg/mL (for pharmacokinetic details refer to § 5.2.5.1.).

The toxicity of reparixin L-lysine salt was evaluated in Sprague Dawley SD rats after 4 consecutive weeks

infusion at 100, 300 and 900 mg/kg/day. No treatment-related death occurred. Several animals from main groups and satellite groups were killed during the study following disconnection or rupture of the implanted catheter. No clinical signs attributable to treatment were observed throughout the study. No significant differences in body weight were observed between treated and control animals during the treatment and recovery periods. Food consumption was similar to controls with the exception of the second week of treatment, when it was reduced in high-dose females. No ophthalmic abnormalities were detected at the end of treatment and recovery periods. Statistically significant decreases in haematocrit, red blood cell count, haemoglobin and mean corpuscular haemoglobin concentration were detected in high-dose males at the end of the treatment, while no differences were observed at the end of the recovery period. Statistically significant increments in aspartate aminotransferase in high-dose males and females and in alkaline phosphatase in high-dose females were detected at the end of the treatment period while no relevant differences were observed at the end of the recovery phase. Terminal body weight of all treated groups was similar to controls at the end of the treatment and recovery periods. Statistically significant increases in absolute and relative liver and kidney weights were noted in high-dose males, while a statistically significant decrease in absolute and relative spleen weights was noted in mid and high-dose females. Organ weights of treated animals were similar to controls at the end of the recovery period. Macroscopical changes in the caudal vena cava were detected in the treated animals when compared with controls at the end of the treatment and recovery periods. Microscopical treatment-related changes were seen including increased incidence and severity of inflammatory/degenerative changes in the wall and perivascular areas of the caudal vena cava in mid and high-dose groups and increased incidence of perivascular/peribronchiolar mixed inflammatory cell (with eosinophils) in the lungs of high-dose group. In conclusion, systemic adverse effects were observed only at 900 mg/kg/day, while local adverse effects were observed only at 300 and 900 mg/kg/day [8]. The plasma levels of reparixin after day 1 ranged between 135.06 and 296.40 µg/mL while after 4 weeks ranged between 108.26 and 181.07µg/mL. (For pharmacokinetic details refer to § 5.2.5.1.).

The toxicity of reparixin L-lysine salt was also evaluated in CD rats after consecutive 4-week infusion at 0, 300, 500 and 1000 mg/kg/day mg/kg/day. During the treatment period a brown staining of the dorsal body surface was reported in males receiving 1000 mg/kg/day. The incidence of this finding during the recovery period was similar to controls. There was no effect of treatment upon splenic lymphocyte numbers or the Natural Killer (NK) cell activity of the splenocytes. At macroscopical examination, an increased incidence of thickening of the proximal vena cava was seen in animals receiving 1000 mg/kg/day. Treatment-related microscopical changes after 4 weeks consisted of minimal centrilobular hepatocytic hypertrophy and perivascular chronic inflammation of the vena cava close to the tip of the cannula in animals at 1000/mg/kg/day. These changes fully reversed after 2 weeks recovery. None of the changes was considered toxicologically significant and therefore the dosage of 1000 mg/kg/day is considered to be the NOAEL in this study [9]. For pharmacokinetic details refer to § 5.2.5.1**.**

DF1681B and DF1681Y were well tolerated when given to male Wistar rats twice daily (6 hrs apart) by oral route at doses ranging between 100 -1000 mg/kg/bid. Indeed, no mortality, clinical signs or body weight changes were observed. A maximum tolerated dose (MTD) of 1000 mg/kg/bid could be established for both compounds [10].

Toxicological profile of reparixin (DF1681Y) was evaluated in male and female SPF-bred Wistar rats after the bid oral administration (8 hrs apart) of 100, 200 and 400 mg/kg body weight, for a period of 14 consecutive days. One male treated with 100 mg/kg bid of the test item was found spontaneously dead on treatment day seven. The cause of death was due to the accidental penetration of the test item in the airways that caused tracheal necrosis. No clinical signs were noted during the acclimation and treatment period in any male and female. The mean absolute and relative food consumption of the treated males was comparable with those of the controls. The mean absolute and relative food consumption of the treated females (200 mg/kg bid or 400 mg/kg bid) was slightly lower compared with the controls between treatment days eight and 14 or ten. The mean absolute body weights and the mean body weight gain of the treated males and females were comparable with these of the control animals during the treatment period. No treatment related changes of parameters of hematology, clinical biochemistry and urinalysis were noted in animals treated with DF1681Y when compared with control animals at the end of the treatment period. In females treated with 400 mg/kg bid the mean liver weights, the mean liver to body weights and the mean liver to brain weights

were increased when compared with controls at the end of treatment. The mean adrenal weights, the mean adrenal to body weight ratios and the mean adrenal to brain weight ratios were also increased in females of this dose groups when compared with controls at the end of treatment. The mean thymus weight, the mean thymus to body weight ratio and the mean thymus to brain weight ratio were decreased in females treated with 400 mg/kg bid when compared with controls at the end of treatment. These changes in the thymus and adrenal gland could be considered to be treatment related but no macroscopic or microscopic correlates were found, therefore no toxicological significance was attributed to them. There were no gross lesions that could be attributed to treatment with the DF1681Y. All gross lesions recorded were considered to be within the range of normal background alterations. Centrilobular hepatocellular hypertrophy was recorded in two females treated with 400 mg/kg bid. There were no further indicators of liver injury such as degeneration, necrosis or inflammation, hence, this lesion is considered to be of metabolic nature and not a result of a toxic effect of DF1681Y. In conclusion, under the conditions of this study a NOEL of 400 mg/kg/bid in males and a NOAEL of 400 mg/kg/bid in females could be determined [1].

A 13-week oral administration of DF1681Y, followed by a 4-week recovery period, was carried out in Wistar rats at the doses of 100, 200 and 400 mg/kg bid [12]. No mortality occurred dusing the study. No changes in food consumption, body weight, ophthalmoscopy, hematology or urine parameters were seen.

At 200 and 400 mg/kg bw/bid salivation was recorded. At 400 mg/kg, increased absolute and relative liver and kidney weights were recorded at necropsy in both sexes and correlated with centrilobular hepatocellular hypertrophy and tubular hypertrophy, respectively; hyaline droplets accumulation was observed in the proximal convoluted tubule of males. Liver weight was also increased in males at the dose of 200 mg/kg without having a microscopic correlate as the organ morphology was normal. In addition a decreased globulin concentration in combination with an increased albumin / globulin ratio in males and increased sodium, potassium, chloride and phosphorus concentration in females as well as decreased protein and albumin concentrations in females were recorded.

As there were no further indicators of liver or kidney injury such as degeneration, necrosis or inflammation, the changes in these organs were considered to be of metabolic nature (metabolism and excretion of the test item) and not a result of a toxic effect. Hyaline droplets accumulation in the proximal convoluted tubules of kidneys of males group 4, are well know of being species and sex-specific with no safety relevance for humans. All effects recorded during and at the end of the treatment period were completely reversible after the 4 weeks recovery phase; therefore, none of the effects was considered to be adverse.

In conclusion, under the conditions of this study, the organ weight changes at 200 or 400 mg/kg/bid, the blood chemistry and microscopical changes seen at 400 mg/kg/bid were not considered adverse but rather a metabolic adaptation; therefore the NOAEL was established at 400 mg/kg/bid for males and females.

The toxicity of DF 2243Y (metabolite of reparixin) was evaluated following continuous i.v. infusion to Sprague-Dawley rats for 2 weeks at 600 and 900 mg/kg/day. Under the conditions of this study, no treatment-related effects at all doses tested and the NOAEL was established at 900 mg/kg/day [13]. For pharmacokinetic details refer to § 5.2.7.1.2.

5.3.3.2. Dog

In a Dose Range-Finding study, male and female dogs were treated with 30, 50, 100 mg/kg/day of reparixin L-lysine salt by i.v. route. No general clinical signs, behavioural changes, body weight or food consumption changes were observed in consequence of treatment with 50 mg/kg/day. All 4 dogs showed hard swelling at the injection sites (cephalic veins) after the first two or three days of treatment. No general signs or behavioural changes and no changes in body weight or food consumption occurred with the dose of 100 mg/kg. At the haematological investigations no changes were observed in any animals. Blood chemistry showed transient (disappearing within 14 days of recovery) increases in ALT (marked) and in alkaline phosphatase (slight) activity in one out of three dogs. Urine examination did not show any change. Neither general clinical signs or behavioural changes nor body weight or food consumption changes were observed at the dose of 30 mg/kg/day. The local reaction observed in cephalic veins after the 50 mg/kg/day dose period, made it necessary to inject the 100 mg/kg/day dose using the posterior legs. Moderate-severe hard swelling was again noted starting from the first injection. The local damage at the injection sites clinically regressed within 21 days of recovery in three out of four dogs, but the fourth was killed because of the difficulty in healing of local damage at the injection site (impossibility of doing further injections due to the

marked local damage). Post-mortem investigations did not reveal systemic changes in all animals. At the injection site all animals showed moderate-severe thrombi formation and perivascular haemorrhage at histological examination. In conclusion, doses of 30 and 50 mg/kg/day did not result in any general clinical signs. The dose of 100 mg/kg/day resulted, only in one dog, in a severe but transient increase in hepatic enzymes. In all animals reparixin clinically induced local changes. The histologic findings indicated that the compound was not well tolerated, inducing chronic vascular damage [14]. Plasma levels after 30 mg/kg/day were evaluated on day 1 and day 14 testifying to a continuous presence of the compound in the blood stream without accumulation or induction phenomena (refer to § 0)**.**

A preliminary toxicity study was performed in males and females dogs treated by continuous infusion for 72h with reparixin L-lysine salt at the dosages of 10, 15, 30 mg/kg/day with a washout period between each dose. Based on the limited findings (clinical signs, food consumption, haematology, biochemistry, urinalysis and faecal occult blood) of this study, dosages of up to and including 30 mg/kg/day would be appropriate for a longer term administration.The plasma levels obtained for both reparixin and ibuprofen were part of the information used to choose the dosages for the main study [15]. For pharmacokinetic details refer to § 5.2.5.2.

A preliminary study was conducted to ascertain the toxicity of reparixin L-lysine salt when given by i.v. continuous infusion to male and female dogs for 2 consecutive weeks at dosages of 0, 50, 100, 200 and 400 mg/kg/day, in order to define the dosages for the main toxicity study. Due to their poor health condition, all high-dose males and two high-dose females were humanely killed. In addition, one mid-high dose female was found dead. Decreased activity, paleness, episodes of emesis, diarrhoea, dark and/or mucoid faeces were generally observed among the high and the mid-high dose group of animals. Decreases in body weight and food consumption were observed in all the high-dose animals and in the mid-high dose females. The animals humanely killed or dead during the study presented the following main changes at clinical pathology and post-mortem investigations: decreases in haematocrit, haemoglobin and red blood cell count and increases in white blood cell count and neutrophils. Increases in alkaline phosphatase, total bilirubin, urea and creatinine levels, and decreases in potassium, calcium, total protein and albumin concentrations. Increments in absolute and relative liver, spleen and kidney weights in the high-dose animals were observed. The majority of the macroscopic changes were localized in the GI tract, kidneys, urinary bladder, thymus and lymphoid tissues and liver. The main microscopic findings were ulcerations of the gastric mucosa and inflammatory reactions of the gastrointestinal tract, renal papillary necrosis with inflammatory reaction, renal congestion, tubular epithelial degenerative changes, inflammatory reaction of the urinary bladder mucosa, depletion of lymphoid organs/tissues, hepatic sinusoidal dilatation, hepatocytic degeneration, hepatic inflammatory changes and bile ducts proliferation. In the animals subjected to terminal kill, clinical pathology analysis revealed decreases in haematocrit, haemoglobin and red blood cell count in mid-low, mid-high and high-dose females. Increases in absolute and relative liver, spleen and kidney weights were noted in treated females. The main macroscopic findings in the animals killed at the end of the treatment period were localised in the GI tract, kidneys, liver and thymus. At the microscopic examination the stomach was clearly identified as a target organ. The thymus was also affected in many animals from the treated groups. The liver, although revealed a few changes, was mainly considered secondary or of minor toxicological importance. It may be concluded that in animals receiving 50 mg/kg/day the only clear evidence of direct toxicity was seen in the stomach of one male, appearing as mucosal (fundic) ulceration. In addition, two males and three females showed mild degree of lymphoid depletion of thymus [20].

Ibuprofen was identified in plasma samples as one of the major metabolites of reparixin. It is well known from a paper of Mills et al. [16] that ibuprofen in dogs plasma remains unchanged and disappears more slowly than from plasma of other species (rats, baboons, human). The particular sensitivity of the dog to nonsteroidal anti-inflammatory drugs is well documented [17, 18] with a typical pathology at the level of the GI tract and kidneys. The macroscopic changes seen in this study were similar to those observed by Adams et al. [19] after oral administration of ibuprofen at 8 mg/kg/b.i.d. suggesting that the findings occurred in the GI tract and kidneys are attributable to the action of the metabolite rather than to the parent compound; as a consequence, the NOEL was not attained in this study [20]. For pharmacokinetic details refer to § 5.2.5.2.

The last toxicity study was performed in males and females dogs treated by 2 weeks continuous infusion

with reparixin L-lysine salt at the dosages of 0, 15, 30, 60 mg/kg/day. Findings considered attributable to the dosing procedure included macro and/or micropathology lesions in the heart, subcutaneous port, jugular vein, superior vena cava and the lungs; incidence and severity of all these findings was considered within normal limits for continuous infusion to dogs.There were no effects on any measured parameter up to 60 mg/kg/day for 14 days infusion [21]. For pharmacokinetic details refer to § 5.2.5.2**.**

5.3.4. Carcinogenicity

No carcinogenicity studies have been conducted.

5.3.5. Special studies

Special studies included local tolerability in rats [22] and rabbits [23, 24].

Local tolerability of reparixin L-lysine salt was evaluated in rats after the administration of 100 mg/kg (2 mL/kg) by i.v. or paravenous route. No mortality was seen after the two routes of administration. Reparixin did not induce compound related clinical abnormalities in animals treated by i.v. Slight swelling of the injection site was observed in animals treated paravenously. Clinical recovery was achieved in 2 out of 3 animals within 4 days after treatment. At the post mortem examination (gross pathology and histology) of animals treated by i.v., no direct treatment-related changes were seen at either killing time. Animals treated paravenously showed slight macroscopic changes at both killing times (dark firm area, scab or whitish area). Histological examination revealed the presence of a moderate increase in subcutaneous inflammatory changes with haemorrhage, degeneration and necrosis, also involving the blood vessels. At day 7, slight perivascular fibrosis or signs of muscle regeneration were also observed. In conclusion, the i.v. route was on the whole well tolerated, while the paravenous route was not well tolerated, since moderate degenerative and/or inflammatory changes were noted in all rats at different sites (muscle, blood vessels) at both examination times [22].

The effects of dose, volume and delivery rate of reparixin L-lysine salt on venous tolerability in rabbits was evaluated. Animals were treated into the right and left ear lateral vein; 48h after treatment, the vein was observed in order to assign the scores and, immediately after, a second administration in the same vein was performed and the perfusion characteristics were evaluated for the score assessment related to the perfusion phase. The two scores were added to obtain the total score. When the compound was given at 5, 6.5, 15, 25, 50 mg/kg in 1 mL/kg volume and at 1 mL/min, the delivery rate caused a dose-dependent venous irritation that decreased until disappearing completely at the dose of 5 mg/kg. Venous irritation was volume dependent and the safe doses were 7.5 mg/kg and 15 mg/kg for the administered volumes of 1 and 2 mL/kg, respectively. The venous irritation was reduced when the delivery rates was constant (by infusion pump) and near to physiological flux (200-500 µL/min) [23].

The histological characterisation of the vein damage was evaluated in the acute phase (48h post treatment) and in chronic phase (7 or 15 days post treatment) in female rabbits after the injection of reparixin L-lysine salt at the concentrations of 5, 7.5, 15 and 50 mg/mL (1mL/kg) into lateral vein of right and left ear. The histopathological examination of the acute damage showed a dose-related severity grade and distribution. Haemorrhage and infiltration of inflammatory cells was observed in 2/3 of animals receiving 15 mg/mL and in all the animals treated with 50 mg/mL. Like to control group, none of the animals treated with 5 and 7.5 mg/mL showed damage signs related to the treatment. The microscopic observation of the specimens collected at 7 and 15 days reported vascular obliteration and focal moderate chronic inflammation in 2/3 of the animals receiving 50 mg/mL and no significant, clear treatment-related abnormality in the remaining experimental groups. In conclusion, the safe concentration of reparixin L-lysine salt was 7.5 mg/mL [24].

5.3.6. Reproductive toxicity

Fertility studies were performed in male [25] and female [26] rats.

In both genders, reparixin L-lysine salt was administered at the doses of 0, 300, 500 and 1000 mg/kg/day, and plasma level collected on the second day of infusion and one day during the last week of treatment indicated that animals were linearly exposed to reparixin in a dose-proportional manner.

In males, the compound was given over a period of 28 days before pairing, throughout mating and up to the day before necropsy to evaluate the effects on gonadal function, mating behaviour and reproductive performance. No adverse effects were reported on mating performance and fertility at all tested doses. Testicular sperm counts and epididymal sperm motility parameters were not influenced by the treatment [25].

In females, the compound was given over a period of 14 days before mating, throughout mating and until day 7 (G7) of gestation (inclusive) to evaluate the effects on gonadal function, mating behaviour, reproductive performance and early pregnancy. No adverse effects were reported on mating performance and fertility at all tested doses [26].

5.3.7. Genotoxicity

The genotoxic potential of reparixin L-lysine salt was evaluated in four studies: a bacterial reverse mutation test [27], a chromosome aberration test [28], a micronucleus assay [29] and a UDS (Unscheduled DNA Synthesis) test [30].

In the first study, reparixin L-lysine salt was examined for its ability to induce gene mutation in strains of Salmonella typhimurium and Escherichia coli. It can be concluded that the compound did not induce any significant increase in the number of mutations, both in the absence and in the presence of metabolic activation up to 5000 µg/plate, in two independent experiments [27].

The potential of reparixin L-lysine to induce chromosomal aberration was assessed in *in vitro* cultured human lymphocytes. The experiment was performed either in the presence or absence of an activating system derived from Aroclor 1254-induced rat liver (S9 mix). The results showed that only in the presence of S9 mix, reparixin caused statistically significant increases in the proportion of cells with chromosomal aberrations at 2500 µg/mL ($P<0.001$), both including and excluding gap-type aberrations, when compared with the solvent control. It is concluded that reparixin showed evidence of clastogenic activity in this in vitro cytogenetic test system, in the presence of S9 mix only, under the experimental conditions described [28].

The third study investigated the capacity of reparixin L-lysine salt to cause in vivo micronuclei formation (secondary nuclei, much smaller that the principal nuclei) as a results of loss of chromosome fragments or whole chromosomes. Sprague Dawley rats were treated by i.v. route with reparixin L-lysine salt at 62.5, 125 and 250 mg/kg. The results showed no significant difference between the micronucleus frequency in the treated groups in comparison with the negative control group, Furthermore, the ratio of polychromatic to normochromatic erythrocytes in both male and female animals remained unaffected by reparixin, indicating that reparixin L-lysine salt is not toxic to bone marrow cells [29].

The last study evaluated the potential DNA-damaging activity of reparixin L-lysine salt, *in vivo*, in the rat liver DNA repair (UDS) system. DNA repair was assessed in hepatocytes following i.v. administration of reparixin L-lysine salt to male rats at dosages of 108 and 325 mg/kg. The results indicated that reparixin did not cause any significant increase in the nuclear grain count at any dose level at either sampling time (2 and 14h) showing no evidence of causing DNA damage in the rat liver [30].

5.3.8. Expert opinion on reparixin genotoxicity data package

This expert opinion was issued by Dr Ian de G Mitchell (Kelvin Associates).
Reparixin L-lysine salt is an *in vitro* genotoxin. Although it was non-genotoxic in bacterial assays, reparixin was clearly genotoxic in cytogenetic assays in human lymphocytes after metabolic activation with S9. The nature of the response with significant genotoxic activity only at high and cytotoxic concentrations after S9 activation suggested that this activity was unlikely to be reproduced *in vivo* for metabolic and pharmacokinetic reasons. This lack of in vivo activity was confirmed in two appropriate tests in rats, one which measured cytogenetic activity and the other DNA repair activity. Concentrations which resulted in genotoxicity in vitro and the estimated maximum plasma concentrations in the *in vivo* genotoxicity tests were about 3 orders of magnitude above the anticipated maximum plasma concentrations in the clinic giving enormous safety margins. It may, therefore, be concluded that reparixin L-lysine salt poses no genotoxic hazard for humans.

A further assessment of the genotoxicity studies carried out with reparixin was done by another expert toxicologist (Dr Virgilio Pace) which resulted in the following statement:

According to the ICH S2(R1) when a positive result occurs in an *in vitro* mammalian cell assay, then clearly negative results in two well conducted *in vivo* assays, in appropriate tissues and with demonstrated adequate exposure, are considered sufficient evidence for lack of genotoxic potential *in vivo*. This was exactly the case of reparixin: the mammalian chromosome aberration test positivity was followed by two appropriate negative studies *in vivo* (micronucleus test in rat using hemopoietic cells and the liver DNA strand breakage assay in rats) in which animals were exposed at high i.v. doses. Furthermore these two *in vivo* tests were very appropriate considering: ***a.*** the low volume of distribution of reparixin thus is concentrating in the blood and bone marrow; ***b.*** the metabolism of reparixin that occurs in the liver.
Furthermore, several recent reviews of the *in vitro* mammalian cell tests showed the oversensitivity and lack of specificity of these tests (Kirkland et al, 2005 and Matthews et al, 2006) particularly when the positivity is associated with cytotoxicity that by itself causes DNA fragmentation as is the gap-type aberration seen in the reparixin study.
The current ICH guideline [S2(R1)] emphasizes the presence or absence of a threshold response in the genotoxicity studies as it is very important for the biological significance of a positivity, particularly *in vitro* where the concentrations of the test item are very high and commonly never reached *in vivo* neither in animals nor in humans. DNA damage often is related to cytotoxicity and not genotoxicity; thus such indirect induction of DNA damage is secondary to other mechanisms and usually occurs above a certain concentration threshold (this is exactly the response induced by reparixin).
Human plasma level concentrations attained in all clinical studies performed to date with reparixin are far lower than those attained in the *in vitro* and *in vivo* genotoxicity studies. In the ongoing pharmacokinetic and safety study in patients with metastatic breast cancer (REP0111) with oral doses of 1200 mg/ t.i.d., the average C_{max} = 48 µg/mL, and the AUC_{INF} = 135 µg/mL. These concentrations when compared to the NOEL concentration of 2000 µg/mL attained in the *in vitro* mammalian chromosome aberration test in human lymphocytes results in a safety factor of 42 and 15, respectively for the C_{max} and $AUC_{INF.}$
In addition, to support the safety of reparixin and the no potential risk of inducing proliferation in terms of cell hyperplasia or any kind of tumors in any organ or tissue, studies in rats up to 3 months duration and followed by a one month recovery period did not induce any cell proliferation in any of the tissues examined.
In conclusion based on the overall genotoxicity results, we can infer that the risk of potential new cancer occurrence in humans exposed to reparixin can be excluded.

5.3.9. References (section 5.3)

1. DF 1681B. Single dose intravenous toxicity study in the mouse [RTC8245]
2. DF 1681B. Single dose oral toxicity study in the mouse [RTC9083]
3. DF 1681B. Single dose intravenous toxicity study in the rat [RTC8244]
4. DF 1681B. Single dose oral toxicity study in the rat [RTC9084]
5. DF 1681B and DF 1785B. Preliminary intravenous infusion toxicity study in rats [RTC7780]
6. DF 1681B and DF 1785B. Preliminary intravenous infusion toxicity study in dogs [RTC7781]
7. DF 1681B. Preliminary intravenous infusion toxicity study in rats [RTC8226]
8. DF 1681B. 4 week intravenous infusion toxicity study in rats followed by a 2-week recovery period [RTC8594]
9. Toxicity study by continuous intravenous infusion to CD rats for 4 weeks followed by a 2 week recovery period [DOM056]
10. DF1681B & DF1681Y: Ascending Dose Pharmacokinetic Study in Male Wistar Rats [Harlan Laboratories C99443]
11. DF1681Y: 14-Day Oral Toxicity (Gavage) Study in the Wistar Rat [Harlan Laboratories D01208]
12. DF1681Y: 13-week Oral (Gavage) Toxicity Study in the Wistar Rat with a 4-week Recovery [Harlan Laboratories D55388]

13. DF 2243Y – 2 week continuous intravenous infusion study in the rat [MDS AA17669]
14. DF 1681B. Dose range-finding study in dogs by intravenous route [RBMR14500]
15. DF 1681B. Preliminary toxicity studies by continuous intravenous infusion. [DOM 046 – DOM 048]
16. Mills RFN. et al. The metabolism of ibuprofen. Xenobiotica 1973;3(9):589-98.
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20. DF 1681B. 2 week intravenous infusion toxicity study in dogs [RTC8850].
21. DF 1681B. Toxicity study by continuous intravenous infusion to Beagle dogs for 2 weeks [DOM047].
22. DF 1681B. Local tolerability study in rats by intravenous and paravenous routes [RBMR14660]
23. Effect of the dose, volume and delivery rate of DF 1681B on venous tolerability in the rabbit [A0126]
24. Evaluation of the venous tolerability of DF 1681B in the rabbit [A0127BPL; RTC9086]
25. DF 1681B – Fertility study by continuous intravenous infusion in the male rat (Segment I) [MDS 35/004-D; A0311BPL]
26. DF 1681B - Fertility study by continuous intravenous infusion in the female rat (Segment I) [MDS 35/005-D; A0311BPL]
27. DF 1681B. Reversion test with *Salmonella typhimurium* and *Escherichia coli* strains [RBMR05500]
28. DF 1681B. *In vitro* mammalian chromosome aberration test in human lymphocytes [DOM 041/012621]
29. Micronucleus induction in bone marrow cells of rats treated by intravenous route with the test article DF 1681B [RBMR14520, A0141BPL]
30. DF 1681B. Rat liver DNA repair (UDS) test [DOM 045/014076]

6. EFFECTS IN HUMANS

To date a total of 404 subjects have been treated in phase 1 and phase 2 completed clinical studies. Among these, 266 have been exposed to reparixin.

During the Phase 1 studies a total of 166 subjects, of whom 103 normal volunteers (including 3 females), 17 patients with different grades of renal impairment (including 5 females), 16 patients undergoing cardiopulmonary by pass (including 6 females) and 30 female patients with metastatic breast cancer were exposed to reparixin. A total of 65 subjects (60 M and 5 F) were treated with placebo.

In Phase 2 studies, 46 patients undergoing lung transplantation (23 M and 23 F) and 48 patients undergoing kidney transplant (including 17 females) have been exposed to reparixin and a total of 81 patients have been treated with placebo. In addition, 6 patients undergoing islet transplantation (out of 9 involved in the trial) have been exposed to reparixin, with 4 patients receving the reparixin twice.

6.1. PHARMACOKINETICS AND PRODUCT METABOLISM IN HUMANS

6.1.1. Summary

The pharmacokinetics of reparixin was investigated during four Phase 1 clinical studies with the intravenous formulation [1, 2, 4, 5],

Reparixin was given either as short (30 min) [1] or long infusion [2, 4, 5], as single agent [1, 2] or in combination [4] in subjects with normal function [1, 2, 4, 5] and subjects with different degrees of renal impairment [5].

Plasma concentrations of reparixin were determined using a validated HPLC method with UV-MS detection. The metabolic profile was studied by reverse phase HPLC/MS analysis in urine samples after β-glucuronidase digestion. Analytical determinations of methansulfonamide in plasma and urine were performed according to validated LC-MS/MS analytical methods [3].

Reparixin was characterised by a low clearance, a low volume of distribution and a short terminal half-life (about 1 h). Up to the dose of 8mg/kg given as 30 min infusion, the pharmacokinetics appeared to be dose independent, whereas when given as 48 h continuous infusion plasma concentrations seemed to increase less than in direct proportion with the dose.

Reparixin was highly bound to plasma protein; the free plasma concentrations accounted for about or less than 1%. As observed in *in vitro* studies, the percentage of free fraction appeared to increase at high concentrations suggesting a saturation of binding.

The excretion of unchanged reparixin in urine was absent and low in faeces.

Reparixin was extensively metabolized: in urine fourtheen different metabolites were detected. The main metabolites were ibuprofen, methansulfonamide, DF 2243Y and DF 2188Y (the hydroxylated and carboxylated metabolite, respectively). The total recovery of reparixin and its metabolites in urine accounted about for 60-75% of the administered dose.

The pharmacokinetics of methansulfonamide, ibuprofen and DF 2243Y was elimination-rate limited, whereas the pharmacokinetics of DF 2188Y was formation-rate limited.

No apparent gender differences in the pharmacokinetics of reparixin and its metabolites were observed.

When reparixin L-lysine salt was administered in combination with midazolam and tolbutamide (probe substrates for CYP3A4 and CYP2C9, respectively), neither the pharmacokinetics of midazolam nor that of tolbutamide was altered to any clinically relevant extent by reparixin. Reparixin free fraction appears to increase by at least 50% over the infusion period, after midazolam/tolbutamide co-administration, while total reparixin concentrations remained stable.

The comparison of the pharmacokinetic profile between normal renal function and renally impaired subjects indicated that the PK profile of reparixin is not influenced, as expected, by the degree of renal function. Viceversa renal function had an effect on plasma concentrations of the major metabolites, which were found to increase over time along with the increase of their elimination t½.

Methodologies and pharmacokinetics results are summarised in the table below and detailed in the subsequent paragraphs.

Pharmacokinetic and Product Metabolism Studies in Humans			
Type of Study	**Dose (mg/kg)*/ Route/Frequency**	**Comments**	**Reference**
Phase 1 Clinical studies			
CLIN/TOL/PK (plasma, urine, faeces) 30 M (4 active+2 placebo per cohort) Age: 27±8 (range 18-50) Double blind, placebo controlled.	reparixin L-lysine salt 1, 2, 4, 8, 16/i.v. infusion/30min	Cmax= 8.28, 20.57, 31.33, 59.21 92.47 µg/mL t½ elim. = 0.98, 0.98, 0.97, 0.94, 1.28 h AUCtot = 10.66, 28.10, 42.89, 80.69, 126.12 µg·h /mL CL =98.4, 74.6, 94.8, 100.0, 129.2 mL/h/kg Vz= 134.9, 102.7, 130.6, 133.8, 233.2 mL/kg Urine: no unchanged reparixin was excreted.	REP0101 (ME0706)[1] (§1) (Safety § 6.2.2.1)
CLIN/TOL/PK (plasma, urine, faeces) 36 M (9 active+3 placebo per cohort) + 4 replacements (3 active + 1 placebo per cohort 2) Age: 27.9±9.5 (range 19-53) Double blind, placebo controlled.	reparixin L-lysine salt) Cohort 1: loading 3.1 mg/kg/h for 30min maintenance: 1 mg/kg/h for 47.5h (Total dose: 49.1 mg/kg) to reach a steady state concenjtration of 10 µg/mL (Cohort 2: loading 3.8 mg/kg/h for 30min maintenance: 2 mg/kg/h for 47.5h (Total dose: 96.9mg/kg) to reach a steady state concenjtration of 20 µg/mL Cohort 3: loading: 6.8 mg/kg/h for 30min maintenance: 4.2 mg/kg/h for 47.5h (Total dose: 202.9 mg/kg) to reach a steady state concenjtration of 30 µg/mL	**Mean±SD for reparixin** Cinitial= 10.70±3.22, 16.63±4.58, 30.06±7.05 µg/mL Cmax= 15.47±3.84, 20.07±2.36, 35.16±7.45 µg/mL t½ elim. = 1.42±0.50, 1.20±0.17, 1.47±0.36 h AUCtot = 519±160, 748±101, 1387±371 h·µg /mL CL = 68.±22, 87±12, 103±26 mL/h/kg Vz = 129±29, 148±19, 211±49 mL/kg **Mean±SD for buprofen** Cinitial= 0.94±0.37, 2.01±0.94, 4.12±1.54 µg/mL Cmax= 1.25±0.59, 2.39±1.20, 4.28±1.56 µg/mL t½ elim. = 2.96±0.56, 2.65±0.38, 2.84±0.53 h AUCtot = 50.6±22.0, 101.2±47.5, 185.6±72.9 h·µg /mL Ae = 0.71±0.37, 0.93±0.59, 1.01±.0.42 % of dose **Mean±SD for DF 2243Y** Cinitial= 2.79±0.58, 6.42±1.19, 15.98±3.15 µg/mL Cmax= 3.20±0.80, 7.38±1.44, 17.34±3.19 µg/mL t½ elim. = 2.03±0.16, 1.93±0.28, 1.84±0.14 h AUCtot = 131±30, 289±51, 658±115 h·µg /mL Ae = 30.47±7.20, 36.21±9.12, 39.38±.9.09 % of dose **Mean±SD for DF 2188Y** Cinitial= 0.57±0.18, 1.31±0.25, 3.25±0.64 µg/mL Cmax= 0.67±0.16, 1.53±0.27, 3.44±0.57 µg/mL t½ elim. = 1.64±0.22, 1.50±0.27, 1.47±0.21 h AUCtot = 27±5, 58±11, 133±21 h·µg /mL Ae = 18.10±4.42, 21.90±5.92, 25.20±.8.16 % of dose	REP0102 (ME0735)[1] (§ 2) (Safety § 6.2.2.2)
MET Analysis of Methanesulfonamide Plasma - ME0735: Cohort 3: 9 male healthy volunteers. Plasma - ME0761: Stage A: 4 ESRD patients (Haemodialysis, 3M+1F) Urine - ME0735: Cohort 1, 2 and 3: 27 male healthy volunteers.	Plasma - ME0735 Cohort 3**:** 6.8 mg/kg/h for 30min, 4.2 mg/kg/h for 47.5h Plasma - REP0761 Stage A: 6.8 mg/kg/h for 30min Urine - ME0735: Cohort 1: 3.1 mg/kg/h for 30min, 1 mg/kg/h for 47.5h; Cohort 2**:** 3.8 mg/kg/h for 30min, 2 mg/kg/h for 47.5h; Cohort 3: 6.8 mg/kg/h for 30min, 4.2 mg/kg/h for 47.5h.	**Healthy volunteers** **Mean±SD for methanesulfonamide** Cmax=4.08±2.87 µg/mL t½ elim. = 12.28±3.62 h AUClast = 130±57.16 h·µg/mL **Urinary excretion:** Ae = 10.84±3.51, 12.25±4.41, 11.20±4.16 % of dose **Total recovery** in urine of reparixin, ibuprofen, DF 2188Y, DF 2243Y and MSA: 60.12 ± 13.46, 71.43 ± 19.96, 75.40 ± 14.78% of dose. **ERSD patients:** pharmacokinetics of MSA appeared to be modified by the degree of renal function. MSA is accumulated in plasma and Cmax corresponds to the last blood sampling time (t_{max} 25.6h) indicating that the main route of elimination of MSA is through the kidney, similarly to the other metabolites, DF 2243Y and DF 2188Y.	M0409 (§ 3) Medeval analytical reports ME0823/001 and ME0823/002

Pharmacokinetic and Product Metabolism Studies in Humans			
Type of Study	**Dose (mg/kg)*/ Route/Frequency**	**Comments**	**Reference**
CLIN/INTERACTION (plasma, urine) 14M (cross over) Age: 27.4±1.8 (range 20-40) Open label	**Day 1:** Midazolam (MDZ) 7.5 mg single oral and Tolbutamide (TLB) 500 mg single oral **Day 3:** Reparixin L-lysine salt /(RPT) loading: 6.8 mg/kg/h for 30min maintenance: 4.2 mg/kg/h for 7.5h then 70min after start of infusion Midazolam 7.5 mg single oral and Tolbutamide 500 mg single oral **Total Dose:** 34.9 mg/kg	**Statistical comparison after MDZ/TLB or MDZ/TLB/RPT administration** **Midazolam — 90% CLM — p-value** Cmax 89.67-120.20 0.658 AUClast 99.50-114.79 0.124 AUCtot 99.79-114.88 0.110 **1-OH Midazolam — 90% CLM — p-value** Cmax 84.60-118.14 0.998 AUClast 105.06-127.76 0.019# AUCtot 106.99-130.30 0.012# MRt 99.00-118.71 0.139 MR 104.35-126.27 0.026# **Tolbutamide- — 90% CLM — p-value** $CCR_{0\text{-}8h}$ 38.88-132.78 0.356 ER_{COOH} 107.77-163.92 0.033# ER_{OH} 68.72-126.61 0.690 No clinically significant pharmacokinetic interaction between midazolam/tolbutamide and reparixin was observed. **$CCR_{0\text{-}8h}$** = combined 0-8 h concentration ratio for (Carboxytolbutamide+Hydroxytolbutamide)/tolbutamide **ER_{COOH}** = excretion ratio for Carboxytolbutamide/Tolbutamide; **ER_{OH}** = Excretion ratio for Hydroxytolbutamide/Tolbutamide # = statistically significant	REP0103 (ME0757)[1] (§ 4) (Safety § 6.2.2.3)
CLIN/RENAL FAILURE (plasma, urine) Stage A: ESRD: 4 Haemodialysis (3M + 1F) Stage B: Normal renal function: 6 (3M+3F) Mild renal impairment: 3 (1M+2F) Moderate renal impairment: 2 (1M+ 1F) Severe renal impairment: (2M) ESRD: 3 CAPD (2M+1F) 3 Haemodialysis (3M) Open label	reparixin L-lysine salt Stage A: 6.8 m/kg/h for 30min: Total Dose 3.4 mg/kg Stage B: 2 mg/kg/h for 6h to achieve a Css in the range of 10-30 µg/mL	The administration of reparixin L-lysine salt as an i.v. infusion of 2.0 mg/kg/h for 6h to patients with mild, moderate, severe renal impairment and to ESRD patients (CAPD and haemodialysis) was safe and well tolerated. The unbound concentration of reparixin in mild, moderate, severe and ESRD patients was similar to that observed in normal subjects. There were no apparent differences in the plasma pharmacokinetics of ibuprofen in patients with renal impairment and normal subjects. The pharmacokinetics of DF 2188Y and DF 2243Y appeared to be modified by the degree of renal function. The elimination of DF 2188Y and DF 2243Y was longer in ESRD patients as compared to normal subjects. There were no apparent gender differences in the pharmacokinetics of reparixin, ibuprofen, DF 2188Y and DF 2243Y. The difference in the pharmacokinetics of DF 2188Y and DF 2243Y had no significant clinical implications regarding the safety and tolerability of reparixin.	REP0203 (ME0761–CM8003)[1] (§ 5) (Safety § 6.2.2.4)

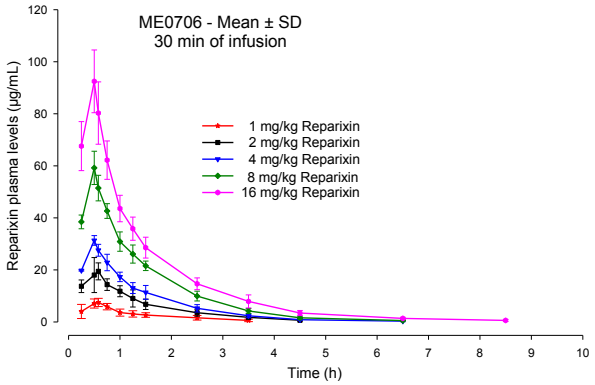
CLIN = clinical; **PK** = pharmacokinetics, **TOL** = tolerability, **MET** = Metabolism, **i.v.** = intravenous, **M** = male, **F** =female, [1] = GCP/GLP; * = dose as reparixin L-lysine salt (DF 1681B)

6.1.2. Pharmacokinetics after infusion

The pharmacokinetics of reparixin have been evaluated in two studies after 30 min [1] and continuous infusion [2].

After 30 min infusion [1], peak plasma levels occurred at the end of infusion (Figure 4).

Figure 4: Mean (+SD) reparixin concentration versus time profiles following a single 30min i.v. infusion of 1, 2, 4, 8, and 16 mg/kg reparixin L-lysine salt to healthy male volunteers (n=4).



Following peaks, concentrations at each dose level were observed to decline similarly with terminal t½ ranging from 0.94 to 1.28 h. As already observed in preclinical studies, the volume of distribution and the clearance were low. Reparixin pharmacokinetic parameters are given in Table 20. Linear kinetics for C_{max}, AUC_t and $AUC_{0-\infty}$ were observed over the dose range 1-8 mg/kg. CL appeared to be independent of dose. Unchanged reparixin was not determinable in urine. A very small amount of unchanged reparixin was excreted in the faeces over 72 hours.

Table 20: Reparixin pharmacokinetic parameters after administration of 1, 2, 4, 8, 16 mg/kg of reparixin L-lysine salt by 30min of i.v. infusion to healthy male volunteers.

REP0101 (ME0706) PK parameters of Reparixin		Group 1 (n=4)		Group 2 (n=4)		Group 3 (n=4)		Group 4 (n=4)		Group 5 (n=4)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dose	mg/kg	**1**		**2**		**4**		**8**		**16**	
Cinitial	µg/mL	**7.09**	1.76	**18.01**	6.73	**31.33**	1.86	**59.21**	6.39	**92.47**	12.06
Cmax	µg/mL	**8.28**	0.53	**20.57**	2.20	**31.33**	1.86	**59.21**	6.39	**92.47**	12.06
Tmax	h	**0.55**	0.03	**0.55**	0.04	**0.50**	0.00	**0.51**	0.01	**0.51**	0.01
$t_{½}$	h	**0.98**	0.27	**0.98**	0.19	**0.97**	0.15	**0.94**	0.13	**1.28**	0.30
tlast	h	**3.50**	1.41	**5.50**	2.00	**7.00**	1.91	**7.50**	1.15	**9.00**	2.52
AUClast	h µg/mL	**9.83**	2.56	**27.35**	6.64	**42.33**	6.76	**80.27**	8.82	**124.96**	17.89
AUCtot	h µg/mL	**10.66**	2.62	**28.10**	6.94	**42.89**	6.65	**80.69**	8.81	**126.12**	17.97
Vz	mL/kg	**134.9**	26.7	**102.7**	15.4	**130.6**	16.0	**133.8**	8.6	**233.2**	34.6
CL	mL/h/kg	**98.4**	25.4	**74.6**	18.6	**94.8**	12.9	**100.0**	11.0	**129.2**	21.4
CL_R	mL/h	No unchanged reparixin was excreted in urine									
Total faecal excretion $_{0-72}$	µg	NP	-	NP	-	**3.786**	73.539	NP	-	NP	-

NP – Not performed. Faecal sampling not performed at the 1, 2, 8 and 16 mg/kg reparixin L-lysine salt dose levels.

From the dose of 2 mg/kg onwards, ibuprofen was detected in plasma. It was observed that as the dose of reparixin increased, the plasma ibuprofen concentrations increased. Following C_{max}, plasma levels were observed to decline with terminal t½ longer than that for reparixin, suggesting an elimination-rate limited pharmacokinetics. Ibuprofen was found in urine but not in faeces. The pharmacokinetics of ibuprofen appears not to be dose proportional. The metabolic ratios (AUC_{last} ibuprofen/AUC_{last} reparixin) ranged between 4.2% and 14.6% (Table 13).

Table 13: Summary ibuprofen pharmacokinetic parameters after administration of 1, 2, 4, 8, 16 mg/kg of reparixin L-lysine salt by 30min of i.v. infusion to healthy male volunteers.

REP0101 (ME0706) PK parameters of Ibuprofen		Group 1 (n=4)		Group 2 (n=4)		Group 3 (n=4)		Group 4 (n=4)		Group 5 (n=4)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dose Reparixin	mg/kg	**1**		**2**		**4**		**8**		**16**	
Cinitial	µg/mL	-	-	-	-	**0.51**	0.11	**0.65**	0.07	**2.33**	0.44
Cmax	µg/mL	-	-	**0.38**	0.05	**0.90**	0.14	**1.00**	0.05	**4.03**	1.08
Tmax	h	-	-	**0.94**	0.24	**1.19**	0.38	**1.38**	0.14	**1.26**	0.21
$t_{½}$	h	-	-	**10.93**	3.15	**3.62**	0.50	**2.60**	0.45	**2.57**	0.46
AUClast	h µg/mL	-	-	**1.16**	0.54	**3.81**	1.12	**4.25**	1.07	**18.25**	6.25
AUCtot	h µg/mL	-	-	**5.49**	1.32	**5.62**	1.14	**5.40**	1.05	**20.12**	6.49
TLast	h	-	-	**4.25**	1.71	**6.50**	1.63	**6.50**	1.63	**9.00**	2.00
Ae_{0-72}	mg	**0.20**	0.06	**0.45**	0.11	**0.97**	0.67	**1.05**	0.44	**6.86**	3.57
CL_R	mL/h	-	-	**85.77**	28.01	**159.75**	93.82	**190.92**	57.03	**361.90**	224.95
Total faecal excretion $_{0-72}$	µg	**NP**	-	**NP**	-	**NC**	-	**NP**	-	**NP**	-
Metabolic Ratio (%)				4.24		8.98		5.29		14.61	

NP – Not performed, faecal sampling not performed at the 1, 2, 8 and 16 mg/kg reparixin L-lysine salt dose levels.
NC – Not calculated, 3 out of 4 faecal excretions calculated equal zero. Metabolic ratios was calculated as AUClast ibuprofen/AUClast reparixin

In urine, thirteen different metabolites were detected (see 5.2.7.1.2, Figure 3). The main route conduces to hydroxylated and carboxylated reparixin, DF 2243Y and DF 2188Y, that accounted for more than 30% of the administered dose, while the second one conduces to ibuprofen and related metabolites.

The metabolites found in urine accounted for about 47% of the total administered dose (Table 14).

Table 14: Summarised metabolites excretion data in humans.

A0303 study Metabolite Identification	Mean Recovery - 4 mg/kg; n = 4		Mean Recovery - 8 mg/kg; n = 3*	
	% of dose	%CV	% of dose	%CV
DF 2243Y	20.7	66.7	20.4	55.3
DF 2188Y	12.7	53.3	11.7	65.1
DF 2260Y	4.0	36.1	3.6	62.5
DF 2184Y#	2.2	56.2	1.5	23.1
DF 2151Y#	2.3	47.8	2.4	63.4
DF 2196Y#	0.1	141.4	0.1	0.0
DF 2233Y#	0.2	66.7	0.1	0.0
Ibuprofen	0.5	69.6	0.3	33.3
Dehydro-Hydroxy-Reparixin	0.1	81.6	0.3	34.6
Carboxy-Hydroxy-Reparixin	0.2	66.7	0.2	24.7
Di-Hydroxy-Ibuprofen#	0.2	40.8	0.2	34.6
Di-Hydroxy-Reparixin	3.4	47.4	3.8	52.5
Dehydro-Carboxy-Reparixin	0.8	50.8	1.0	60.6
Total	**47.2**		**45.6**	

* - Excretion data of subject N° 19 were not included in the calculations due to lack of the 0-6h period urinary sample
# - metabolites related to ibuprofen

After 48 h continuous infusion of reparixin L-lysine salt given at doses suitable to reach steady state concentrations of 10, 20 and 30 µg/mL [2], the compound declined rapidly in a mono-exponential fashion. There were no quantifiable concentrations beyond 24h post-infusion termination. As observed in the previous study, the volume of distribution and the clearance were low. C_{max}, AUC_{last} and $AUC_{0-\infty}$ increased less than in direct proportion with the dose, even if the small number of subjects prevented any statistical comparison. Very small amounts of reparixin were excreted in urine and the CL_R appears to increase more than in direct proportion with the dose. The amount excreted in the faeces over 72h was only estimable for few subjects and ranged from 1.71 to 21.93 µg and no mean was calculated (Table 15).

Table 15: Reparixin pharmacokinetic parameters after i.v. infusion of reparixin L-lysine salt for 48h to healthy male volunteers.

REP0102 (ME0735) Reparixin - PK parameters		Cohort 1 (n=9)		Cohort 2 (n=9)		Cohort 3 (n=9)	
		Mean	SD	Mean	SD	Mean	SD
Target group	µg/mL	**10**		**20**		**30**	
Cinitial	µg/mL	**10.70**	3.22	**16.63**	4.58	**30.06**	7.05
Cmax	µg/mL	**15.47**	3.84	**20.07**	2.36	**35.16**	7.45
Tmax	h	**0.50**[1]	0.50-24.00	**24.12**[1]	0.75-48.28	**12.07**[1]	0.75-48.17
$t_{½}$	h	**1.42**	0.50	**1.20**	0.17	**1.47**	0.36
tlast	h	**55.11**	3.02	**54.00**	1.73	**56.22**	2.33
AUClast	h µg/mL	**518.28**	159.39	**746.75**	101.25	**1386.14**	370.99
AUCtot	h µg/mL	**519.05**	159.56	**747.78**	101.23	**1387.27**	371.33
Vz	mL/kg	**129.4**	28.7	**148.2**	18.6	**211.0**	49.0
CL	mL/h/kg	**68.2**	22.2	**86.9**	12.1	**102.5**	26.3
Ae_{0-60}	mg	**0.0101**	0.0052	**0.0243**	0.01132	**0.0632**	0.0207
CL_R	mL/h/kg	**0.0213**	0.0108	**0.0321**	0.01431	**0.0486**	0.0210
Total faecal excretion $_{0-60}$	µg	**NC (N = 4)**	-	**NC (N = 2)**	-	**NC (N = 1)**	-

[1] median and range values; NC – Not calculated, very low amounts.

Reparixin appeared to be highly bound to plasma protein, since the free plasma concentrations accounted for about or less than 1%. Free concentrations expressed as absolute values and percentage are reported in Table 16. The percentage of free fraction appears to increase with target SS increasing, in agreement with *in vitro* data where it was observed a saturation of binding at high concentrations [5.2.6].

Table 16: Mean summary free reparixin concentrations (ng/mL) and free fraction (%).

Target steady state concentrations		0.5 h		24 h		48 h	
		(ng/mL)	(%)	(ng/mL)	(%)	(ng/mL)	(%)
10 µg/mL	**Mean**	**3.672**	**0.0250**	**3.619**	**0.0326**	**3.690**	**0.0350**
	SD	0.803	0.0083	0.765	0.0094	0.767	0.0101
20 µg/mL	**Mean**	**7.222**	**0.0517**	**9.502**	**0.0569**	**7.696**	**0.0482**
	SD	1.389	0.0261	2.905	0.0174	2.003	0.0155
30 µg/mL	**Mean**	**19.480**	**0.0740**	**35.037**	**0.1191**	**31.401**	**0.1096**
	SD	6.936	0.0247	8.422	0.0379	9.836	0.0173

Four metabolites were detected in plasma and urine: ibuprofen, DF 2243Y, DF 2188Y and methansulfonamide.
The concentrations of ibuprofen, DF 2243Y, DF 2188Y increased with the dose of reparixin L-lysine salt, but not in direct proportion. No information regarding the linearity of methansulfonamide pharmacokinetics was available, since this metabolite was analysed only at the highest dose [3]. The terminal t½ for DF 2188Y was similar to reparixin suggesting formation-rate limited pharmacokinetics. On the contrary, the terminal t½ of ibuprofen, DF 2243Y and methansulfonamide was longer than that for reparixin, suggesting an elimination-rate limited pharmacokinetics for these three metabolites.

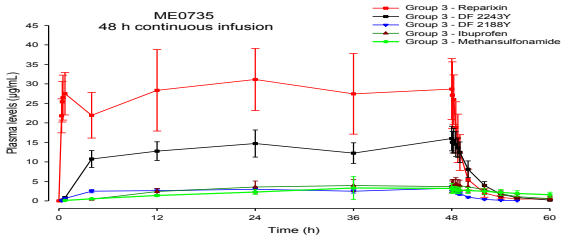
In comparison to reparixin, the higher metabolic ratio was observed for DF 2243Y and the lowest for DF 2188Y (Table 17).

Table 17: Metabolic ratio (MR = AUCtot metabolite/AUCtot reparixin) of ibuprofen, DF 2188Y, DF 2243Y and methansulfonamide after reparixin L-lysine salt administration i.v. 48h infusion.

Target Group	Ibuprofen MR (%)	DF 2188Y MR (%)	DF 2243Y MR (%)	Methansulfonamide (%)
10 µg/mL	9.75	5.16	25.31	
20 µg/mL	13.53	7.80	38.60	
30 µg/mL	13.38	9.59	47.46	11.55

The comparison between the plasma profile of reparixin and its metabolites is shown in Figure 5 and the corresponding pharmacokinetic parameters are given in Table 18.

Figure 5: Mean (±SD) plasma levels of reparixin and its metabolites after administration of reparixin L-lysine salt (Cohort 3: loading dose of 6.8 mg/kg/h for 30min, maintenance dose of 4.2 mg/kg/h for 47.5h).



The four metabolites were excreted in urine in different proportion: the urinary excretions of DF 2243Y, DF 2188Y, methanesulfonamide and ibuprofen ranged between 30-40%, 18-25%, 10-12% and 0-7-1% of the dose, respectively. Total recovery in urine of reparixin and its metabolites (ibuprofen, DF 2188Y, DF 2243Y and methanesulfonamide) accounted for 60.12 ± 13.46%, 71.43 ± 19.96%, 75.40 ± 14.78% of administered doses, for the three doses, respectively. Reparixin and ibuprofen were detectable in the faeces over 60h at very low amounts and only in few subjects (Table 18).

Table 18: Ibuprofen, DF 2243Y, DF 2188Y and methansulfonamide pharmacokinetic parameters after after i.v. infusion of reparixin L-lysine salt in healthy volunteers.

REP0102 (ME0735), M0409			Cohort 1 (n=9)		Cohort 2 (n=9)		Cohort 3 (n=9)	
	Dose regimen		dose: 3.1 mg/kg/h for 30min, 1 mg/kg/h for 47.5h		dose 3.8 mg/kg/h for 30min, 2 mg/kg/h for 47.5h		Dose: 6.8 mg/kg/h for 30min, 4.2 mg/kg/h for 47.5h	
	Target group	µg/mL	10		20		30	
			Mean	SD	Mean	SD	Mean	SD
Ibuprofen	**Cinitial**	µg/mL	**0.94**	0.37	**2.01**	0.94	**4.12**	1.54
	Cmax	µg/mL	**1.25**	0.59	**2.39**	1.20	**4.28**	1.56
	Tmax	h	**24.00**[1]	23.98-47.88	**36.07**[1]	0.75-48.28	**48.23**[1]	24.02-48.62
	$t_{½}$	h	**2.96**	0.56	**2.65**	0.38	**2.84**	0.53
	AUClast	h µg/mL	**49.04**	22.10	**96.45**	44.70	**183.84**	72.51
	AUCtot	h µg/mL	**50.59**	22.04	**101.17**	47.47	**185.58**	72.93
	Ae_{0-60}	mg	**12.40**	7.27	**32.52**	24.74	**72.83**	33.21
	Ae_{0-60}	% of dose	**0.71**	0.37	**0.93**	0.59	**1.01**	0.42
	CL_R	mL/h	**244.4**	76.1	**317.2**	89.77	**397.5**	103.5
	Total faecal excretion $_{0-60}$	µg	**NC (N = 2)-**		**NC (N = 0)-**		**NC (N = 1)-**	
DF 2243Y	**Cinitial**	µg/mL	**2.79**	0.58	**6.42**	1.19	**15.98**	3.15
	Cmax	µg/mL	**3.20**	0.80	**7.38**	1.44	**17.34**	3.19
	Tmax	h	**24.17**[1]	18.0-48.5	**48.07**[1]	24.0-48.3	**48.12**[1]	24.0-48.9
	$t_{½}$	h	**2.03**	0.16	**1.93**	0.28	**1.84**	0.14
	AUClast	h µg/mL	**131.03**	29.66	**288.29**	50.45	**657.80**	115.25
	AUCtot	h µg/mL	**131.35**	29.64	**288.66**	50.48	**658.35**	115.41
	Ae_{0-60}	mg	**792.73**	200.26	**1843.14**	494.95	**4302.34**	1231.71
	Ae_{0-60}	% of dose	**30.47**	7.20	**36.21**	9.12	**39.38**	9.09
	CL_R	L/h	**6.14**	1.59	**6.60**	2.23	**6.55**	1.63
DF 2188Y	**Cinitial**	µg/mL	**0.57**	0.18	**1.31**	0.25	**3.25**	0.64
	Cmax	µg/mL	**0.67**	0.16	**1.53**	0.27	**3.44**	0.57
	Tmax	h	**24.02**[1]	4.00-48.08	**35.95**[1]	24.03-48.08	**48.12**[1]	4.00-48.37
	$t_{½}$	h	**1.64**	0.22	**1.50**	0.27	**1.47**	0.21
	AUClast	h µg/mL	**26.65**	4.93	**58.26**	11.01	**132.91**	21.45
	AUCtot	h µg/mL	**26.76**	4.98	**58.35**	11.03	**133.00**	21.45
	Ae_{0-60}	mg	**450.92**	121.52	**1070.36**	341.84	**2628.33**	945.39
	Ae_{0-60}	% of dose	**18.10**	4.42	**21.90**	5.92	**25.20**	8.16
	CL_R	L/h	**17.24**	4.95	**18.89**	7.00	**19.36**	4.85
Methane sulfonamide	**$C_{initial}$**	(µg/mL)	**NA**	NA	**NA**	NA	**3.19**	1.32
	C_{max}	(µg/mL)	**NA**	NA	**NA**	NA	**4.08**	2.87
	t_{max}	(h)	**NA**	NA	**NA**	NA	**45.62**	5.4
	AUClast	h µg/mL	**NA**	NA	**NA**	NA	**129.59**	57.16
	AUCtot	h µg/mL	**NA**	NA	**NA**	NA	**160.17**	78.57
	AUCextra	(%)	**NA**	NA	**NA**	NA	**17.49**	4.67
	t½	(h)	**NA**	NA	**NA**	NA	**12.28**	3.62
	Ae_{0-60}	mg	**86.16**	30.94	**190.98***	72.35	**361.47#**	123.04
	Ae_{0-60}	% of dose	**10.84**	3.51	**12.25***	4.41	**11.20#**	4.16

[1] median and range values, NC – Not calculated very low amounts, NA : not applicable because this metabolite was analysed only at the highest dose, * subject 17 excluded from the mean calculations (if included 244.03±172.96 mg; 16.13±12.36%), # subject 36 excluded from the mean calculations (if included 325.84±157.08 mg; 10.09±5.14%)

6.1.3. Drug-drug interaction

Since in an *in vitro* study it was observed that reparixin was a slight inhibitor of CYP3A4, CYP2C9 [5.2.9], an *in vivo* study was performed to evaluate if reparixin L-lysine salt alters the pharmacokinetics of midazolam, a probe substrate for CYP3A4, and tolbutamide, a probe substrate for CYP2C9 [4].

The systemic exposure and the t_{max} of midazolam were not statistically different in the absence and presence of reparixin. Besides, for the 1-hydroxymidazolam (the main midazolam metabolite) the C_{max}, MR_t and t_{max} were not statistically different. AUC_t, AUC and MR were statistically different (15-18% higher) in the presence *vs.* absence of reparixin, however, this increase was not considered clinically relevant.

Plasma midazolam and 1-hydroxymidazolam pharmacokinetic parameters in absence or presence of reparixin are given in Table 19.

Table 19: Summary midazolam and 1-hydroxymidazolam pharmacokinetic and statistical results.

REP0103 (ME0757) n =14		MIDAZOLAM				Analysis of Variance (n=28)		
		MDZ/TLB		MDZ/TLB/RPT		90% CLM		
PK parameters/units		Mean	SD	Mean	SD	Lower (%)	Upper (%)	p-value
Cmax	ng/mL	**32.54**	13.18	**34.30**	15.18	89.67	120.20	0.658
Tmax[1]	h	**0.63**	0.25-3.00[1]	**0.75**	0.25-4.00[1]	-0.625	0.625	0.772[#]
$T_{½}$	h	**2.78**	0.37	**2.71**	0.55			
AUClast	h ng/mL	**126.19**	39.82	**133.29**	37.82	99.50	114.79	0.124
AUCtot	h ng/mL	**135.74**	44.32	**143.95**	43.69	99.79	114.88	0.110
PK parameters/units		**1-HYDROXYMIDAZOLAM**						
		MDZ/TLB		**MDZ/TLB/RPT**				
Cmax	ng/mL	**9.41**	3.88	**9.99**	5.17	84.60	118.14	0.998
Tmax[1]	h	**0.75**	0.28-2.00[1]	**0.88**	0.25-4.02[1]	-0.015	-0.25	0.914[#]
$t_{½}$	h	**2.06**	0.605	**3.04**[2]	1.37			
AUClast	h ng/mL	**26.34**	10.85	**30.96**	13.81	105.06	127.76	0.019*
AUCtot	h ng/mL	**30.67**	11.33	**40.22**[2]	14.78	106.99	130.30	0.012*
MRt	-	**0.212**	0.063	**0.239**	0.110	99.00	118.71	0.139
MR	-	**0.231**	0.068	**0.278**	0.128	104.35	126.27	0.026*

[1] median and range values; [2] λ_z and derived parameters not estimable in presence of reparixin for subjects 5, 7, 8; [#] Wilcoxon matched pairs test for t_{max},; **NA** - Not applicable; ***** - statistically significant; **MR** - metabolic ratio defined as AUCtot $_{1\text{-hydroxymidazolam}}$/AUCtot $_{midazolam}$; **MR_t** - metabolic ratio between defined as AUClast $_{1\text{-hydroxymidazolam}}$/AUClast $_{midazolam}$

The influence of reparixin on tolbutamide was evaluated by measuring the concentrations of tolbutamide and its metabolites (hydroxytolbutamide and carboxytolbutamide) in urine.

Tolbutamide cumulative amount, hydroxytolbutamide cumulative amount and urinary excretion ratio appeared to be similar in the absence and presence of reparixin. There appeared to be differences in the urinary cumulative amount and excretion ratio for carboxytolbutamide and mean $CCR_{0\text{-}8h}$ (combined 0-8 h concentration ratio for (metabolite1+metabolite2)/parent) in the absence and presence of reparixin, however the variability associated with these indices was very large.

The ER_{OH} (excretion ratio for hydroxytolbutamide/tolbutamide), and the combined concentration ratio $CCR_{0\text{-}8h}$ were not statistically different in the absence and presence of reparixin. There was a tendency for ER_{COOH} (excretion ratio for carboxytolbutamide/tolbutamide) to be higher (33%) in the presence *vs.* absence of reparixin and the confidence limits for $CCR_{0\text{-}8h}$ were very broad, outside the 80 to 125 range.

Css concentrations of total reparixin at 0.5 (in absence of midazolam and tolbutamide) and 8h (in presence of midazolam and tolbutamide) indicated that the targeted Css 30 µg/mL was achieved. Corresponding free reparixin concentrations were considerable higher in presence of midazolam and tolbutamide than prior to midazolam and tolbutamide administration.

Concentrations of ibuprofen were also 5 times higher after midazolam and tolbutamide administration than prior to midazolam and tolbutamide administration. This behaviour is not different from that seen in a previous study [2].

Reparixin free fraction appeared to increase by 50% after midazolam and tolbutamide administration. However, this increase did not appear to correlate with a corresponding increase in total concentrations of reparixin. It is likely that the 50% increase in reparixin free fraction between the 0.5 and 8h is a result of a displacement by midazolam, tolbutamide and/or their metabolites (Table 20).

Table 20: Total, free reparixin and ibuprofen plasma concentrations.

REP0103 (ME0757)	Plasma concentrations in absence (0.5h) or in presence (8h) of midazolam and tolbutamide							
Nominal time after start of infusion (h)	Reparixin (n = 14)						Ibuprofen (n = 14)	
	Total reparixin (µg/mL)		Free reparixin (µg/mL)		Free Fraction (%)		(µg/mL)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5	28.98	4.22	0.026031	0.006333	0.0920	0.0284	0.52	0.17
8.0	27.71	8.93	0.037846	0.011337	0.1511	0.0688	2.60	0.62

In conclusion, the pharmacokinetics of midazolam, 1-hydroxymidazolam and tolbutamide were not altered to any clinically relevant extent by reparixin. Reparixin free fraction appears to increase by at least 50% over the infusion period, after midazolam/tolbutamide co-administration, while total reparixin concentrations remained stable. No clinically significant pharmacokinetic interaction between midazolam/tolbutamide and reparixin was observed.

6.1.4. Special population: renal impairment

The effect of renal impairment on pharmacokinetics of reparixin L-lysine salt was evaluated after the administration of 6.8 mg/kg/h over 30min (3.4 mg/kg) to ESRD patients and 2 mg/kg/h for 6h of i.v. infusion to patients with normal renal function and mild, moderate and severe renal impairment [5].

In ESRD patients, after the administration of reparixin at 6.8 mg/kg/h over 30min, the pharmacokinetic of reparixin was not influenced, as expected, by the degree of renal function. Viceversa renal function had a profound effect on plasma concentrations of the major metabolites (DF 2188Y and DF 2243Y) that were found to increase over time along with the increase of their elimination t½ (Figure 6- Table 21).

The pharmacokinetics of MSA (methansulfonamide) [3] appeared to be modified by the degree of renal function. MSA in ESRD patients was accumulated in plasma; Cmax corresponded to the last blood sampling time (t_{max} 25.6h) indicating that the main route of elimination of MSA is through the kidney, similarly to DF 2243Y and DF 2188Y (Table 21).

Figure 6: Mean plasma levels of reparixin and its metabolites after administration of reparixin L-lysine salt 6.8 mg/kg/h for 30min to end stage renal disease patients (haemodialysis).

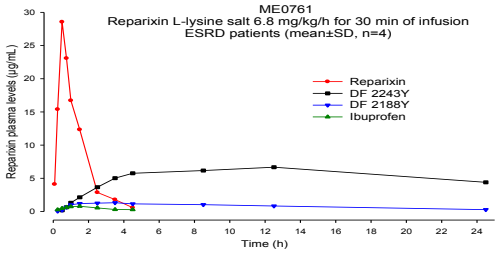


Table 21: Mean±SD reparixin, ibuprofen, DF 2243Y, DF 2188Y and methanesulfonamide pharmacokinetic parameters after administration of reparixin L-lysine salt 6.8 mg/kg/h for 30min to End Stage Renal Disease (ESRD) patients.

ME0761, M0409		$C_{initial}$ (µg/mL)	C_{max} (µg/mL)	t_{max}[1] (h)	AUClast (h)•(µg/mL)	AUCtot (h)•(µg/mL)	t½ (h)	$Ae_{0\text{-}24.5h}$ %	CL (mL/h/kg)	V_z (mL/kg)
Reparixin	**Mean**	**28.56**	**28.68**	**0.50**	**39.51**	**40.61**	**1.18**	**<0.001**	**55.9**	**99.8**
	SD	8.61	8.50	0.50-0.78	4.46	5.47	0.71	-	7.4	69.4
Ibuprofen	**Mean**	**0.47**	**0.80**	**1.31**	**2.28**	**4.19**	**2.67**	**0.003**	NA	NA
	SD	0.20	0.19	1.02-1.50	0.99	0.64	1.45	0.005	NA	NA
DF 2243Y	**Mean**	**0.18**	**6.89**	**12.51**	**127.60**	**NC**	**NC**	**4.85**	NA	NA
	SD	0.13	1.09	8.50-12.62	21.33	-	-	3.05	NA	NA
DF 2188Y	**Mean**	**0.20**	**1.47**	**3.54**	**19.77**	**23.38**	**8.66**	**1.60**	NA	NA
	SD	0.13	0.36	1.50-4.62	4.09	4.32	2.06	0.91	NA	NA
methanesulfonamide	**Mean**	**NQ**	**0.38**	**24.56**	**6.85**	**NC**	**NC**		NA	NA
	SD	-	0.11	0.06	2.20	-	-		NA	NA

NC = not calculable as C_{max} would have been included in the linear regression; [1] median and range values, NA= not applicable

After the administration of 2 mg/kg/h for 6h of i.v. infusion to normal volunteers and patients with different degree of renal impairment, the pharmacokinetics of total reparixin was quite similar. A slight decrease in elimination t½ was seen in mild, moderate, severe and CAPD patients but not in haemodialysis patients as compared to normal subjects (Table 30). The pharmacokinetics of unbound reparixin did not appear to be significantly affected by renal function. The fraction of unbound reparixin in plasma for normal subjects and in patients ranged between 0.0955 to 0.1334% (Table 22).

Table 30: Pharmacokinetic parameters of reparixin (mean±SD) after infusion of reparixin L-lysine salt 2 mg/kg/h for 6h in normal volunteers and in patients with different degree of renal function.

ME0761/CM8003/REP0203 – Renal impairment						
Reparixin PK Parameters	**Normal vol.** CLcr >80 mL/min (n=6)	**MILD** CLcr 80-51 mL/min (n=3)	**MODERATE** CLcr 50-30 mL/min (n=2)	**SEVERE** CLcr <30 mL/min (n=2)	**ESRD CAPD** (n=3)	**ESRD Haemodialysis** (n=3)
Dose (infusion duration)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)
$C_{initial}$ (µg/mL)	**11.70**±2.75	**7.13**±0.83	**8.27**	**6.45**	**7.01**±1.61	**6.66**±2.51
C_{max} (µg/mL)	**12.36**±2.47	**8.54**±1.15	**11.84**	**8.58**	**7.89**±2.09	**8.88**±0.58
AUC_{last} (h•µg/mL)	**74.83**±14.50	**49.54**±8.67	**68.71**	**43.74**	**44.17**±13.35	**46.13**±7.419
$AUC_{0-\infty}$ (h•µg/mL)	**75.44**±14.67	**50.23**±8.94	**69.34**	**44.31**	**44.87**±13.47	**46.85**±7.34
t½ (h)	**1.20**±0.43	**0.96**±0.78	**0.91**	**0.53**	**0.62**±0.08	**1.48**±0.58
CL (mL/h/kg)	**107.58**±16.42	**161.70**±26.25	**114.29**	**179.13**	**188.90**±66.71	**171.57**±24.87
Vz (mL/kg)	**181.17**±52.8	**201.84**±129.28	**152.72**	**133.73**	**168.63**±69.10	**362.57**±153.65
$Ae_{(0-30h)}$ (µg)	**2.24**±1.99	**3.27**±3.68	**0.70**	**3.27**	**6.83**±9.14	**0.49**±0.693
Urinary excretion (% of dose)	**0.0004**±0.0004	**0.0006**±0.0006	**0.0002**	**0.0005**	**0.013**±0.018	**0.00090**±0.000 13

Table 22: Unbound concentrations of reparixin (ng/mL) in normal subjects and in patients with different degree of renal impairment after reparixin L-lysine salt i.v. infusion of 2 mg/kg/h for 6h.

ME0761/CM8003/REP0203 – Renal impairment												
	Normal CLcr >80 mL/min) **(n=6)**		**Mild** CLcr 80-51 mL/min **(n=3)**		**Moderate** CLcr 50-30 mL/min **(n=2)**		**Severe** CLcr <30 mL/min **(n=2)**		**ESRD CAPD (n=3)**		**ESRD Haemodialysis (n=3)**	
Time	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
30 min	**4.51**	1.09	**4.64**	0.63	**4.05**	-	**3.94**	-	**3.70**	0.55	**4.19**	1.62
3 h	**11.13**	2.85	**14.73**	1.67	**15.84**	-	**14.95**	-	**9.17**	1.42	**11.30**	9.04
6 h	**14.37**	9.06	**16.59**	6.93	**15.46**	-	**12.62**	-	**8.47**	1.42	**13.04**	7.87
fu (%) (range)	**0.0955**±0.0290 (0.039-0.139)		**0.1574**±0.0884 (0.0488-0.2776)		**0.1148** (0.0362-0.1835)		**0.1655** (0.0321-0.3691)		**0.1124**±0.0643 (0.0643-0.2457)		**0.1332**±0.0864 (0.0562-0.2867)	

The plasma pharmacokinetics of ibuprofen was variable but did not appear to be significantly affected by renal function (Table 23).

The elimination of DF 2188Y and DF 2243Y was prolonged in renal disease patients resulting in accumulation of plasma levels, as shown by statistically significant increases in exposure (AUC) with deteriorating renal function (Table 23).

The degree of renal function had a substantial effect on the excretion of metabolites in urine, with excretion of ibuprofen, DF 2188Y and DF 2243Y as a percentage of dose considerably lower in ESRD patients as compared to normal subjects.

There were no apparent gender differences in the pharmacokinetics of reparixin, ibuprofen, DF 2188Y and DF 2243Y.

The method of dialysis in ESRD patients did not appear to affect the pharmacokinetics of reparixin, unbound reparixin and ibuprofen. On the contrary, DF 2188Y and DF 2243Y exposure (AUC) appeared to be greater and % excreted in urine appeared to be lower in haemodialysis patients compared to CAPD patients.

The difference in the pharmacokinetics of reparixin, ibuprofen, DF 2188Y and DF 2243Y had no significant clinical implications regarding the safety and tolerability of reparixin.

Table 23: Pharmacokinetic parameters of ibuprofen, DF 2243Y and DF 2188Y (mean±SD) after infusion of reparixin L-lysine salt 2 mg/kg/h for 6h in normal subjects and in patients with different degree of renal function.

ME0761/CM8003/REP0203 – Renal impairment						
	Normal vol. CLcr >80 mL/min (n=6)	**MILD** CLcr 80-51 mL/min (n=3)	**MODERATE** CLcr 50-30 mL/min (n=2)	**SEVERE** CLcr <30 mL/min (n=2)	**ESRD CAPD** (n=3)	**ESRD Haemodialysis** (n=3)
Dose (infusion duration)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)	**2** mg/kg/h (6 h)
Ibuprofen						
$C_{initial}$ (µg/mL)	**1.46**±0.31	**1.66**±0.50	**1.76**	**1.69**	**1.40**±0.48	**1.59+**±0.58
C_{max} (µg/mL)	**1.47**±0.33	**1.75** ±0.60	**1.79**	**2.10**	**1.40**±0.48	**1.73**±0.43
AUC_{last} (h•µg/mL)	**8.94**±2.56	**12.85**±5.73	**19.87**	**14.42**	**12.87**±9.73	**10.10**±3.59
$AUC_{0-\infty}$ (h•µg/mL)	**10.05**±2.45	**13.53**±2.85	**21.92**	**NC**	**NC**	**13.99**±3.37
t½ (h)	**2.44**±0.46	**3.96**±0.75	**5.95**	**NC**	**NC**	**4.40**±3.32
Ae (% of dose)	**0.75**±0.16	**1.90**±1.63	**0.41**	**0.70**	**0.01**±0.01	**0.05**±0.03
DF 2243Y						
$C_{initial}$ (µg/mL)	**4.30**±1.13	**8.64**±2.18	**20.23**	**14.67**	**17.32±**1.91	**11.16±**0.58
C_{max} (µg/mL)	**4.74**±1.15	**9.23**±2.89	**21.88**	**18.46**	**20.89**±4.09	**16.20**±0.84
AUC_{last} (h•µg/mL)	**26.81**±5.64	**59.76**±11.85	**218.43**	**253.40**	**369.99**±45.61	**340.51**±23.15
$AUC_{0-\infty}$(h•µg/mL)	**27.01**±5.61	**61.32**±12.46	**227.80**	**309.64**	**714.52**±91.00	**1055.79**±360.65
t½ (h)	**1.50**±0.19	**4.28**±3.88	**5.29**	**10.06**	**25.96**±0.91	**44.32**±15.82
Ae (% of dose)	**35.94**±3.84	**31.06**±4.63	**36.28**	**24.03**	**9.04**±3.25	**4.30**±3.24
DF 2188Y						
$C_{initial}$ (µg/mL)	**0.72**±0.15	**1.73**±0.23	**3.62**	**3.89**	**4.20**±0.92	**4.85**±0.14
C_{max} (µg/mL)	**0.74**±0.15	**1.87**±0.14	**3.70**	**3.92**	**4.92**±1.19	**5.22**±0.48
AUC_{last} (h•µg/mL)	**4.32**±0.92	**10.87**±1.28	**31.56**	**36.52**	**57.26**±18.73	**82.17**±20.76
$AUC_{0-\infty}$ (h•µg/mL)	**4.38**±0.93	**11.06**±1.23	**31.73**	**37.06**	**60.60**±21.02	**104.91**±49.41
t½ (h)	**1.21**±0.28	**3.89**±3.72	**3.36**	**4.16**	**6.16**±0.68	**10.54±**6.18
Ae (% of dose)	**19.32**±3.46	**17.76±**3.58	**16.28**	**11.81**	**3.22±**1.50	**1.40±**064

( ) - range; NC - Not calculable (only two data points in terminal phase**);** NQ = not quantifiable (LLOQ 0.1µg/mL); NC not calculated

6.2. SAFETY AND EFFICACY

A total of 404 subjects have been treated in phase 1 and phase 2 completed (CSR issued) clinical studies. Among these, 266 have been exposed to reparixin.

During the Phase 1 studies a total of 166 subjects of whom 103 normal volunteers (including 3 females) and 17 patients with different grade of renal impairment (including 5 females),16 patients undergoing cardiopulmonary by pass (including 6 females) and 30 patients with metastatic breast cancer were exposed to reparixin. A total of 65 subjects were treated with placebo.

In phase 2 studies, a total of 100 patients, 46 undergoing lung transplantation (23 M and 23 F), 48 patients undergoing kidney transplant (including 17 females) and 6 patients undergoing intrahepatic islet transplantation (3 M and 3 F) have been treated with reparixin. In the islet transplantation study, 4 patients received reparixin twice.

Out of the 166 subjects exposed to reparixin in Phase 1 studies, 30 female patients with metastatic breast cancer received reparixin oral tablets in combination with paclitaxel in a phase 1b clinical trial.

6.2.1. Summary

The first Phase 1 study (REP0101) was conducted in healthy male volunteers receiving ascending doses of reparixin L-lysine salt (1-16 mg/kg) administered by short i.v. infusion (30min). Reparixin resulted well tolerated at all doses, with minor and unspecific AEs not dose correlated. Pharmacodynamics, assessed by measuring migration of human isolated PMN in response to CXCL8 pre treatment and at 1h after the end of infusion, did not reveal any statistically significant treatment related relationship, apart an effect between pre and post dose values for specific migration at the highest dose level (16 mg/kg). It should be borne in mind that the migration test was used as an explorative tool, being aware that it has never been used as routine test to show the clinical effect and/or the dose response relationship of a drug. Besides, the low number of subjects exposed at each dose level would make any comparisons between treatments solely speculative.

The second Phase 1 study (REP0102), conducted in healthy male volunteers, was drug Css driven, and for each dose level, the infusion rate was calculated upon the PK results from the previous study or dose level in order to reach within 30min from the start of the infusion and then to maintain a prefixed target Css up to 48h total. Twelve subjects (9 active, 3 placebo) were dosed in each dose level. Target Css were 10, 20 and 30 µg/mL. Reparixin resulted well tolerated; again AEs were minor and not dose related. A clear indication of local toxicity, with cannula site reactions and infusion site thrombosis were observed in the subjects treated with reparixin in the first dose level. Local toxicity was clearly related to the drug concentration of the infused solution and to the extremely low rate of infusion as the two subsequent and higher doses were given with no apparent signs of venous toxicity, by administering a more diluted solution at a higher infusion rate. Also in this study no relevant statistically significant relationship was observed for the inhibition of CXCL8 mediated PMN chemotaxis and CXCL8 and FLMP induced CD11b expression, apart from a statistically significant reduction in the 24h, baseline corrected, specific migration for the 30 µg/mL group. However, an a posteriori interpretation of these data suggests that it must be taken into account that the *ex-vivo* chemotaxis assay presents a major drawback. PMN were exposed to reparixin during the *in vivo* infusion period, but not during the purification procedure, because of extensive cell washings and gradient step centrifugation. Thus PMN were not exposed to reparixin for at least 40min immediately before activation with CXCL8. Reparixin is a reversible inhibitor of CXCL8 receptors. Thus, it is conceivable that the *ex vivo* chemotaxis assay underestimates the actual inhibition of CXCL8 activity obtained by exposing PMN to reparixin. The wash out effect does not obviously apply to the *in vivo* condition, in which PMN were continuously exposed to reparixin.

A third Phase 1 study (REP0103) was performed in 14 healthy male volunteers in order to verify whether reparixin can interact with the metabolism of drugs metabolized by CYP3A4 and CYP2C9 in a clinically significant manner, using midazolam and tolbutamide, respectively, as probe substrates. There were no deaths, serious AEs or withdrawals during the study. There were no clinically relevant changes in clinical laboratory measurements, ECGs, vital signs, or physical examinations during the course of the study and no clinical relevant differences between treatments. In conclusion, no safety concerns were raised during co-administration of midazolam/tolbutamide with reparixin.

The fourth Phase 1 study (REP0203) was designed in order to obtain information on safety and pharmacokinetic parameters of reparixin in male and female subjects with different degree of renal impairment. The study was performed in 2 stages, firstly a pilot phase in Stage A in which a single i.v. infusion of 6.8 mg/kg/h was given to 4 patients with ESRD over 30min (i.e. 3.4 mg/kg). Based on the data obtained from Stage A, the dose used in Stage B was 2 mg/kg/h for 6h of i.v. infusion with the aim of achieving Css in the range of 10-30 µg/mL. There were 38 subjects planned (4 for Stage A and 34 for Stage B). The study was discontinued after a total of 23 subjects were enrolled and completed the study (6 normal renal function, 3 mild, 2 moderate, 2 severe renal impairment patients and 10 ESRD), as described in 6.2.2.4. The results of Stage A and B, despite the reduced number of subjects, provided a comprehensive and accurate evaluation of the effect of renal function on the pharmacokinetics of reparixin and its main metabolites. The i.v. infusion of 2 mg/kg/h of reparixin L-lysine salt both in patients with different degree of renal impairment and in subjects with normal renal function was safe and well tolerated. Very few AEs were reported, the majority of which were mild in intensity and unlikely to be due to the study drug.

The fifth study (REP0107) was a pilot study designed to assess if a 24 h continuous infusion of reparixin can reduce the PMNs and the inflammatory mediators influx, during acute inflammation into cantharidin blister. There was a very high variability in response to cantharidin and only two out of 8 subjects were evaluable. The analysis did not detect any significant difference between treatments. Nevertheless, the total cell count in the blister fluid of subjects treated with reparixin was on average one half of the total cells counted in the blister fluid of placebo-recipients which might be explained by the inhibitory action displayed by reparixin, although the proportion of PMN, eosinophils and mononuclear cells seemed to be unaffected. Unfortunately the blister volumes were not sufficient to evaluate the differences in the inflammatory mediators. Reparixin was present in the cantharidin-induced blister at a concentration similar to that found in plasma. No meaningful effect of reparixin on vital signs, ECGs or laboratory parameters was observed. No treatment-related AE occurred in subjects treated with reparixin.

The sixth study (REP0109) was designed to assess the reproducibility of the cantharidin blister model and determine the potential of the model for detecting putative efficacy of novel anti-inflammatory compound by evaluating the effect of dexamethasone and reparixin. Seven male volunteers were enrolled into part A of the study and 14 into part B (13 of whom received study drug). There were no statistically significant differences observed in the inflammatory mediators from fluid taken from different blister sites at the same time point without treatment. Due to errors in sample handling with flow cytometry samples, it was not possible to perform a scientifically meaningful statistical analysis on or analytic interpretation of any flow cytometry data. Therefore, no conclusion could be made regarding these data. The cantharidin blister model was safe and well tolerated in Part A of the study, and cantharidin plus co-administration of dexamethasone, reparixin and placebo were well tolerated in Part B.

For the first US-Canada Phase 2 study (REP0104), 100 patients undergoing single or bilateral lung transplant were planned. The study was completed in September 2007. One hundred and one patients (46 on reparixin, 55 on placebo) were included in the ITT and safety population out of 114 enrolled. The patients were randomized to receive 48 h i.v. continuous infusion (loading: 4.488 mg/kg/h for 30min, maintenance: 2.772 mg/kg/h for 47.5h) of either reparixin or placebo. At Month 1, there was no statistically significant difference in the primary efficacy variable, PaO2/FiO2 ratio, between the reparixin and placebo groups, considering both the measured values and values corrected for altitude (Denver, Center 03). No significant differences between the reparixin and placebo groups were reported for any of the secondary efficacy variables assessed. At Month 12, there were no statistically significant differences in any of the other secondary variables, i.e. lung function tests, BOS scores and acute rejection episodes. Lack of statistical difference was maintained regardless of the transplant type. Death occurred in 7 patients, all in the placebo group. One-year survival analysis demonstrated that there was a statistically significant difference in patient survival between the reparixin and placebo groups. A total of 28 patients experienced SAEs. Only 8 SAEs in 6 patients were judged possibly related to study drug. No particular safety concerns were raised. The AE profile was similar for both reparixin and the placebo group. PMN counts in BAL specimens evaluated in 37 available slides were similar between the two treatment groups. PK analysis was performed on samples from 45 patients. Plasma levels of unbound reparixin were in the range of those expected to be pharmacologically active.

The second Phase 2 study (REP0204) was conducted in US, Italy, Spain and France. Seventy-two patients undergoing kidney transplantation, at increased risk of developing DGF, were planned for inclusion. The study was completed in June 2008. Out of 80 patients randomized, 74 patients (25 reparixin continuous

infusion; 23 reparixin intermittent infusion; 26 pooled placebo) and 73 patients (24 reparixin continuous infusion; 23 reparixin intermittent infusion; 26 pooled placebo) were included in the safety and ITT analysis, respectively. The patients were randomized to receive reparixin or placebo as continuous infusion of 2.772 mg/kg/h for 12h or 12 intermittent i.v. infusions of 2.244 mg/kg for 30min with 1.5h intervals over a total period of 22.5h. At Month 1, there was no significant difference between the treatment groups in the primary efficacy variable (CrCl values measured 1–3 hours and 10–12 hours post-transplant) and in the secondary efficacy variables assessed. At Month 12, there were no differences in any of the secondary variables, i.e. renal function tests, biopsy proven acute rejection episodes, patient and graft survival. Lack of difference was maintained regardless of the biological induction type. SAEs were reported in 21 patients. SAEs possibly related to study drug were reported for only 2 patients, both in the reparixin continuous infusion group. Overall, the AE profile was similar for both reparixin groups and the placebo group. An assessment performed by DMC on a possible study-related higher incidence of thrombosis, excluded the potential relationship of reparixin with these events. The plasma concentrations of reparixin (total and unbound) and its major metabolite, DF2243Y, were derived from 46 patients. The concentration of total reparixin and its metabolite were consistent with the expected values. Unbound reparixin concentrations were about 2–3 times higher than those found in healthy volunteers and the corresponding free fractions were increased with respect to the healthy volunteers.

A phase 2 study (REP0110) was conducted at one center in Italy (another site in Germany was planned to participate, but did not enrol patients). 10 T1D patients undergoing intra-hepatic transplantation of pancreatic islets were planned. The study was completed in April 2013. Out of 9 patients randomized, 6 were assigned to receive reparixin treatment (2.772 mg/kg body weight/hour i.v. continuous infusion for 7 days); 3 were randomized to receive no additional experimental intervention (control group). Improved outcome was shown in the patients on reparixin, when glycemic control was measured by decreases from pre-transplant in mean daily insulin requirement and in mean percentage fasted HbA1c. Improved β-cell function was also shown by greater C-peptide AUC/IEQ/kg values in the patients on reparixin compared with patients from the control group. Similarly, mean values for TEF/IEQ in the patients on reparixin were greater than those from the control group. Out of 4 patients on reparixin who received the second islet transplant, 3 reached insulin-independence which was maintained in 2 patients up to at least 12 months. There were 11 reports of SAEs in 3 patients taking reparixin compared with 1 report in 1 patient in the control group. One patient (Patient 0110) on reparixin experienced several related SAEs after a medication error (anemia, gastrointestinal hemorrhage, nausea, vomiting). No other SAEs were considered to be drug-related. No ADR led to withdrawal of patients on the study. Reparixin concentrations and unbound reparixin concentrations in plasma were similar to those obtained in healthy volunteers.

Based on the results from the pilot trial, a phase 3 study (REP0211) is ongoing in 8 sites in 4 EU countries and 1 site in the US to further evaluate the efficacy of reparixin in pancreatic islet transplantation. To date, 49 patients have been randomized, and 44 underwent treatment and transplant. 23 patients have received a second islet infusion. 13 patients have completed one year follow-up after last islet infusion.

In addition, a phase 2/3 study (REP0112) is being conducted at 6 centers in the US to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation. One Canadian center is going to be opened. To date, 34 patients have been randomized and transplanted.

A phase 2, multicenter open-label randomized pilot clinical has been authorized in the Russian Federation to assess the efficacy and safety of Reparixin in patients undergoing liver transplantation (TPL-RPX-01). Patients will be treated with Reparixin for 7 days in combination with standard immunosuppressive regimen. The primary efficacy end point will be the determination of the proportion of patients that will develop early allograft dysfunction. Secondary endpoints will investigate liver function parameters and patients outcome. The safety of Reparixin in this setting will be also analyzed by comparing adverse events, laboratory and vitals in Reparixin treated patients and in patients not treated with Reparixin. The trial is currently open to enrolment.

Two academic and independent clinical trials with i.v. reparixin were implemented by the Medical University of Vienna. Upon Ethics Committee approval Dompé provided only the study drug under a Material Transfer Agreement.

In the first academic study (EK 116/2004) the effects of reparixin on humoral and cellular parameters in LPS-induced acute systemic inflammation were tested. The study was a randomized, double-blind, placebo-

controlled parallel group trial. Twenty healthy volunteers were randomized. The results indicated that LPS-induced neutrophilia was not significantly affected by reparixin in human volunteers. Consistently, reparixin did not alter the lymphocyte or monocyte counts and had no effect on LPS-induced systemic inflammation as measured by tumor necrosis factor alpha (TNF-α) or interleukin-6 (IL-6) release. Regulation of IL-8 receptors CXCR1 and 2 and the degranulation marker CD11b showed the expected kinetics. Reparixin had no effect on thrombin formation as measured by prothrombin fragment (F_{1+2}). Finally, reparixin, i.e. its metabolite ibuprofen effectively suppressed the cyclo-oxygenase pathway as assessed by serum thromboxane levels. In conclusion, the study showed that reparixin was safe but had no impact on endotoxin induced inflammation. No AEs were reported.

The second academic study (EK 231/2004), a placebo-controlled, randomized pilot trial, was conducted in 32 patients undergoing elective coronary artery bypass grafting with cardiopulmonary bypass (CPB), treated with infusion of reparixin (loading: 4.488 mg/kg/h for 30min; maintenance: 2.772 mg/kg/h for 8h). The study was designed to investigate if reparixin attenuates/decreases CPB induced neutrophilia and/or neutrophil migration into the alveolar space and if there is a trend towards decreased release of standard markers of myocardial injury under CXCL8 antagonism by reparixin. Reparixin was well tolerated and no serious AEs were reported. No significant differences were reported in regard to patient's demographics, operating room time, duration of CPB, cross clamp time, time to extubation, the amount of transfused blood products, core temperature, hemoglobin and lactate levels, administered catecholamines as well as in additional parameters. Neutrophil count declined in both groups with the beginning of CPB, with higher levels being determined in the placebo group ($p > 0.05$). After CPB, neutrophil count exceeded baseline levels in the two groups with a greater increase in the placebo group as compared to the reparixin group ($p < 0.05$). Significant group differences were also detected at 4 hours post-CPB. The rise of the neutrophil count after CPB was less marked in the reparixin group. This may indicate that inflammatory induced ischemia-reperfusion injury is less severe in patients after surgery on CPB when IL-8 is inhibited.

In breast cancer (oral formulation), one phase 1 study has been completed, one pilot biomarker study is ongoing and a phase 2 study is in the process of start up.

A phase 1b study (REP0111) was conducted at five centers in the US to evaluate the safety, PK profile, and effects of reparixin oral tablets in combination with paclitaxel on Cancer Stem Cell (CSC) markers. 33 patients were enrolled and 30 of those received the combination of reparixin oral tablets and paclitaxel infusion for the treatment of metastatic breast cancer. Three cohorts were completed in sequence with increasing reparixin doses of 400 mg t.i.d, 800 mg t.i.d, and 1200 mg t.i.d. on days 1-21 of 28-day cycles. An additional expansion cohort at the 1200mg t.i.d. was also completed. The data cut-off for the main analysis was June 25, 2014. At that time, seven patients were still actively receiving treatment.

All patients reported at least one TEAE during the reporting period (up to 60 days post-LPI). The majority of the study population (76.7%) reported at least one TEAE that was considered by the investigator to be related to the combination treatment. Six patients reported at least one treatment-emergent serious adverse event (none of them related to reparixin) and one patient had a TEAE (progressive disease) that resulted in death. There were no > grade 3 AE. There was no evident dose-effect of increasing reparixin dose on the incidence, severity or profile of TEAEs experienced by the treatment groups. Overall, the combination treatment was safe and well tolerated across the three dose levels explored.

Blood sampling was performed for all patients in cohort 1 and 2 and in 12 patients of cohort 3 (this cohort was expanded). Blood was collected at the first cycle only. Over the range of 400 to 1200 mg (5.7 to 17.1 mg/kg), reparixin C_{max} and AUC_{0-8} increased as dose increased, in a manner that was approximately dose proportional. The fraction of unbound to total drug appeared to be independent on cycle day, but dependent on dose level. For 400, 800 and 1200 mg dose groups, the average ratio of unbound to total drug (as %) was 0.07%, 0.11%, and 0.14%, respectively. There appeared to be little or no accumulation of reparixin over the 21 day dose period. Reparixin $t_{1/2}$ after oral administration was about 2 hours and was independent of dose level or cycle day. Paclitaxel levels were essentially the same in each treatment group and on each treatment day. C_{max} and AUC_{0-inf} were comparable to levels previously reported. There was little or no accumulation from Day 1 to Day 8.

Objective responses were observed across the three reparixin dose levels explored, with a sizeable median Time to Progression (TTP). However, in this study, the numbers of patients in each treatment group were too small to make definitive clinical interpretations regarding the differences in tumor responses between or

within treatment groups over the course of the study until data cut-off date for first analysis. Some interesting long term responses have been observed that, in combination with the safety and tolerability profile, warrant further investigation of the combination.
Circulating tumor cells (CTC) decreased from baseline or remained the same in 17/20 patients for whom at least two samples were available, The protocol foresaw optional tumor biopsies before and after treatment, but no biopsies were obtained.

A pilot "window of opportunity" clinical study (REP0210) is ongoing in patients with operable breast cancer where reparixin is administered as single agent in the time period between clinical diagnosis and surgery. This study aims to evaluate the effects of orally administered reparixin on CSCs in the primary tumor and the tumoral microenvironment in an early breast cancer population. Enrolment to this study began in April 2013 and is ongoing.

The study is an open label, single agent study and is currently ongoing at 9 centers in the US. Patients with early stage breast cancer who are scheduled to have definitive breast surgery and who are not eligible for neoadjuvant treatment are enrolled to the trial and receive single agent oral reparixin 1000mg t.i.d. for 21 days prior to surgery.

Patients enrolled are stratified in to 2 groups:

Group A: ER+ and/or progesterone receptor positive/HER-2–
Group B: ER negative/progesterone receptor negative/HER-2–

Biopsies are obtained at prestudy and on Day 21 (after completion of the last dose of reparixin). In addition to collecting biopsies, a sample of the surgically removed tissue is also collected for analysis. Pharmacokinetic blood samples (PK's) are drawn on Day 1 and Day 21 on selected patients and at selected centers.

To date, 20 patients have been enrolled to study; 18 patients in Group A with ER+ and/or progesterone receptor positive/HER2– breast cancer, and 2 patients in Group B with ER negative/progesterone receptor negative/HER-2– breast cancer. So far, only one SAE (not related) has been reported.

A phase 2 randomized, double-blind, placebo-controlled study of the combination of reparixin and paclitaxel versus paclitaxel alone as front-line therapy for Metastatic TNBC is currently being set up. The primary objective of the study is to evaluate Progression-Free Survival (PFS) of the combination in this patient population. Secondary objectives include: determining median PFS (mPFS) and Overall Survival (OS), evaluating Objective Response Rates (ORR), and assessing the safety of the combination treatment. Additional exploratory objectives include: determining median Time to New Metastasis (TTM) and proportion of patients progressing with new metastatic lesions; comparing the incidence and severity of peripheral neuropathy between the two arms, and evaluating CSCs in metastatic tissue. The study is expected to activate in April 2015, and will be conducted in the US and EU.

Patients with pathologically documented metastatic TNBC, eligible for treatment with paclitaxel, and who have received prior (neo)adjuvant chemotherapy will be eligible for the study. Patients enrolled will be randomized 1:1 to receive either paclitaxel and reparixin or paclitaxel and placebo. The dose of paclitaxel will be 80mg/m^2 IV on days 1, 8, and 15 of a 28 day cycle. The dose of reparixin or placebo oral tablets will be 1200mg t.i.d. on days 1-21 of a 28 day cycle. New metastatic tissue or archival primary tissue will be collected to confirm TNBC and to evaluate CSCs.

Safety and Efficacy in Humans			
Type of Study	**Dose (mg/kg)/ Route/Frequency**	**Comments**	**Reference**
Phase 1 Clinical studies			
CLIN/TOL/PK Ascending dose, tolerability and PK 30 M Volunteers Age: 27±8 y (18-50) (4 active+ 2 placebo per cohort) Double-blind, placebo-controlled, ascending dose	1, 2, 4, 8, 16/i.v. infusion/30min * * = dose as reparixin L-lysine salt	There were no clinically significant changes in vital signs and laboratory parameters. There were no abnormalities in vital signs, physical examinations, ECGs and safety laboratory tests. In conclusion, the administration of reparixin L-lysine salt as a single 30min i.v. infusion was safe and well tolerated.	ME0706 (REP0101)[1] (§ 6.2.2.1) (PK § 6.1.2)
CLIN/TOL/PK Ascending dose, tolerability and PK 40 M volunteers Age: 27.9±9.5 (19-53) (9 active+3 placebo per cohort + 4 replacements for cohort 2) Double blind, placebo controlled, ascending dose.	**Cohort 1:** loading 3.1 mg/kg/h for 30min. * maintenance: 1 mg/kg/h for 47.5h. * **Cohort 2:** loading 3.8 mg/kg/h for 30min* maintenance: 2 mg/kg/h for 47.5h * **Cohort 3:** loading: 6.8 mg/kg/h for 30min* maintenance: 4.2 mg/kg/h for 47.5h * * = dose as reparixin L-lysine salt	Reparixin, administered as a 48h i.v. infusion targeting to reparixin SS concentrations of 10, 20 and 30 µg/mL, was well tolerated. There were no deaths, serious AEs or withdrawals. There were no clinically relevant changes in clinical LAB measurements, ECGs, vital signs, or physical examinations. No clear treatment-emergent pattern emerged for any of the immune cell surface markers measured. The most common AEs considered probably or possibly related to reparixin were cannula site reaction, injection site thrombosis, infusion site oedema, infusion site erythema, headache, hypoaesthesia, and flatulence, followed by one incidence each of lymphadenopathy, abdominal pain, dyspepsia, nausea, lethargy, athralgia, dizziness, somnolence, productive cough, erythema and pruritis. The majority of treatment-emergent AEs (98.0%) were mild, with only 2.0% being moderate in severity. There were no relevant statistically significant relationships in inhibition of CXCL8 mediated neutrophil chemotaxis or CXCL8 and FMLP induced CD11 expression in response to reparixin and placebo, apart from a statistically significant reduction in baseline corrected 24h specific migration for the highest target SS.	ME0735 (REP0102)[1] (§ 6.2.2.2) (PK § 6.1.2)
CLIN/INTERACTION Interaction study 14M volunteers Age: 27.4±1.8 y (20-40) Open label, cross over	**Day 1:** Midazolam 7.5 mg and Tolbutamide 500 mg **Day 3:** Reparixin L-lysine salt loading: 6.8 mg/kg/h for 30min maintenance: 4.2 mg/kg/h for 7.5h 70min after start of infusion Midazolam 7.5 mg and Tolbutamide 500 mg	The objectives of the study were to establish if reparixin alters the pharmacokinetics of midazolam, a probe substrate for CYP3A4, and tolbutamide, a probe substrate for CYP2C9. The most common AE considered probably or possibly related to study drugs was somnolence, followed by dizziness and one incidence each of cannula site reaction, dry throat, abnormal dreams and euphoric mood. The majority of treatment-emergent AEs (84.6%) were mild, with only 15.4% being moderate in severity. In conclusion, no safety concerns were raised during co-administration of midazolam/tolbutamide with reparixin.	ME0757 (REP0103)[1] (§ 6.2.2.3) (PK § 0)

Safety and Efficacy in Humans			
Type of Study	**Dose (mg/kg)/ Route/Frequency**	**Comments**	**Reference**
CLIN/TOL/PK Renal failure Volunteers Stage A: ESRD: 4 Haemodialysis (3M + 1F) Stage B: Normal renal function: 6 (3M+3F) Mild renal impairment: 3 (1M+2F) Moderate renal impairment: 2 (1M+ 1F) Severe renal impairment: (2M) ESRD: 3 CAPD (2M+1F) 3 Haemodialysis (3M) Open label	Stage A: 6.8 mg/kg/h for 30min * Stage B: 2 mg/kg/h for 6h* * = dose as reparixin L-lysine salt	There were no deaths, serious AEs or withdrawals. There were no clinically relevant changes in clinical LAB parameters, ECGs, vital signs or physical examinations. Reparixin both in Stage A (ESRD patients) and Stage B (normal subjects and patients with different degree of renal impairment, varying from mild to ESRD) was safe and well tolerated. Treatment emergent AEs were very few, mild or moderate in intensity, not related to the degree of renal impairment and comparable both in terms of frequency and nature to those observed in normal subjects. Very few AEs were reported, the majority of which were mild in intensity and unlikely due to reparixin. Only three AEs were classified as possibly related to reparixin.	ME0761 – CM8003 (REP0203)[1] (§ 6.2.2.4) (PK § 6.1.4)
CLIN/PD Pilot single-centre, phase 1, study to investigate leukocyte trafficking and cytokine production at site of inflammation using the cantharidin blister method. 8 M healthy vol.s Age: 32.3 ±7.9 y Randomised, double blind, cross-over, placebo-controlled study	Continuous infusion 2.772 mg/kg/h for 24 h* *dose as reparixin	The study was designed to assess if a 24 h continuous infusion of reparixin can reduce the PMNs and the inflammatory mediators influx, during acute inflammation into cantharidin blister. Only two subjects reacted to cantharidin both after treatment with reparixin and matching placebo. The volume of the blister fluid collected from the blisters was higher after treatment with reparixin. In particular after infusion of placebo only 4 exudate specimens could be processed and counted. Similarly after infusion of reparixin only 3 samples of exudate were processed and counted for the primary analysis The analysis did not detect any significant difference between treatments. Nevertheless the total cell count in the blister fluid of subjects treated with reparixin was on average one half of the total cells counted in the blister fluid of placebo-recipients which might be explained with the inhibitory action displayed by reparixin, although the proportion of PMN, eosinophils and mononuclear seemed to be unaffected. Unfortunately the blister volumes were not sufficient to evaluate the differences in the inflammatory mediators. All subjects showed detectable plasma levels of reparixin by the end of infusion. Mean (±SD) plasma concentration was 37.42±4.13 µg/mL. Reparixin was dosed also in the blister fluid samples of 3 subjects. The active substance was present in the cantharidin-induced blister at a mean concentration of 28.28±3.82 µg/mL. No meaningful effect of reparixin on vital signs, ECGs or laboratory parameters was observed. No treatment-related AE occurred in subjects treated with reparixin	CRO-PK-07-197 Dompé: REP0107 (§ 6.2.2.5)

Safety and Efficacy in Humans			
Type of Study	**Dose (mg/kg)/ Route/Frequency**	**Comments**	**Reference**
CLIN/PD Pilot single-centre, phase 1, study to investigate the reproducibility of the inflammatory response in the cantharidin blister model and the effect of dexamethasone and reparixin. 21 M healthy vol.s Part A: single visit. Part B: Randomised, open-label, placebo-controlled, 3-way crossover study.	Reparixin: Continuous infusion 2.772 mg/kg/h for 24 h* strating at the time of cantharidin application *dose as reparixin Dexamethasone: 6 mg single oral dose before and at the time of cantharidin application	This two-part study was designed to investigate the reproducibility of the inflammatory response in the cantharidin blister model (part A) and to determine the potential of the model for detecting putative efficacy of novel anti-inflammatory compound by evaluating the effect of dexamethasone and reparixin (part B). The study was completed in July 2009 There were no statistically significant differences observed in the inflammatory mediators from fluid taken from different blister sites at the same time point without treatment. Due to errors in sample handling with flow cytometry samples, it was not possible to perform a scientifically meaningful statistical analysis on or analytic interpretation of any flow cytometry data. Therefore, no conclusion could be made regarding these data. The cantharidin blister model was safe and well tolerated in Part A of the study, and cantharidin plus co-administration of dexamethasone, reparixin and placebo were well tolerated in Part B..	Dompé: REP0109 (§6.2.2.6)
CLIN/PK/TOL/PD/EFF Multicenter phase 1b dose escalation study to evaluate safety, PK profile, and as secondary objectives antitumor activity and effects on cancer stem cells markers of reparixin oral tablets in combination with paclitaxel in patients with metastatic breast cancer safety 30 F PK Plasma 19 F	3 Cohorts 400mg* oral tablets t.i.d. 800mg* oral tablets t.i.d. 1200mg* oral tablets t.i.d. Oral tablets taken continously for 21 days for each 28-day cycle. Each cohort in combination with paclitaxel 80mg/m2 on Days 1, 8, and 15 of each 28 day cycle PK only at cycle 1 * dose as reparixin	The study shows that oral reparixin administered with paclitaxel was safe and well tolerated in patients with HER-2 negative MBC at all dose levels explored. Neither SAEs nor AEs grade >3 were recorded. No evident dose effect of increasing reparixin dose on the incidence, severity of profile of TEAEs experienced by patients in the three cohorts. Reparixin C_{max} and AUC_{0-8} increased in approximately dose proportional manner over the range of 400 to 1200 mg. Within a given dose level, there was not a consistent difference in either Cmax or AUC0-8 for a given dose over the span of cycle from Day -3 to Day 21. There was no consistent trend in Rac0-8, so there appeared to be little or no accumulation of reparixin over the 21 day dose period. The mean t1/2 was about 2 hours and did not vary either as dose increased from 400 mg to 1200 mg, or over Days -3 to 21. Similarly, mean CL/F and Vz/F were about 5.4 L/hr and 15 L across all dose levels and study days. The fraction of unbound to total drug appeared to be independent on cycle day, but dependent on dose level. For 400, 800 and 1200 mg dose groups, the average ratio of unbound to total drug (as %) was 0.07%, 0.11%, and 0.14%, respectively. DF 2243Y C_{max} and AUC_{0-8} increased in a dose proportional manner, whereas DF 2188Y C_{max} and AUC_{0-8} increased in a greater than dose proportional manner. Ibuprofen C_{max} and AUC_{0-8} increased in a dose-related manner Several objective responses were recorded; however, due to the small numbers of patients within each treatment group and considering that single agent weekly paclitaxel has established activity against MBC, the response rate should be interpreted with caution. Some long term responses have been observed until the time of data cut off for analysis when 7 patients were still actively receiving treatment.	Dompé: REP0111 (§6.2.4.1)
Phase 2 Clinical studies			

Safety and Efficacy in Humans			
Type of Study	**Dose (mg/kg)/ Route/Frequency**	**Comments**	**Reference**
CLIN/EFF Prevention of primary graft dysfunction after lung transplantation Planned 100 (M/F) Randomized: 114 Included in the ITT & safety population: 101 (51M/50F) Randomised, parallel group, placebo controlled, multicentre (US, Canada).	loading: 4.488 mg/kg/h for 30min* maintenance: 2.772 mg/kg/h for 47.5h* *dose as reparixin	This clinical trial evaluated whether CXCL8 inhibition with reparixin leads to reduced severity of PGD in lung transplant patients. The primary efficacy endpoint was PaO_2/FiO_2 ratio measured on ICU admission and at 24h after ICU admission. Secondary efficacy endpoints were: the time profile of PaO_2/FiO_2 ratio; PGD score; time to freedom from mechanical ventilation; duration of ICU stay; ICU mortality; mortality in the first 30 days post-transplant; FEV_1 and FVC; BOS score; cumulative acute rejection episodes; patient survival rate. The safety and the pharmacokinetics of reparixin in the specific clinical setting were also evaluated. PMN count in BAL specimens was evaluated as a "mechanism of action-targeted" endpoint. The study was completed on 13 September 2007. No significant differences between the reparixin and placebo groups were reported for the primary efficacy variable and for any of the secondary efficacy variables assessed. Lack of statistical difference was maintained regardless of the transplant type. Death occurred in 7 patients, all in the placebo group. A total of 28 patients experienced SAEs. Only 8 SAEs in 6 patients were judged possibly related to study drug. The AE profile was similar for both groups. PMN counts in BAL specimens evaluated in 37 available slides were similar between the two treatment groups. PK analysis was performed on samples from 45 patients. Plasma levels of unbound reparixin were in the range of those expected to be pharmacologically active.	REP0104 (§ 6.2.3.1)
CLIN/EFF Prevention of delayed graft function after kidney transplantation Planned 72 (M/F) Randomized: 80 Included in the safety population: 74 (50M/24F) Included in the ITT population: 73 (50M/23F) Pilot, randomised, parallel group (3 arms), placebo controlled, multicentre (US, France, Italy, Spain).	Continuous infusion: 2.772 mg/kg/h for 12h * Intermittent i.v. infusion: 2.244 mg/kg for 30min* followed by 1.5h interval (12 doses over a total period of 22.5h) *dose as reparixin	This clinical trial evaluated whether CXCL8 inhibition with reparixin leads to improved functional and clinical outcomes in kidney transplant patients at increased risk of developing DGF. The primary efficacy endpoint was creatinine clearance (CrCl) in the immediate post-transplant period (1-3 and 10-12h after allograft reperfusion). Secondary efficacy endpoints were: SrCr, GFR, Urine output, dialysis within 7 days post-transplant, graft function, on the basis of SrCr on post operative Day 5. Iohexol clearance, duration of hospital stay, and mortality in the first 30 days post-transplant, SrCr and calculated CrCl at Month 1, 6 and 12 post-transplant, cumulative acute rejection episodes, and patient and graft survival rate. The safety and the pharmacokinetics of reparixin were also evaluated. The study was completed on 19 June 2008. There was no significant difference between the treatment groups in the primary efficacy variable and in the secondary efficacy variables assessed. Lack of difference was maintained regardless of the biological induction type. SAEs were reported in 21 patients. SAEs possibly related to study drug were reported for only 2 patients, both in the reparixin continuous infusion group. Overall, the AE profile was similar for both reparixin groups and the placebo group. The concentration of total reparixin and its metabolite were consistent with the expected values. Unbound reparixin concentrations were about 2–3 times higher than those found in healthy volunteers and the corresponding free fractions were increased with respect to the healthy volunteers.	REP0204[1] (§ 6.2.3.2)

Safety and Efficacy in Humans			
Type of Study	**Dose (mg/kg)/ Route/Frequency**	**Comments**	**Reference**
CLIN/EFF Improved functional and clinical outcome in intrahepatic islet transplantation patients. Planned 10 (M/F) Randomized :9 Pilot, open label, parallel group (2 arms), multicentre (Italy, (Germany)).	Continuous infusion: 2.772 mg/kg /h for 7 days* (168hrs), starting approximately 12hrs (6-16 hrs) before pancreatic islet infusion (4 patients received reparixin course twice) *dose as reparixin	This clinical trial evaluated whether reparixin leads to improved transplant outcome as measured by glycemic control following infusion of pancreatic islets. The primary efficacy endpoint was insulin-independence in the 75 +/- 5 day timeframe post-transplant. Secondary endpoints included insulin-independence up to 1 year post-transplant, time to achieve insulin-independence, total time of insulin-independence, change in average daily insulin requirements, absolute and % decrease in HbA1cA from pre-transplant levels, proportion of patients free of severe hypoglycemic events, proportion of patients free of hypoglycemic events with reduced awareness, Basal (fasting) and -10 to 120 minute time course of glucose, C-peptide, and insulin derived from the mixed meal tolerance test (MMTT), and β-cell function. The safety of reparixin in the specific clinical setting was also evaluated. The study was completed on 30 April 2013. Improved outcome was shown in the patients on reparixin, when glycemic control was measured by decreases from pre-transplant in mean daily insulin requirement and in mean percentage fasted HbA1c. Improved β-cell function was also shown by greater C-peptide AUC/IEQ/kg values in the patients on reparixin compared with patients from the control group. SAE's were reported in 11 patients. SAE's related to study drug were reported in only 1 patient.Overall, reparixin was found to be safe and well-tolerated for patients in this study. Reparixin concentrations and unbound reparixin concentrations in plasma were similar to those obtained in healthy volunteers.	REP0110[1] (§ 6.2.3.3)
Cinical studies not sponsorized by Dompé			
CLIN/PD Effects of reparixin on endotoxin induced inflammation Planned 20 volunteers (12 active + 8 placebo) Enrolled completed: 20 M Randomised, double-blind, placebo-controlled	Continuous infusion: 4.2 mg/kg/h for 8.5h * * = dose as reparixin L-lysine salt	This independent, pilot study was to explore the effect of reparixin on humoral and cellular parameters in LPS-induced acute systemic inflammation. There were no severe, serious or unexpected AEs and all enrolled volunteers completed the study. There were no clinically relevant changes in clinical LAB parameters, ECGs or vital signs physical examinations. LPS-induced neutrophilia was not significantly affected by reparixin in human volunteers. Consistently, reparixin did not alter the lymphocyte or monocyte counts and had no effect on LPS-induced systemic inflammation as measured by tumor necrosis factor alpha (TNF-α) or interleukin-6 (IL-6) release. Regulation of IL-8 receptors CXCR1 and CXCR2 and the degranulation marker CD11b showed the expected kinetics. Reparixin had no effect on thrombin formation as measured by prothrombin fragment (F1+2). Finally, reparixin, possibly via its metabolite ibuprofen, influenced the cyclo-oxygenase pathway as assessed by serum thromboxane levels, which were reduced up to 80% vs baseline.	EK 116/2004[1] (§ 6.2.5.1)
CLIN/EFF Pilot study on the effect of reparixin during cardiopulmonary bypass. Planned: 32 (16 active + 16 placebo) Enrolled completed: 32 (21M/11F) Randomised, parallel group (3 arms), placebo controlled.	Continuous infusion: loading: 4.488 mg/kg/h for 30min maintenance: 2.772 mg/kg/h for 8 h* *dose as reparixin	This academic pilot trial was conducted in patients undergoing elective coronary artery bypass grafting with cardiopulmonary bypass (CPB). It was designed to investigate if reparixin attenuates/decreases CPB-induced neutrophilia and/or neutrophil migration into the alveolar space and if there is a trend towards decreased release of standard markers of myocardial injury under CXCL8 antagonism by reparixin. Reparixin was well tolerated and no serious AEs were reported. No significant differences were reported in regard to patient's demographics, operating room time, duration of CPB, cross clamp time, time to extubation, the amount of transfused blood products, core temperature, hemoglobin and lactate levels, administered catecholamines as well as in additional parameters. The efficay analysis of the clinical results is still ongoing.	EK 231/2004[1] (§ 6.2.5.2)

CLIN = clinical, **PK** = pharmacokinetics; **PD** = Pharmacodynamics; **TOL** = tolerability, **EFF** = efficacy, **i.v.** = intravenous, **M** = male, **F** = female, [1] = GCP/GLP; * = dose as reparixin L-lysine salt.

6.2.2. Phase 1 clinical studies

6.2.2.1. Ascending dose tolerability and preliminary pharmacokinetic assessment of DF 1681B in healthy male volunteers after intravenous administration. [REP0101 (ME0706)]

The study was a parallel group, randomised, double–blind, placebo-controlled, ascending dose design. Reparixin L-lysine salt was tested at 1, 2, 4, 8 and 16 mg/kg by i.v. infusion of 300 mL over 30min. Each dose level was administered to cohorts of six subjects of whom four received reparixin and two placebo.

All subjects were included in the safety evaluation. Moreover, leukocyte subset assessment by flow cytometry was performed (CD3, CD4, CD8 for T lymphocytes; CD19 for B lymphocytes; CD45 for PMN leucocytes; CD14 for monocytes; CD56 for NK cells).

The effect of reparixin on human PMN chemotaxis induced by CXCL8 has been evaluated in order to establish a relationship between the *in vivo* i.v. administration of the compound and an inhibited capacity of human PMN to migrate *in vitro* in response to CXCL8. There were no relevant statistically significant treatment-related relationships in inhibition of CXCL8 mediated neutrophil chemotaxis in response to reparixin and placebo. However, it should be borne in mind that only 4 subjects were investigated at each dose level. Hence any comparisons between treatments remain somewhat speculative. Reparixin at all doses was well tolerated. There were no deaths, no serious AEs or withdrawals during the study. There were no clinically relevant changes in clinical laboratory measurements, ECGs, vital signs, and physical examinations. No clear treatment-emergent pattern emerged for any of the immune cell surface markers measured. There were a total of 25 AEs reported by 43.3% of subjects (13/30), of which 12.0% (3/25) from 3 subjects were assigned to pre-treatment and the remaining 88.0% (22/25) from 10 subjects were study drug treatment-emergent. A summary of the AEs is given in Table 24, Table 25 and Table 26.

The majority of AEs were of mild intensity. The most frequent was headache. Of the 3 AEs classified at injection site (bruising, erythema. pain) all were mild and related to the cannula sampling site.

Five AEs required a concomitant medication: 3 incidences of headache (paracetamol administered) and one each of sore throat and nasopharyngitis (concomitant medication unknown).

Subjects taking part in the study had recovered completely or were with mild intensity of AEs when they were discharged from the Clinical Unit. AEs were followed-up until resolution.

Table 24: Summary of AEs (number of events)

Total Adverse venEts	Pre-treatment	Placebo	Dose of reparixin L-lysine salt				
			1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg	16 mg/kg
	3 from 3 subjects	1 from 1 subject	2 from 2 subjects	1 from 1 subject	4 from 2 subjects	7 from 2 subjects	7 from 3 subjects
Relationship to Study Drug:							
Possible	0	0	1	1	2	4	4
Unlikely	0	1	1	0	2	3	3
Not related	3	0	0	0	0	0	0
Severity of Adverse Event:							
Mild	3	1	1	1	4	7	7
Moderate	0	0	1	0	0	0	0

Table 25: Summary of Treatment-emergent AEs

Adverse Event	Reparixin L-lysine salt					Placebo	Total
	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg	16 mg/kg		
No of subjects exposed	**4**	**4**	**4**	**4**	**4**	**10**	**30**
Cough	-	-	-	-	1(1)	-	1(1)
Dizziness (exc vertigo)	-	-	-	-	1(1)	-	1(1)
Earache	-	-	-	1(1)	-	-	1(1)
Euphoric mood	-	-	-	-	1(1)	-	1(1)
Fatigue	-	-	-	1(1)	1(1)	-	2(2)
Headache NOS	2(2)	1(1)	1(1)	1(1)	-	1(1)	6(6)
Injection site bruising	-	-	-	-	1(1)	-	1(1)
Injection site erythema	-	-	-	-	1(1)	-	1(1)
Injection site pain	-	-	-	-	1(1)	-	1(1)
Nasopharyngitis	-	-	2(2)	-	-	-	2(2)
Nausea	-	-	-	2(1)	-	-	2(1)
Somnolence	-	-	-	1(1)	-	-	1(1)
Sore throat NOS	-	-	1(1)	1(1)	-	-	2(2)

NOS = Not otherwise specified; x(y) = number of events (number of subjects

Table 26: Summary of Treatment-emergent AEs that were "possibly" related to study treatment

	Reparixin L-lysine salt						
Adverse Event	**1 mg/kg**	**2 mg/kg**	**4 mg/kg**	**8 mg/kg**	**16 mg/kg**	**Placebo**	**Total**
No of subjects exposed	**4**	**4**	**4**	**4**	**4**	**10**	**30**
Cough	-	-	-	-	1(1)	-	1(1)
Dizziness (exc vertigo)	-	-	-	-	1(1)	-	1(1)
Euphoric mood	-	-	-	-	1(1)	-	1(1)
Fatigue	-	-	-	-	1(1)	-	1(1)
Headache NOS	1(1)	1(1)	-	-	-	-	2(2)
Nasopharyngitis	-	-	2(2)	-	-	-	2(2)
Nausea	-	-	-	2(1)	-	-	2(1)
Somnolence	-	-	-	1(1)	-	-	1(1)
Sore throat NOS	-	-	-	1(1)	-	-	1(1)

NOS = Not otherwise specified; x(y) = number of events (number of subjects)

6.2.2.2. The tolerability, pharmacokinetics and pharmacodynamics of a continuous infusion of reparixin L-lysine salt in healthy male volunteers [REP0102 (ME0735)]

This Phase 1, single centre, randomised within dose group, double-blind, placebo-controlled, ascending single dose, parallel group study was designed to characterise the pharmacokinetics, to examine the safety and tolerability of reparixin and to assess the relationship between plasma concentration of reparixin and the pharmacological effects of inhibition of CXCL8 induced neutrophil chemotaxis and CXCL8 induced CD11b expression.

Four groups of male healthy volunteers (n=12) were treated (9 active+3 placebo) by 48h infusion with ascending doses to reach plasma Css of reparixin ranging from 10 to 40 µg/mL that should include clinically effective concentrations.

Following completion of the 30 µg/mL SS target group, a decision was made not to proceed to any further dose escalations. The reasons that led to this decision were: 1) daily dose (and actual drug exposure as per AUC24) reached a level which was not far from the safety level in some of the toxicology studies; 2) the target plasma concentration of 30 µg/mL was obtained by administering a substantial amount of product and although safety did not seem to be an issue, so it was possible that the administration of larger amounts of reparixin might induce non-specific effects that would be difficult to interpret; 3) a few subjects in this group had plasma concentrations well above the target concentration, which could be a sign of increased PK variability, possibly of non linearity due to saturation of plasma binding. This would have led to difficulties in achieving suitable target concentrations in all the volunteers in any further dose escalations.

Upon blinded review of the pharmacokinetic data for 20 µg/mL SS target group, it was noted a dosing error (much lower levels of reparixin than expected) in three volunteers. This was traced back to a dispensing error in pharmacy where the study drug to be dosed was made from a more diluted solution. Four volunteers, one subject receiving placebo and 3 subjects receiving active drug, were replaced. Therefore a total of 40 subjects were randomised into the study (Table 27). There were no discontinued subjects.

Table 27: Disposition of Subjects

N = 40 subjects receiving double-blind medication			
N = 10[1] Placebo	N = 9 Reparixin L-lysine salt 1.0 mg/kg/h	N = 12[1] Reparixin L-lysine salt 2.0 mg/kg/h	N = 9 Reparixin L-lysine salt 4.2 mg/kg/h
N = 10 completed N = 0 withdrawn	N = 9 completed N = 0 withdrawn	N = 12 completed N = 0 withdrawn	N = 9 completed N = 0 withdrawn

[1] A dosing error was discovered in one subject receiving placebo and 3 subjects receiving active drug and they were replaced.

The infusion treatment schedules for cohort 1, 2 and 3 are presented in Table 28.

Table 28: Treatments administered

Cohort	Target Plasma Concentration	Dose (reparixin L-lysine salt) Loading/Maintenance dose	Total Dose per kg BW (reparixin L-lysine salt)
1	**10 µg/mL**	3.1 mg/kg/h for 30 minutes/ 1.0 mg/kg/h for 47.5 hours	49.1 mg/kg
2	**20 µg/mL**	3.8 mg/kg/h for 30 minutes/ 2.0 mg/kg/h for 47.5 hours	96.9 mg/kg
3	**30 µg/mL**	6.8 mg/kg/h for 30 minutes/ 4.2 mg/kg/h for 47.5 hours	202.9 mg/kg

The first dose regimen was well tolerated, with very few systemic AEs. The most common AEs were related to local tolerability, i.e. infusion site reactions, mainly erythema or aseptic thrombophlebitis, with this being reported in 10 out of the 12 volunteers. This would improve with resiting of the infusion cannula, and tended to settle over the next week or so. It was not associated with any systemic symptoms or signs of sepsis.
In order to reduce the possibility of inducing infusion site reactions, the second cohort of subjects received a less concentrated solution (3.38 mg/mL instead of 6 mg/mL considering a body weight of 70 kg) by increasing the administration volume from 300 mL/24h to 1000 mL/24h for cohort 2 and 2100 mL/24h for cohort 3 and by cannula resiting every 24h. These changes improved local tolerability as no infusion site reactions were observed.

AEs were coded using MedDRA. They were summarised and expressed in terms of maximum severity and relationship to study drug and also in terms of frequency with respect to the dose (Table 29).

Table 29: Summary of Adverse Events

Total Adverse Events	Pre-treatment	Treatment-emergent	Placebo	Reparixin L-lysine salt target group		
				10 µg/mL	20 µg/mL	30 µg/mL
	3 from 3 subjects	51 from 24 subjects	4 from 4 subjects	24 from 9 subjects	9 from 6 subjects	14 from 5 subjects
Relationship to Study Drug:						
Probable	0	16[10]	0	14[8]	1[1]	1[1]
Possible	0	22[13]	2[2]	8[4]	6[3]	6[4]
Unlikely	1[1]	13[9]	2[2]	2[2]	2[2]	7[3]
Not related	2[2]	0	0	0	0	0
Severity of Adverse Event:						
Mild	3[3]	50[24]	4[4]	23[9]	9[6]	14[5]
Moderate	0	1[1]	0	1[1]	0	0
x[y] = number of events [number of subjects]						

There was a total of 54 AEs reported by 60% of subjects (24/40), of which 5.6% (3/54) from 3 subjects were assigned to pre-treatment and the remaining 94.4% (51/54) from 24 subjects were study drug treatment-emergent (Table 30 and Table 31). A summary of the AEs is given in Table 31 and Table 32.

Table 30: Overall Summary of Pre-treatment related AEs (number of events [number of subjects])

MedDRA Body System	Adverse Event	Pre-treatment
Number of subjects treated		**40**
General disorders and administration site conditions	Fatigue	1[1]
Nervous system disorders	Headache	1[1]
Skin and subcutaneous system disorders	Psoriasis	1[1]
Total		**3[3]**
x[y] = number of events [number of subjects]		

The relationship to study drug was “probable” for 31.4% (16/51) of events, “possible” for 43.1% (22/51) of events and “unlikely” for 25.5% (13/51) of events. The maximum severity of these AEs was classified as mild for 98.0% (50/51) of events and moderate for 2.0% (1/51).

All but one of the AEs reported during the study were of mild intensity, indicating that study treatment was well tolerated. The most frequent AE was cannula site reaction (14). In total 22 AEs, all mild, were related to sampling or administration site reactions: cannula site reaction (14), injection site thrombosis (3), infusion site oedema (2), injection site erythema (2) and pruritis (1).

AEs were followed-up until resolution. Subjects taking part in the study had recovered completely or were with mild intensity of adverse events when they were discharged from the Clinical Unit.

Table 31: Overall summary of Study Drug Treatment-emergent AEs (number of events).

MedDRA Body System	Adverse Event	Reparixin L-lysine salt target group			Placebo	Total
		10 µg/mL	20 µg/mL	30 µg/mL		
Number of subjects treated		**9**	**12**	**9**	**10**	**40**
Blood and lymphatic system disorders	Lymphadenopathy	1[1]				**1[1]**
Gastrointestinal disorders	Abdominal Pain NOS		1[1]			**1[1]**
	Dyspepsia	1[1]		1[1]		**2[2]**
	Flatulence		2[1]			**2[1]**
	Nausea		1[1]			**1[1]**
General disorders and administration site conditions	Cannula site reaction	10[7]		2[2]	3[3]	**15[12]**
	Chest pain			1[1]		**1[1]**
	Injection site thrombosis	3[3]				**3[3]**
	Infusion site oedema		1[1]	1[1]		**2[2]**
	Lethargy			1[1]		**1[1]**
Infections and infestations	Herpes simplex			1[1]		**1[1]**
Musculoskeletal and connective tissue disorders	Arthralgia	1[1]				**1[1]**
	Back pain	1[1]				**1[1]**
Nervous system disorders	Dizziness		1[1]			**1[1]**
	Headache			6[3]		**6[3]**
	Hypoaesthesia	1[1]		1[1]		**2[2]**
	Somnolence		1[1]			**1[1]**
Respiratory, thoracic and mediastinal disorders	Epistaxis	1[1]				**1[1]**
	Productive cough	1[1]	1[1]			**2[2]**
Skin and subcutaneous system disorders	Contusion		1[1]			**1[1]**
	Dermatitis contact				1[1]	**1[1]**
	Erythema	1[1]				**1[1]**
	Infusion site erythema	2[2]				**2[2]**
	Pruritis	1[1]				**1[1]**
Total		**24[9]**	**9[6]**	**14[5]**	**4[4]**	**51[24]**
x[y] = number of events [number of subjects]						

Table 32: Summary of Treatment-emergent Adverse Events "probably" or "possibly" related to study treatment.

MedDRA Body System	Adverse Event	Reparixin L-lysine salt target group			Placebo	Total
		10 µg/mL	20 µg/mL	30 µg/mL		
Number of subjects treated		**9**	**12**	**9**	**10**	**40**
Blood and lymphatic system disorders	Lymphadenopathy	1[1]				**1[1]**
Gastrointestinal disorders	Abdominal Pain NOS		1[1]			**1[1]**
	Dyspepsia	1[1]				**1[1]**
	Flatulence		2[1]			**2[1]**
	Nausea		1[1]			**1[1]**
General disorders and administration site conditions	Cannula site reaction	10[7]		2[2]	2[2]	**14[11]**
	Injection site thrombosis	3[3]				**3[3]**
	Infusion site oedema		1[1]	1[1]		**2[2]**
	Lethargy			1[1]		**1[1]**
Musculoskeletal and connective tissue disorders	Arthralgia	1[1]				**1[1]**
Nervous system disorders	Dizziness		1[1]			**1[1]**
	Headache			2[2]		**2[2]**
	Hypoaesthesia	1[1]		1[1]		**2[2]**
	Somnolence		1[1]			**1[1]**
Respiratory, thoracic and mediastinal disorders	Productive cough	1[1]				**1[1]**
Skin and subcutaneous system disorders	Erythema	1[1]				**1[1]**
	Infusion site erythema	2[2]				**2[2]**
	Pruritis	1[1]				**1[1]**
Total		**22[9]**	**7[4]**	**7[3]**	**2[2]**	**38[18]**
x[y] = number of events [number of subjects]						

With regard to the clinical laboratory evaluations, additional coagulation checks were performed on subjects 5 to 12 due to local tolerability issues that were observed around the infusion cannula site.
There were 2 values outside the normal range, both classified as being of no clinical significance.
Ten abnormal clinical laboratory results were classified as being potentially clinically significant (Table 33), due to concomitant illness (2) or cause unknown (8).

Table 33: Summary of potentially clinically significant abnormal laboratory values.

Subject Number	Laboratory Parameter	Visit	Result	Units	Interpretation
6	**Bilirubin**	Post-Study	34	µmol/L	Cause unknown
	AST	Post-Study	58	IU/L	Concomitant illness
	ALT	Post-Study	50	IU/L	Concomitant illness
9	**Calcium**	Day-1	1.88	mmol/L	Cause unknown
21	**Lymphocytes**	Post-Study	0.60	E+9/L	Cause unknown
24	**White Cell Count**	Post-Study	3.1	E^{+9}/L	Cause unknown
	Neutrophils	Post-Study	1.62	E^{+9}/L	Cause unknown
27	**Calcium**	Day 3	2.05	mmol/L	Cause unknown
28	**White Cell Count**	Post-Study	2.8	E^{+9}/L	Cause unknown
	Neutrophils	Post-Study	1.27	E^{+9}/L	Cause unknown

In conclusion, reparixin was well tolerated. There were no deaths, serious AEs or withdrawals. There were no clinically relevant changes in clinical LAB measurements, ECGs, vital signs, or physical examinations.
No clear treatment-emergent pattern emerged for any of the immune cell surface markers measured. The most common AEs considered probably or possibly related to reparixin were cannula site reaction, injection site thrombosis, infusion site oedema, infusion site erythema, headache, hypoaesthesia, and flatulence, followed by one incidence each of lymphadenopathy, abdominal pain NOS, dyspepsia, nausea, lethargy, athralgia, dizziness, somnolence, productive cough, erythema and pruritis. The majority of treatment-emergent AEs (98.0%) were mild in severity, with only 2.0% being moderate in severity.
There were no relevant statistically significant treatment-related relationships in inhibition of CXCL8 mediated neutrophil chemotaxis or CXCL8 and FMLP induced CD11b expression in response to reparixin and placebo, apart from a statistically significant reduction in baseline corrected 24h specific migration for the highest target SS group. However, it should be borne in mind that only 9 subjects were investigated at each dose level, hence any comparisons between treatments remain somewhat speculative. Moreover, the *ex vivo* chemotaxis assay had an intrinsic drawback which must be taken into account, as suggested by an a posteriori interpretation of these results. PMN isolated from the blood of volunteers previously exposed *in vivo* to reparixin undergo extensive washings and a gradient step purification procedure in order to obtain a purified PMN population to be used in the chemotaxis assay. *In vitro* data show that, after incubation, removal of reparixin by cell washing impairs its inhibitory activity. After cell washing, as much as 58% of reparixin inhibitory activity is lost as soon as 30min after washing. In the *ex vivo* chemotaxis assay, PMN were not exposed to reparixin for at least 40min before activation with CXCL8 in the chemotaxis chamber. Thus reparixin, in keeping with its molecular mechanism of action [6], is a reversible inhibitor of CXCL8 activity. As a consequence, it is likely that the chemotaxis assay underestimates the actual inhibition of CXCL8 activity obtained by exposing PMN to reparixin *in vivo*.

6.2.2.3. Study to assess the potential pharmacokinetic interaction between reparixin L-lysine salt and probe substrates for CYP3A4 (midazolam) and CYP2C9 (tolbutamide) in adult healthy male volunteers [REP0103 (ME0757)]

In this Phase 1, single centre, open-label, drug-interaction study, 14 healthy male volunteers received concomitant single oral doses of midazolam and tolbutamide on Day 1 and Day 3, with single i.v. administration of reparixin L-lysine salt on Day 3. The objectives of the study were to establish if reparixin, at plasma concentrations equal to or exceeding that thought to be required for therapeutic efficacy, alters the pharmacokinetics of midazolam, a probe substrate for CYP3A4, and tolbutamide, a probe substrate for CYP2C9.

Midazolam 7.5 mg and tolbutamide 500 mg as single oral doses administered alone and concomitantly with an 8-hour i.v. infusion of reparixin L-lysine salt at a total dose of 34.9 mg/kg (targeted Css of 30 µg/mL) were safe and well tolerated.

There were no deaths, serious AEs or withdrawals. There were no clinically relevant changes in clinical LAB measurements, ECGs, vital signs, or physical examinations and no clinical relevant differences between treatments.

Similar numbers of AEs were reported by 13 subjects receiving midazolam/tolbutamide alone and by 12 subjects receiving midazolam/tolbutamide/reparixin. The most common AEs considered probably or possibly related to study drugs was somnolence, followed by dizziness and one incidence each of cannula

site reaction, dry throat, abnormal dreams and euphoric mood. The majority of treatment-emergent AEs (84.6%) were mild in severity, with only 15.4% being moderate (Table 34, Table 35, Table 36). Twenty-seven of the 30 "probably" or "possibly" treatment-related AEs were of mild intensity, the remaining 3 being of moderate, indicating that the study treatments were well tolerated. All subjects taking part in the study had recovered completely or were with mild intensity of AEs when they were discharged from the Clinical Unit. All AEs were successfully followed-up until resolution, apart from the occasion of cannula site reaction for Subject 9. After clinical phase completion the subject was repeatedly reminded to report AE status but failed to comply.

In conclusion, no safety concerns were raised during co-administration of midazolam/tolbutamide with reparixin L-lysine salt.

Table 34: Summary of AEs.

Total Adverse Events	**Pre- study**	**Total treatment-emergent**			**Midazolam / Tolbutamide alone**			**Midazolam/Tolbutamide/ Reparixin L-lysine salt**		
	0 from 0 subject	**39 from 14 subjects**			**19 from 13 subjects**			**20 from 12 subjects**		
Relationship to Study Drug:		**MDZ**	**TLB**	**RPT**	**MDZ**	**TLB**	**RPT**	**MDZ**	**TLB**	**RPT**
Probable	0	27[13]	0	0	13[12]	0	0	14[12]	0	0
Possible	0	2[1]	29[13]	13[10]	1[1]	14[12]	0	1[1]	15[12]	13[10]
Unlikely	0	7[5]	7[5]	7[7]	2[2]	2[2]	0	5[5]	5[5]	7[7]
Not related	0	3[2]	3[2]	19[13]	3[2]	3[2]	19[13]	0	0	0
Severity of AEs										
Mild	0	33 from 13 subjects			14 from 10 subjects			19 from 12 subjects		
Moderate	0	6 from 5 subjects			5 from 4 subjects			1 from 1 subject		
MDZ: Midazolam; TLB: Tolbutamide; RPT: Reparixin; L-lysine salt; x[y] = number of events [number of subjects]										

Table 35: Overall summary of study drug Treatment-emergent AEs (number of events).

MedDRA Body System	**Adverse Event**	**Treatment**		**Total**
		Midazolam/ Tolbutamide alone	**Midazolam/Tolbutamide/ Reparixin L-lysine salt**	
Number of subjects treated		**14**	**14**	**14**
Eye disorders	Blepharospasm	-	1[1]	**1[1]**
General disorders and administration site conditions	Cannula site reaction	1[1]	1[1]	**2[2]**
	Feeling hot	1[1]	-	**1[1]**
Immune system disorders	Seasonal allergy	-	2[2]	**2[2]**
Musculoskeletal and connective tissue disorders	Back pain	1[1]	1[1]	**2[2]**
Nervous system disorders	Dizziness	1[1]	1[1]	**2[2]**
	Headache	1[1]	-	**1[1]**
	Somnolence	13[12]	12[12]	**25[13]**
Respiratory, thoracic and mediastinal disorders	Dry throat	1[1]	-	**1[1]**
Psychiatric disorders	Abnormal dreams	-	1[1]	**1[1]**
	Euphoric mood	-	1[1]	**1[1]**
Total		**19[13]**	**20[12]**	**39[14]**
x[y] = number of events [number of subjects]				

Table 36: Treatment-emergent AEs "probably" or "possibly" related to any of the study treatments.

MedDRA Body System	**Adverse Event**	**Treatment**		**Total**
Number of subjects treated		**Midazolam/ Tolbutamide alone**	**Midazolam/ Tolbutamide/ Reparixin L-lysine salt**	
		14	**14**	**14**
General disorders and administration site conditions	Cannula site reaction	-	1[1] [RPT]	**1[1]**
Nervous system disorders	Dizziness	1[1]	1[1]	**2[2]**
	Somnolence	12[12]	9[9] + 3[3] [MDZ,TLB]	**24[13]**
Respiratory, thoracic and mediastinal disorders	Dry throat	1[1]	-	**1[1]**
Psychiatric disorders	Abnormal dreams	-	1[1]	**1[1]**
	Euphoric mood	-	1[1]	**1[1]**
Total		**14[12]**[1]	**16[12]**[2]	**30 [13]**
[1] probably or possibly related to midazolam (MDZ) and tolbutamide (TLB); [2] probably or possibly related to midazolam, tolbutamide and reparixin (RPT), unless otherwise indicated; x[y] = number of events [number of subjects]				

6.2.2.4. The pharmacokinetics and tolerability assessment of I.V. infusion of reparixin L lysine salt in adult patients with chronic impaired renal function [REP0203-CM8003-ME0761]

This was an open, single dose, two-centre study to investigate the effect of renal impairment on the pharmacokinetics and safety of reparixin in male and female subjects. The study was performed in 2 stages, firstly a pilot phase in Stage A in which a single loading i.v. infusion of 6.8 mg/kg/h was given to 4 patients with ESRD over 30min, i.e. a dose of 3.4 mg/kg. Based on the data obtained from Stage A, the dose used in Stage B was 2 mg/kg/h for 6h of i.v. infusion with the aim of achieving Css in the range of 10-30 µg/mL. There were 38 subjects planned (4 for Stage A and 34 for Stage B). The study was discontinued after a total of 23 subjects were enrolled and completed the study (6 normal renal function, 3 mild, 2 moderate, 2 severe renal impairment patients and 10 ESRD) as the results of Stage A and B, despite the reduced number of subjects, provided a comprehensive and accurate evaluation of the effect of renal function on the pharmacokinetics of reparixin and its main metabolites.

The decision to interrupt the study during Stage B due to a low recruitment rate was taken considering that the original protocol was aimed at obtaining an accurate evaluation of the effect of renal function on the pharmacokinetic profile of reparixin. Indeed, the results of Stage A, further confirmed by the subjects recruited in Stage B up to the study discontinuation, indicated that the pharmacokinetic profile of reparixin was not affected by the degree of renal function and also indicated a predictable pharmacokinetic profile of the metabolites for each degree of renal function. Besides, there was a clear indication that the pharmacokinetic profile of the major metabolite, DF 2243Y, was significantly altered starting from a moderate degree of renal function.

Stage A – ESRD patients (3 males, 1 female; Table 37)
There were no deaths, serious AEs or withdrawals. There were a total of 5 AEs reported by 50% of subjects (2/4), of which 40% (2/5) from 2 subjects were assigned to pre-treatment and the remaining 60% (3/5) from 2 subjects were study drug treatment-emergent. Of the treatment-emergent AEs, the relationship to study drug was "not related" for 100% (3/3) and maximum severity was classified as mild for 66.6% (2/3) and unknown for 33.3% (1/3) of events. All AEs reported were mild in intensity except 1 which was unknown in intensity. All AEs were regarded as not related to study drug, indicating that reparixin was well tolerated by ESRD patients.

Table 37: Pre-treatment and Study Drug Treatment-Emergent AEs (Stage A; n=4).

MedDRA Body System	Pre-treatment	MedDRA Body System	ESRD
Gastrointestinal disorders	1(1)	General disorders and administration site conditions	1(1)
Metabolism and nutrition disorders	1(1)	Eye disorders	1(1)
		Musculoskeletal and connective tissue disorders	1(1)
Total	**2(2)**	**Total**	**3(2)**
x(y) = number of events (number of subjects)			

Stage B – Overall (19 subjects)
There were no deaths, serious AEs or withdrawals. There were no clinically relevant changes in clinical LAB parameters, ECGs, vital signs or physical examinations. Pre-treatment and treatment-emergent AEs are presented in Table 38.

Table 38: Pre-treatment and Study Drug Treatment-Emergent AEs (Stage B).

MedDRA Body System	Pre-treatment	NORMAL CLcr >80 mL/min	MILD CLcr 80-51 mL/min	MODERATE CLcr 50-30 mL/min	SEVERE CLcr <30 mL/min	ESRD	Total
Number of subjects treated	**19**	**6**	**3**	**2**	**2**	**6**	
Cardiac disorders	-	-	-	-	-	1(1)	1(1)
Gastrointestinal disorders	3(3)	3(2)	1(1)	-	-	1(1)	5(4)
General disorders and administration site conditions	-	-	-	-	-	2(1)	2(1)
Infections and infestations	2(2)	-	-	-	-	1(1)	1(1)
Injury, poisoning and procedural complications	-	-	-	-	1(1)	-	1(1)
Nervous system disorders	3(3)	4(2)	1(1)	-	2(1)	1(1)	8(5)
Psychiatric disorders	1(1)	-	-	-	-	1(1)	1(1)
Renal and urinary disorders	1(1)	-	-	-	-	-	-
Musculoskeletal and connective tissue disorders	1(1)	-	-	-	-	-	-
Total	**11(7)**	**7(4)**	**2(2)**	**-**	**3(2)**	**7(3)**	**19(11)**
x(y) = number of events (number of subjects)							

There were no treatment-emergent AEs classified with the relationships "certain" or "probable/likely" related to reparixin. All subjects taking part in the study had recovered. All AEs were successfully followed-up until resolution.

Stage B - Normal Renal Function Volunteers (3 males, 3 females)
In total 7 treatment-emergent AEs were reported. The majority of AEs were mild in intensity and 5 of them were considered possibly related to reparixin (Table 39).

Table 39: Treatment-Emergent AEs "possibly" related to Study Drug (Stage B - Normal Renal Function).

Adverse Event (MedDRA term)	Maximum severity		
	Mild	Moderate	Total
Headache	2(2)	-	**2(2)**
Nausea	2(2)	-	**2(2)**
Vomiting	-	1(1)	**1(1)**
Total	**4(4)**	**1(1)**	**5(4)**
x(y) = number of events (number of subjects)			

Stage B - Mild renal impairment (1 male, 2 females)
In total 2 treatment-emergent AEs were reported. These AEs were mild in intensity and only 1 event, headcold (headache), was considered possibly related to reparixin.

Stage B - Moderate renal impairment (1 male, 1 female)
There were no treatment-emergent AEs reported by the subjects.

Stage B - Severe renal impairment (2 males)
In total 3 treatment-emergent AEs were reported; these adverse events were mild in intensity and only 1 event (headache) was considered possibly related to reparixin.

Stage B - End Stage Renal Disease (CAPD: 2 males, 1 female - Haemodialysis: 3 males)
In total 7 treatment-emergent AEs were reported. The majority was moderate in intensity (4 in 1 subject), only 1 event (restlessness) was considered possibly related to reparixin which was mild in intensity.

In conclusions, the overall results of the study suggest that, during both stages, reparixin administered to normal renal function volunteers, renally impaired and ESRD patients was safe and well tolerated. Moreover, there were no deaths, serious AEs or withdrawals during both stages of the study. The most commonly reported treatment-emergent AEs were headache (4 possible and 2 unlikely) and nausea (2 possible and 2 unlikely). There were no clinically relevant changes in clinical laboratory parameters, ECGs, vital signs or physical examinations.

6.2.2.5. Pilot single-centre, phase 1, randomised, double blind, cross-over, placebo-controlled study to investigate leukocyte trafficking and cytokine production at site of inflammation using cantharidin blister after reparixin 24 h continuous infusion [REP0107 (CRO-PK-07-197)]

The study was designed to assess if a 24 h continuous infusion of reparixin can reduce the PMNs and the inflammatory mediators influx into cantharidin blister, during acute inflammation. Eight healthy males volunteers (32.3±7.9 y) were stimulated with cantharidin for 24 h on the forearm in 2 consecutive periods (with a wash-out interval of at least 15 days but not more than 40 days) and were infused concomitantly with either 2.772 mg/kg/h of reparixin injectable solution or matching placebo (9 mg/mL injectable solution of NaCl). The main criteria of efficacy and pharmacokinetic evaluations were:

- collected blister fluid volume,
- total blister cell count,
- differential cell count (PMN, eosinophils and mononuclear cells).
- reparixin concentration in plasma and in blister fluid,

The main criteria for safety evalution were:

- CXCL8 (IL-8) blister fluid concentration (if feasible),
- TNF-α blister fluid concentration (if feasible),
- C3a blister fluid concentration (if feasible),
- C5a blister fluid concentration (if feasible),
- Comparative tolerability and safety of the treatments..Collected blister fluid volume.

In each Period 6 out of 8 subjects reacted to cantharidin. One subject was a non responder to cantharidin, whilst all other subjects reacted to cantharidin. Three of them developed a blister from 49 min to about 4 h after the end of the stimulation. Only two subjects reacted to cantharidin both after treatment with reparixin and matching placebo. The volume of the fluid collected from the blisters was higher after treatment with reparixin. In particular, after infusion of placebo only 4 exudate specimens could be processed and counted. Similarly, after infusion of reparixin only 3 samples of exudate were processed and counted for the primary analysis.

Due to the low number of subjects that could be kept in the analysis, both total cell count and differential cell count as well as blister fluid volume were analysed by investigating the treatment effect only. No difference between treatments was detected. Nevertheless, data from the two subjects that developed blisters after both treatments revealed that the total cell count was higher with infusion of placebo than with reparixin. This quantitative result is not specific for the PMN, as the whole cellular population of exudate resulted subdivided into similar proportions of cell types after infusion with both reparixin and placebo, as shown in the following table:

Table 40: Total and differential cell counts in blister fluid of evaluable subjects.

Treatment	Subject	Total cell count	PMN (%)	Eosinophils (%)	Mononuclear cells (%)	Volume (µL)
Placebo	**4**	514675.0	78.5	2.9	18.7	119.0
Reparixin		221610.0	81.3	0.9	17.8	267.0
Placebo	**8**	796050.0	42.1	0.1	57.8	183.0
Reparixin		51840.0	66.9	0.6	32.6	288.0

The analysis did not detect any significant difference between treatments. Nevertheless, the total cell count in the blister fluid of subjects treated with reparixin was, on average, one half of the total cells counted in the blister fluid of placebo-recipients which might be explained with the inhibitory action displayed by reparixin, although the proportion of PMN, eosinophils and mononuclear cells seemed to be unaffected.

Unfortunately the blister volumes were not sufficient to evaluate the differences in the inflammatory mediators.

All subjects showed detectable plasma levels of reparixin by the end of infusion. Mean (±SD) plasma concentration was 37.42±4.13 µg/mL. Reparixin was dosed also in the blister fluid samples of 3 subjects. The active substance was present in the cantharidin-induced blister at a mean concentration of 28.28±3.82 µg/mL.

No meaningful effect of reparixin on vital signs, ECGs or laboratory parameters was observed. No treatment-related AE occurred to subjects treated with reparixin (Table 41).

Table 41: AEs occurring during the study

Treatment	Subject	MedDRA description*	Type	Duration	Intensity	Relationship to treatment
Placebo	1	Headache	Single episode	7:00 h	Mild	Possible
	3	Headache	Single episode	10:30 h	Mild	Possible
Reparixin	1	Cantharidin application site erythema	Single episode	9 days and 23:00 h	Mild	Unlikely/none

6.2.2.6. A two-part, phase I study to investigate the reproducibility of the cantharidin blister model and to determine the effect of dexamethasone and reparixin on the inflammatory response in this model [REP0109]

This two-part study was designed to investigate the reproducibility of the inflammatory response in the cantharidin blister model (part A) and to determine the potential of the model for detecting putative efficacy of novel anti-inflammatory compound by evaluating the effect of dexamethasone and reparixin (part B). The following treatments were used in part B, a randomized, open-label, placebo-controlled, 3-way crossover study: dexamethasone, 6 mg single oral dose before and at the time of cantharidin application; reparixin, 24-hours i.v. infusion of 2.772 mg/kg/hour, starting at the time of cantharidin application; placebo, 24-hours i.v. infusion, strating at the time of cantharidin application. Seven healthy male subjects were enrolled into part A and 14 healthy male subjects were enrolled into part B (13 of whom were treated).

All subjects enrolled in Part A showed sufficient response to cantharidin, and the cellular composition of the blister fluid was similar between the three blister sites (A, B and C).

Overall, there were no statistically significant differences observed when measuring the inflammatory mediators from fluid taken from different blister sites at the same time point without treatment.

In part B, statistically significant differences were seen between different time points (notably IL-8, TNF-α and C5a). For IL-8, although large differences were seen between placebo and both dexamethasone and reparixin groups, these were not statistically significant. However, the data set contained a high proportion of results that had been truncated to the upper limit of quantification – a larger proportion of the placebo group than the others. Therefore, it may be that had repeat analysis of these samples been completed and actual (higher) results entered into the database that the differences for IL-8 could also be statistically significant.

VEGF concentrations were statistically significantly lower after reparixin administration compared with placebo (ratio of geometric means 0.6083; 95% CI: 0.3988 to 0.9279).

All study drugs were well tolerated, with very few TEAEs reported in both parts of this study. Only one TEAE was reported in Part A and eight TEAEs in Part B of the study. In Part B, most of the TEAEs were reported by subjects who received reparixin + cantharidin (four TEAEs), two TEAEs were reported by subjects who received dexamethasone + cantharidin, one TEAE was experienced by one subject who received dexamethasone alone, and one TEAE was experienced by one subject who received placebo + cantharidin. No TEAE occurred more than once and only two subjects reported more than one TEAE.No TEAE occurred more than once and only two subjects reported more than one TEAE.

Two of the eight TEAEs reported in Part B, were considered possibly related to reparixin + cantharidin, one TEAE was considered possibly related to dexamethasone and one TEAE considered possibly related to cantharidin. The maximum severity of TEAEs was classified as mild for seven TEAEs and moderate for one TEAE.

In conclusion there were no statistically significant differences observed in the inflammatory mediators from fluid taken from different blister sites at the same time point without treatment. Due to errors in sample handling with flow cytometry samples, it was not possible to perform a scientifically meaningful statistical analysis on or analytic interpretation of any flow cytometry data. Therefore, no conclusion could be made regarding these data. The cantharidin blister model was safe and well tolerated in Part A of the study, and cantharidin plus co-administration of dexamethasone, reparixin and placebo were well tolerated in Part B.

6.2.3. Phase 2-3 clinical studies

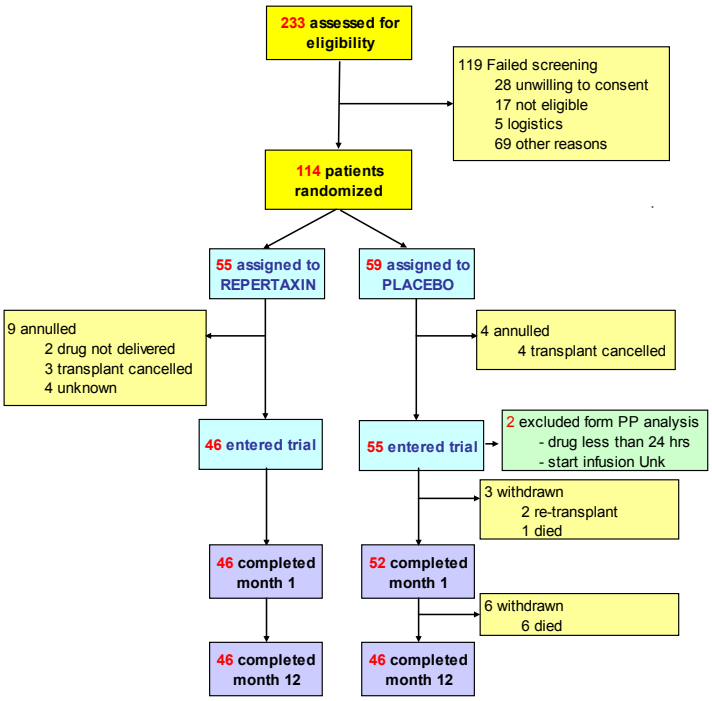
6.2.3.1. A phase 2, multi-center, randomized, double-blind, placebo-controlled, parallel-group study of reparixin in the prevention of primary graft dysfunction after lung transplantation [REP0104]

The objective of this study was to evaluate whether CXCL8 inhibition with reparixin leads to reduced severity of PGD, as the result of improved functional and clinical outcomes in lung transplant patients. The primary efficacy endpoint was the PaO2/FiO2 ratio measured on ICU admission and at 24h after ICU admission. Secondary endpoints were: Time profile of PaO2/FiO2 ratio (ICU admission then q24 hours up to extubation or up to 72 hours); PGD score; Time to freedom from mechanical ventilation; Duration of ICU stay; Mortality within 30 days post-transplant; Pulmonary Function Tests at month 1, 6 and 12 post-transplant; BOS at Month 6 and 12 post-transplant; Acute Rejection Episodes at month 1, 6 and 12 post-transplant; Survival Rate up to month 12. The safety of reparixin in the specific clinical setting was also evaluated. The ability of reparixin to reduce target cells (PMN) infiltration into the graft was evaluated to confirm its mechanism of action. Plasma levels of reparixin and its major metabolite were measured at SS conditions to provide population pharmacokinetic profile.

The study was conducted at six centers in the US and Canada in adult male and female patients (18-65 years) accepted and listed for lung transplantation (planned isolated lung transplant from a non-living donor with brain death) with exclusion of pregnant or breast feeding women. One-hundred patients were planned for inclusion in the study.

Patients were randomized to receive 48h intravenous continuous infusion (loading: 4.488 mg/kg/h for 30 min, maintenance: 2.772 mg/kg/h for 47.5h) of either reparixin or placebo. As per protocol, the blind code was broken after database lock of month 1 data.

The study was completed on 13 September 2007. Patient disposition is reported in the flow chart below:



Demographic and baseline characteristics of both patients and donors were comparable between the two treatment groups. Results all indicate that there were no statistically significant differences between the reparixin and the placebo groups in PaO2/FiO2 values (both measured and corrected for altitude) measured at ICU admission (time 0) and 24 hours post-ICU admission for both the ITT and PP populations (primary variable). In the secondary analysis, both corrected and measured PaO2/FiO2 variables after ICU admission indicate that there were no statistically significant differences between the placebo and reparixin groups from ICU admission (time 0) to 72 hours post-ICU admission for the ITT and PP populations. There were no statistically significant differences (p-value > 0.05) in the PGD scores between the two groups when stratified by the type of transplant (either single or double transplant) at all time points measured. A total of 15 (27.3%) and 9 patients (19.6%) in the placebo and reparixin groups, respectively, had decreased PaO2/FiO2 ratios and X-rays consistent with a PGD score of 3 and were diagnosed as having clinically severe PGD during Month 1 of the study. There was no statistically significant difference between the two treatment groups in the time to freedom from mechanical ventilation and the duration of ICU stay, regardless of the type of transplant. Mean FEV1 and FVC values, BOS scores, total number of acute rejection episodes and time to first biopsy-proven acute rejection episode were comparable between the treatment groups, regardless of the type of transplant. A total of 7 patients in the placebo group died before Month 12 post-transplant. All patients in the reparixin group were alive at the Month 12 visit. There was a statistically significant difference in patient survival at Month 12 post-transplant between the placebo and reparixin groups.

The extent of study drug exposure was similar for both treatment groups; for patients in the reparixin group, a mean value of 8344 mg reparixin was infused.

The adverse event (AE) profile was similar for both placebo and reparixin groups. AEs were reported for 55 patients (100.0%) in the placebo group and 46 patients (100.0%) in the reparixin group. SAEs were reported for 14 patients (25.5%) and 14 patients (30.4%) in the placebo and reparixin groups, respectively. A total of 8 SAEs possibly related to study drug (coagulopathy, thrombocytopenia, cytokine release syndrome, lung transplant rejection, acute renal failure, respiratory failure, pulmonary oedema, hemorrhage) were reported

for 2 patients (3.6%) in the placebo group and 4 patients (8.7%) in the reparixin group. There were no AE related withdrawals or deaths in this study for either placebo or reparixin groups. Summary of SAEs is reported in Table 42.

One patient in the placebo group died before the end of Month 1. The proximate cause of death was cardiovascular accident. Other problems that might have affected outcomes were identified as being atrial fibrillation and atrial flutter. Six (6) patients in the placebo group died between Month 1 and Month 12 of the study. Death was due to infections in 4 patients leading to sepsis/bacteraemia (3 patients) or pneumonia (1 patient). Coronary artery disease or unknown origin were the cause of death reported for the other 2 patients for whom minimal information was available. None of the deaths were judged related to study drug administration.

Clinical laboratory data (haematology, clinical chemistry and coagulation results), vital signs and cardiopulmonary measurement results were comparable between the placebo and reparixin groups.

All 55 (100.0%) patients in the placebo group and 46 (100.0%) patients in the reparixin group received concomitant medications during the course of the study and the use of concomitant medications was similar between the two groups.

PMN counts in BAL specimens evaluated in 37 available slides were similar between the two treatment groups.

PK analysis was performed on samples from 45 patients. Plasma levels of unbound reparixin were in the range of those expected to be pharmacologically active.

Overall, the study was unable to show a statistically significant effect of reparixin on short and long term functional and clinical outcomes after lung transplantation. Reparixin was found to be safe and well tolerated in patients undergoing lung transplantation.

Table 42: Treatment Emergent Serious AEs - Cumulative Summary Tabulation

Number of Reports by Terms for Trial: REP0104 (Reparixin / Placebo) (An * indicates a SUSAR)			
MedDRA Body System / LLT ADR term		**Reparixin**	**Placebo**
Blood and lymphatic system disorders		**4**	**3**
	Coagulopathy	2 + 1*	2
	Thrombocytopenia		1*
Cardiac disorders		**4**	**6**
	Atrial fibrillation	1	3
	Atrial flutter		1
	Cardiac arrest	1	
	Cardiac tamponade		1
	Cardiopulmonary failure	1	
	Pericarditis		1
	Supraventricular tachycardia	1	
Immune system disorders		**1**	**3**
	Cytokine release syndrome		1*
	Lung transplant rejection	1*	2
Infections and infestations			**1**
	Pneumonia		1
Injury, poisoning and procedural complications		**2**	**2**
	Graft dysfunction	1	1
	Post Procedural Haemorrhage	1	1
Investigations		**1**	
	Oxygen saturation decreased	1	
Nervous system disorders		**1**	**2**
	Depressed level of consciousness		1
	Hemiparesis		1
	Vocal cord paralysis	1	
Renal and urinary disorders			**2**
	Renal failure		1*
	Renal failure acute		1
Respiratory, thoracic and mediastinal disorders		**5**	**6**
	Pleural haemorrhage	1	
	Pneumothorax	3	
	Pulmonary oedema		1 + 1*
	Respiratory distress		1
	Respiratory failure	1*	2
	Vocal cord disorder		1
Vascular disorders		**2**	**9**
	Haemorrhage	1 + 1*	3
	Hypotension		3
	Reperfusion injury		2
	Vena cava thrombosis		1
	TOTAL	**19**	**34**

6.2.3.2. A phase 2, multi-centre, randomized, double-blind, placebo-controlled, parallel-group (3 arms) pilot study to assess the efficacy, the safety and the pharmacokinetics of two treatment schedules of reparixin in the prevention of delayed graft function after kidney transplantation in high risk patients [REP0204]

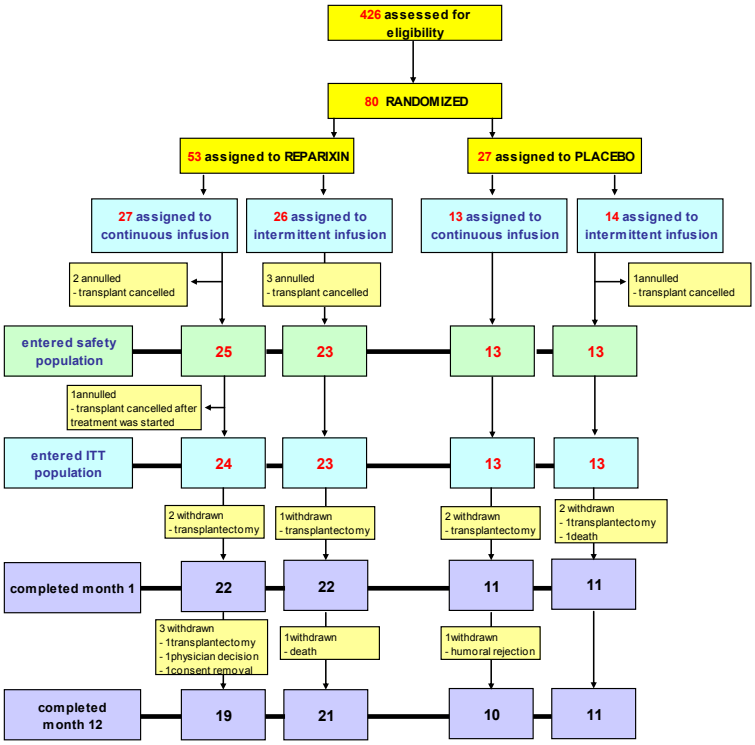
The objective of this pilot clinical trial was to evaluate whether CXCL8 inhibition with reparixin leads to improved functional and clinical outcomes in kidney transplant patients. The primary efficacy endpoint was creatinine clearance (CrCl) in the immediate post-transplant period, as determined by 2x60min urine collections during the time intervals 1-3 and 10-12h after allograft reperfusion. Secondary endpoints were: Serum creatinine (SrCr), calculated glomerular filtration rate (GFR) and Urine output measured daily from Day 1 up to 7 days post-transplant; Dialysis within 7 days post-transplant; Graft function, on the basis of SrCr on post operative Day 5; Iohexol clearance (facultative); Duration of hospital stay and mortality in the first 30 days post-transplant; SrCr and calculated CrCl (Cockcroft-Gault formula, 1976) at Month 1, 6 and 12 post-transplant; Cumulative acute rejection episodes, and patient and graft survival rate evaluated at Months 6 and 12 post-transplant. The safety and the pharmacokinetics of reparixin in the specific clinical setting was also evaluated.

The study was conducted at 9 centers in Europe (Italy, Spain and France) and the US in adult male and female patients (18-65 years) accepted and listed for kidney transplantation due to ESRD (planned isolated single kidney transplant from a non-living donor with brain death; kidney maintained in cold storage).

Patients must have been at increased risk of developing DGF (scoring ≥3 as per the modified Novartis scoring system). They also were to receive an induction with steroids + mycophenolate mofetil (or mycophenolic acid) + either RATG or IL-2-receptor antibodies. Pregnant or breast feeding women were excluded. Seventy-two patients were planned for inclusion in the study.

Patients were randomized (2:2:1:1) to receive either 12h i.v. infusion (2.772 mg/kg/h) or 24h intermittent i.v. infusion (30min; 2.244 mg/kg) of reparixin or matching placebo. As per protocol, the blind code was broken after database lock of month 1 data.

The study was completed on 19 June 2008. Patient disposition is reported in the flow-chart below:



Demographic and baseline characteristics of both patients and donors were comparable between the treatment groups.

Differences between means and p-values (all p-values > 0.05) all indicate that there was no statistically significant difference between the reparixin continuous infusion, reparixin intermittent infusion and the pooled placebo groups in CrCl values measured 1–3 hours and 10–12 hours post-transplant for the ITT and PP populations (primary variable). In the secondary analysis, differences between means and p-values (all p-values > 0.05) all indicate that there was no statistically significant difference between the three treatment groups in SrCr, GFR and Urine Output measured from Day 1 to Day 7 post-transplant or hospital discharge. Mean iohexol clearance values and duration of hospital stay were all comparable between treatment groups. The number of patients who required dialysis within 7 days post-transplant, the number of days the patient was on dialysis before kidney function was resumed and the number of patients who had resumption of kidney function within 7 days post-transplant were comparable between the three treatment groups. The majority of patients in the reparixin intermittent infusion group had immediate graft function. The numbers of patients with immediate graft function in the reparixin continuous and placebo groups were similar; more patients in these groups had slow or delayed graft function than immediate graft function. SrCr and calculated CrCl at Month 1, Month 6 and Month 12 were comparable between treatment groups. Results were not affected by the type of biological induction. Total number of biopsy proven acute rejections and number of patients reporting at least one acute rejection episode by Month 6 and Month 12 were comparable between the treatment groups, even when data were stratified by the type of biological induction. Similarly,

the incidence of graft failure was comparable between treatment groups. One patient in the pooled placebo groups died within the first 30 days post-transplant. One patient in the reparixin intermittent infusion group died between Month 6 and Month 12.
The total amount of fluid infused (reparixin or placebo solution) was comparable between the three treatment groups. Mean values of 2178.3 mg (reparixin continuous infusion) and 1912.3 mg (reparixin intermittent infusion) of reparixin were infused. The great majority of patients had treatment compliance >80%. Four patients in the reparixin continuous infusion group and 2 patients in the placebo group received less than 50% of the predicted dose. Two patients in the reparixin continuous infusion group received 286% and 128% of the predicted dose, respectively.
The AE profile was similar for both reparixin groups and the placebo group. AEs were reported for 24 patients (96.0%) in the reparixin continuous group, 23 patients (100%) in the reparixin intermittent infusion group and 25 patients (96.2%) in the pooled placebo group. Only 1 AE in the reparixin intermittent infusion group with highly probable relationship to study drug was reported. AEs leading to discontinuation of study drug were reported for 3 patients in the reparixin continuous infusion group and for 1 patient in placebo group. There was 1 AE-related withdrawal (1.4%) in the reparixin continuous infusion group. SAEs were reported for 9 patients (36.0%) in the reparixin continuous infusion group; 4 patients (17.4%) in the reparixin intermittent infusion group; and 8 patients (30.8%) in the placebo group. Only 2 SAEs possibly related to study drug (Retroperitoneal haemorrhage) were reported for 2 patients, both in the reparixin continuous infusion group. One patient in the placebo group had an AE leading to death. Summary of SAE is reported in the table below.

Table 43: Treatment Emergent Serious AEs - Cumulative Summary Tabulation

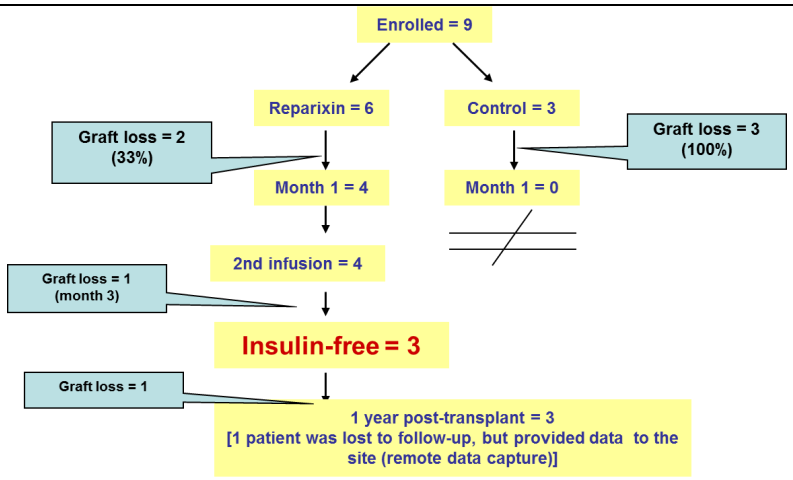
Number of Reports by Terms for Trial: REP0204 (Reparixin / Placebo) (A *denotes a SUSAR)		
MedDRA Body System / LLT ADR term	**Reparixin**	**Placebo**
Gastro intestinal Disorders	**1**	
Ileus	1	
Immune system disorders	**1**	**2**
Hypersensitivity		1
Kidney transplant rejection	1	1
Infections and infestations		**3**
Pneumonia		1
Sepsis		1
Septic shock		1
Injury, poisoning and procedural complications	**3**	
Operative haemorrhage	1	
Perirenal haematoma	1	
Urinary anastomotic leak	1	
Metabolic and nutrition disorders	**3**	
Diabetes Mellitus	1	
Metabolic acidosis	2	
Renal and urinary disorders	**8**	**7**
Complications of transplanted kidney	1	
Renal vein thrombosis	1	3
Renal artery thrombosis	1	1
Renal tubular necrosis	2	1
Ureteral necrosis	1	
Ureteric fistula	2	1
Urinary tract obstruction		1
Respiratory, thoracic and mediastinal disorders	**1**	
Pneumonia aspiration	1	
Vascular disorders	**5**	
Deep vein thrombosis	1	
Retroperitoneal haemorrhage	2*	
Hypotension	2	
TOTAL	**22**	**12**

During the conduct of the study, an apparent cluster of cases of thrombosis occurred, which raised a possible safety concern. The cases of thrombosis were observed in patients recruited at three different sites and were observed over a time span of about 3 months. None was judged related to the study drug. Upon review by the DMC of the SAE narratives and actual treatment received by the four patients, the panel felt comfortable with continuing enrolment in the study. Two additional cases of thrombosis leading to graft loss were observed, which were reviewed by the DMC. No concern was raised as the rate of early thrombosis had apparently dropped and did not appear to be associated with a treatment group or a particular centre.

One patient in the placebo group died before the end of Month 1. The proximate cause of death was septic shock. The antecedent condition was identified to be pneumonia. One patient in the reparixin intermittent infusion group died between Month 6 and Month 12. No information is available.
Clinical laboratory data (haematology, clinical chemistry and coagulation results) and vital signs were comparable between the three treatment groups, with no trends observed.
All 25 patients in the reparixin continuous infusion group, 23 patients in the reparixin intermittent infusion group and 26 patients in the placebo group, received concomitant medication during the course of the study and the use of concomitant medication was similar between the groups.
The plasma concentrations of reparixin (total and unbound) and its major metabolite, DF2243Y, were derived from 46 patients. The concentration of total reparixin and its metabolite were consistent with the expected values. Unbound reparixin concentrations were about 2–3 times higher than those found in healthy volunteers and the corresponding free fractions were increased with respect to the healthy volunteers.
Overall, the study was unable to demonstrate a statistically significant effect of reparixin on short and long term functional and clinical outcomes after kidney transplantation in patients at increased risk of developing DGF. Reparixin was found to be safe and well tolerated in patients undergoing kidney transplantation.

6.2.3.3. A phase 2 multicenter, randomized, open label, parallel assignment, pilot study to assess the efficacy and safety of reparixin following a single-infusion islet transplantation in patients with type 1 diabetes mellitus. [REP0110 - EudraCT# 2010-019424-31]

The objective of this clinical trial was to evaluate whether reparixin leads to improved transplant outcome as measured by glycaemic control following single intra-hepatic infusion of pancreatic islets in type 1 diabetes patients. The primary efficacy endpoint was insulin-independence in the 75 +/- 5 day timeframe post-transplant. Secondary endpoints included insulin-independence up to 1 year post-transplant, time to achieve insulin-independence, total time of insulin-independence, change in average daily insulin requirements, absolute and % decrease in HbA1cA from pre-transplant levels, proportion of patients free of severe hypoglycemic events, proportion of patients free of hypoglycemic events with reduced awareness, Basal (fasting) and -10 to 120 minute time course of glucose, C-peptide, and insulin derived from the mixed meal tolerance test (MMTT), and β-cell function. The safety of reparixin in the specific clinical setting was also evaluated.
The study was conducted at two centers (Italy and Germany); however, only the Italian sites recruited patients into the trial. The study was initiated in July 2010 (first patient in). Patients were on an immunosuppression regimen consisting of induction with ATG followed by maintenance immunosuppression. 10 patients were planned for inclusion in the study. Eligible patients included adult patients (aged 18-65) with a clinical history of type 1 diabetes (T1D) with insulin-dependence for $\geq$ 5 years and undetected stimulated C-Peptide in the 12 months before transplant. Inclusion criteria restricted enrolment to patients who were expected to receive an islet mass (4000 to 7000 islet equivalent (IEQ)/kg body weight) in the lower range of the commonly accepted transplantable islet amount.
Originally, patients were randomly (1:1) assigned to receive either no additional experimental intervention (control group) or reparixin treatment (2.772 mg/kg body weight/hour i.v. continuous infusion for 7 days) – treatment group). Due to preliminary results obtained in 7 patients, protocol REP0110 was amended to allow randomization to the reparixin treatment group only. Also, patients already treated with reparixin with a functioning graft were allowed to receive a 2nd islet infusion. Follow-up was re-scheduled to provide measurements up to one year after the 2nd islet infusion. The schedule of post-transplant visits was re-set on the basis of the date of the second islet infusion, if any.
The study was completed 30 April 2013 (last patient, last visit). All together, a total of 9 patients were enrolled, 6 randomized to reparixin treatment and 3 to the control group. Patient disposition is reported in the flow-chart below:



Demographic and baseline characteristics of patients were comparable between the 2 treatment groups.
All patients in the control group and 2 patients in the reparixin group were withdrawn after Transplant 1 due to graft loss. Four patients in the reparixin group received Transplant 2. Thereafter, 1 patient was withdrawn due to graft loss. One patient was lost to follow-up. Two patients in the reparixin group completed the study (observation up to Month 12 after the second transplant).

No patients in the study reached insulin-independence at Day 75±5 after a single islet transplant. Although the primary efficacy endpoint was not reached, most markers of graft function were notably improved in the reparixin group when compared to the control group, even after transplantation of a marginal islet mass. Indeed, all of the 6 patients treated with reparixin experienced improved transplant outcome, as measured by glycemic control, increased insulin requirement, and appearance of detectable levels of C-peptide well above 0.3 ng/mL. Only 2 (33.3%) patients in the reparixin group were withdrawn from the study due to graft failure after the 1st transplant. On the contrary, none of the patients in the control group expressed a β-cell function at Month 1 post-transplant and all were withdrawn as a result of graft loss. Out of 6 patients on reparixin, 4 received the second islet transplant and 3 then reached insulin-independence. From these, 2 patients were insulin-independent at least after 1 year. This trial was not statistically powered to measure differences between treatments in efficacy. Nevertheless, patients in the reparixin group had greater improvement in efficacy outcomes following the first islet transplantation, particularly when compared to the control group, even after transplantation of a marginal islet mass. Moreover, this improvement was further strengthened by the second transplant with insulin-independence observed in 50.0%, 75.0%, and 50.0% of patients at Month 3, 6 and 12, respectively.

Apart from Patient 0110 who, due to an error in drug administration, received a 3-fold dose of reparixin during Transplant 2 and was exposed to 8.316 mg/kg/hour of reparixin for 24.03 hours, all the patients were administered reparixin at a dose of 2.772 mg/kg/hour for 7 consecutive days. In 4 patients, reparixin administration was repeated during the 2nd islet infusion. The sample size was too small to determine whether reparixin had an effect on the incidence of AEs during the trial. Most of the AEs reported during the study were known complications resulting from islet infusion procedure or general surgical procedures (e.g. diarrhea, nausea, pain, oedema, intraperitoneal bleeding) and were judged unrelated to reparixin.

A total of 11 SAEs were reported in 4 patients (1 patient had 3 different sets of SAEs) overall, 10 in 3 (50.0%) patients from the reparixin group and 1 (33.3%) in the control group. There were no deaths and no withdrawals from the study due to TEAEs or SAEs. Five (50%) out of 10 SAEs experienced by the patients receiving reparixin were judged to be related to the study drug. These 5 SAEs (anemia, gastrointestinal bleeding, nausea, vomiting, and medication error) all occurred in 1 patient (patient 0110 mentioned above) and were directly related to over-exposure (3 times the foreseen dose administered for 24 hours) to reparixin. Infusion of reparixin was immediately discontinued upon detection of the drug administration error and the patient recovered.

No trends were observed in vital signs and laboratory parameters between the treatment groups. All changes in hematology and clinical chemistry values observed within the study period were considered by the Investigator not to be clinically relevant. A summary of the SAE's reported is in the table below.

Table 44: Treatment Emergent Serious AEs - Cumulative Summary Tabulation

Number of Reports by Terms for Trial: REP0110 (Reparixin / Control group)		
MedDRA SOC Preferred Term	**Reparixin**	**Control group**
Blood and lymphatic system Disorders	**1**	
- Anemia	1	0
Gastrointestinal Disorders	**6**	
- Diarrhea	2	0
- Gastrointestinal haemorrhage	1	0
- Nausea	1	0
- Peritoneal haemorrhage	1	0
- Vomiting	1	
Injury, poisoning and procedural complications	**1**	
- Drug administration error	1	0
Metabolism and nutrition disorders	**1**	
- Diabetic ketoacidosis	1	0
Renal and urinary disorders	**2**	
- Renal impairment	2	0
Vascular disorders	0	1
- Hemorrhage		1

MedDRA=Medical Dictionary for Regulatory Activities; SOC=system organ class

Overall, reparixin was found to be safe and well-tolerated in T1D patients undergoing islet transplantation, even when administered for 7 days in up to 2 treatment sessions. And, although the sample size was too small to be statistically significant, the results suggest a clinical benefit of reparixin in terms of improved islet transplant outcomes and provide further evidence of its potential.

Follow-up of these patients is being extended up to 3 years post-last transplant through an extension study (REP0113).

6.2.3.4 A phase 3, multicenter, randomized, double-blind, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet transplantation. [REP0211]

A phase 3 study is ongoing in 8 sites in 4 EU countries and 1 site in the US to further evaluate the efficacy and safety of reparixin in pancreatic islet transplantation in T1D. A total of at least 42 (up to a maximum of 72) pancreatic islet transplant recipients will be randomly (2:1) assigned to receive either reparixin [continuous i.v. infusion for 7 days (168hrs), starting approximately 12hrs before each islet transplant (treatment group)] or matched placebo (control group), administered as an added on treatment to the immunosuppressant regimen. Patients may receive up to 2 islet transplants, the 2nd one to occur between 3 and 12 months after the 1st one in patients who retain graft function (fasting C-peptide > 0.3 ng/mL). Primary endpoint is the AUC for the serum C-peptide level during the first 2 hours of a Mixed Meal Tolerance Test (MMTT), normalized by the number of IEQ/kg, calculated at day 75$\pm$5 after the 1st islet infusion and day 365$\pm$14 after the last islet infusion.

To date, 49 patients have been randomized, and 44 have gone on to treatment and transplant. 23 patients have received a second islet infusion. 13 patients have completed one year follow-up after last transplant (either the 1st or the 2nd). 66 SAEs (excluding co-manifestations) have been reported to date in 30 patients. Most of the events involved the gastrointestinal system (e.g. abdominal pain, nausea, vomiting); blood and lymphatic system disorders (leukopenia, neutropenia, anemia, hemoperitoneum), other body system disorders (increased liver enzyme, hepatic hematoma, bleeding, raise in donor specifyc antibodies): overall the kind of SAEs reported were well known events associated to the transplant procedure. The following cases were considered by the investigator to be at least possibly related to the IP, either reparixin or placebo: one case of neutropenia and leukopenia, one case of anemia, intraabdominal haemorrhage and abdominal pain lower; one case of adenocarcinoma and cytomegalovirus colitis, two cases of transplant rejection, one case of pneumonia, one case of implant site haemorrhage.

6.2.3.5 A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation. **[**REP0112**]**

This phase 2/3 is being conducted at 6 centers in the US to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation (IAT) in patients undergoing total pancreatectomy due to chronic pancreatitis. One Canadian center is going to be opened. The goal of this study is to reach a total of 100 adult patients who are randomized and receive IAT. Patients will be randomly (1:1) assigned to receive either reparixin [continuous i.v. infusion for 7 days (168hrs)], or matched placebo (control group),starting approximately 12hrs before islet infusion. The primary endpoint is the proportion of insulin-independent patients at one year after the transplant. To date, 34 patients have been randomized and transplanted. 41 SAEs have been reported to date in 26 patients. Most of the events involved the gastrointestinal system (e.g. abdominal pain, nausea, vomiting, clostridium difficile) and are known events associated to the transplant procedure. All cases were considered unrelated.

6.2.4. Studies in breast cancer

The first study in oncology was opened in July 2011 and the primary objective of the initial clinical investigations was to achieve the Proof of Concept that reparixin targets CSCs in patients and that such targeting translates into potential for clinical benefit. Subsequent proof of benefit studies will then be performed. Based on the aforementioned preclinical data [6], a Phase 1b study to evaluate the safety and pharmacokinetic profile of reparixin oral tablets in combination with paclitaxel, and to explore the effects of reparixin on disease response and breast CSCs and the tumor microenvironment was initiated and has now completed enrollment. A Phase 2 study has also been initiated to evaluate the use of single agent reparixin in the selective targeting of CSCs in breast cancer in the early stage setting. In addition, a new phase 2 study is in start up to evaluate reparixin in combination with paclitaxel in metastatic TNBC.

6.2.4.1. Phase 1b pilot study to evaluate reparixin in combination with chemotherapy with weekly paclitaxel in patients with HER-2 negative metastatic breast cancer (MBC) [REP0111]

The primary objective of this study was to evaluate the safety and pharmacokinetic (PK) profile of orally administered reparixin in combination with weekly paclitaxel and, as secondary objectives, to evaluate the antitumor activity of the combination and its effects on cancer stem cell markers. The study was conducted at 5 centers in the US, with enrollment beginning in February 2012. Enrollment completed in February 2014, and all patients are now off study. Eligible patients included adult female patients with histologic or cytologic diagnosis of breast cancer with evidence of metastatic disease with documented human epidermal growth factor receptor-2 (HER-2) negative status and who were eligible for treatment with paclitaxel. Patients must have had a least one measurable lesion according to RECIST criteria 1.1, and must not have received more than three prior chemotherapy lines for advanced breast cancer (not including neo/adjuvant chemotherapy). If prior treatment with paclitaxel, PD must have occurred > 12 months from the end of previous neo/adjuvant treatment or, for previous metastatic treatment, no PD must have occurred during treatment or within 3 months of the end of treatment. In this study patients received a three-day run-in with single agent reparixin oral tablets 3 times daily (t.i.d.) followed by paclitaxel 80 mg/m2/week (days 1, 8, and 15 for 28-day cycle) + reparixin oral tablets t.i.d. days 1-21. Three reparixin dose levels (400 mg oral t.i.d.; 800 mg t.i.d.; 1200 mg t.i.d.) were planned to be explored in cohorts of 3-6 patients. A Dose Limiting Toxicity assessment was made for each patient following the first cycle of treatment. Treatment continued for each patient as long as clinical benefit was observed. An additional 6 subjects were planned to be enrolled at the highest tolerated dose. This study was further amended to allow an additional 11 subjects (total of 20 evaluable patients) to be enrolled at the 1200mg dose to obtain additional correlative study data and to perform ECG measurements at Cmax after the first dose and at steady state, as requested by FDA on initial approval of the study (clinical pharmacology non-hold comments dated 07 July 2011). It was not previously possible to estimate Cmax as the PK profile of the oral tablet formulation has been studied for the first time in this trial. PK sampling was performed for reparixin and metabolites and paclitaxel during cycle 1.A total of 33 patients with HER-2 negative MBC were enrolled into the study. Of these 33 patients, 30

(90.9%) were included in the Safety Population and 19 (63.3%) were included in the PK Population. Of the 30 patients who were included in the Safety Population, 4 patients were in Group 1 (400 mg reparixin + paclitaxel 80 mg/m^2), 3 were in Group 2 (800 mg reparixin + paclitaxel 80 mg/m^2), and 23 patients were in Group 3 (1200 mg reparixin + paclitaxel 80 mg/m^2).

All patients reported at least one TEAE. In total, 23 patients (76.7%) reported at least one TEAE that was considered by the investigator to be related to the combination treatment. Six patients reported at least one treatment-emergent SAE (all of which were judged as unrelated or unlikely related to reparixin) (Table 45: Number of patients with Treatment-Emergent Serious Adverse Events by System Organ Class and Preferred Term (Safety Population)) and one patient had an unrelated TEAE (disease progression) that resulted in death. Three patients discontinued the study due to a TEAE. In two of them the reason was hypersensitivity reaction to paclitaxel infusion. The third patient discontinued the study due to grade ≤2 gastrointestinal disorder and nausea related to the study drugs. The system organ class most commonly associated with TEAEs was gastrointestinal disorders. The most common events reported under gastrointestinal disorders were constipation and nausea. No gastrointestinal AEs were reported during the three-day reparixin single agent run-in period. There seems to be a temporal relationship of gastrointestinal AEs to paclitaxel. Regardless of system organ class, the most commonly reported TEAEs were fatigue, alopecia, constipation, nausea, decreased appetite, diarrhoea, dyspnoea, peripheral edema, and cough. No treatment-related Grade 4 or Grade 5 TEAEs were recorded during the study. There were no apparent differences between the treatment groups with regards to the distribution of treatment-related TEAEs within the system organs.

Table 45: Number of patients with Treatment-Emergent Serious Adverse Events by System Organ Class and Preferred Term (Safety Population)

System Organ Class Preferred Term	Group 1 (N = 4) n (%)	Group 2 (N = 3) n (%)	Group 3 (N = 23) n (%)	Total (N = 30) n (%)
Number of Patients with at Least One Serious TEAE	2 (50.0)	1 (33.3)	3 (13.0)	6 (20.0)
Metabolism and Nutrition Disorders	2 (50.0)	0	0	2 (6.7)
Dehydration	2 (50.0)	0	0	2 (6.7)
Hyponatraemia	1 (25.0)	0	0	1 (3.3)
Respiratory, Thoracic and Mediastinal disorders	0	1 (33.3)	1 (4.3)	2 (6.7)
Dyspnoea	0	0	1 (4.3)	1 (3.3)
Hypoxia	0	0	1 (4.3)	1 (3.3)
Pneumothorax	0	1 (33.3)	0	1 (3.3)
Gastrointestinal Disorders	0	0	1 (4.3)	1 (3.3)
Abdominal Pain	0	0	1 (4.3)	1 (3.3)
General Disorders and Administration Site Conditions	0	0	1 (4.3)	1 (3.3)
Disease progression	0	0	1 (4.3)	1 (3.3)
Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)	1 (25.0)	0	0	1 (3.3)
Metastases to Central Nervous System	1 (25.0)	0	0	1 (3.3)
Nervous System Disorders	0	0	1 (4.3)	1 (3.3)
Intracranial Hypotension	0	0	1 (4.3)	1 (3.3)

Note: Group 1 = Paclitaxel 80 mg/m^2 i.v. (Days 1, 8, and 15 of 28-day cycle) + reparixin oral 400 mg t.i.d. three weeks on one week off; Group 2 = Paclitaxel 80 mg/m^2 i.v. (Days 1, 8, and 15 of 28-day cycle) + reparixin oral 800 mg t.i.d. three weeks on one week off; Group 3 = Paclitaxel 80 mg/m^2 i.v. (Days 1, 8, and 15 of 28-day cycle) + reparixin oral 1200 mg t.i.d. three weeks on one week off. AEs were coded using MedDRA version 14.1. For each level of patient summarization, patients were counted only once if they experienced multiple events.

Sixteen SAEs were reported in 10 patients.. All reported SAEs were considered unrelated or unlikely to be related to reparixin by the treating investigator. No DLTs were observed and the Safety Monitoring Committee approved escalation of the reparixin dose at each of the dose levels to reach the maximum studied dose of 1200 mg t.i.d. No formal Maximum Tolerated Dose was reached.

The PK of reparixin, its metabolites and paclitaxel were investigated during the first cycle of treatment [Table 46, Table 47, Table 48, Table 49]. At all dose levels, patients entered a three-day run-in period (day -3 to day -1) during which reparixin was administered at the dose level for each cohort as single agent, for pharmacokinetic purposes. On days 1,8 and 15 of each cycle, reparixin was administered with 250 mL water and food prior to the start of paclitaxel infusion and then every 8 hours. Blood sampling was performed for reparixin and its metabolites on day -3, 1, 8 and 21 whereas for paclitaxel on day 1 and 8. Unbound reparixin was measured 1 and 2 hours after reparixin administration on days -3, 1, 8 and 21.

Reparixin Cmax and AUC0-8 increased in an approximately dose proportional manner over the range of 400 mg to 1200 mg.Within each dose level, there was not a consistent difference in either Cmax or AUC0-8 over cycle Days -3 to 21. There was no consistent trend in Rac0-8, so there appeared to be little or no accumulation of reparixin over the 21 day dose period.After 4 days of t.i.d. dosing, within the variability observed, concentration of reparixin at each dose level appeared to reach a constant value, which is evidence that steady may have been reached.Mean reparixin t1/2 was about 2 hours and was independent of dose level or cycle day. Mean CL/F and Vz/F were about 5 L/hr and 15 L, respectively, and also were independent of dose level or cycle day.

The fraction of unbound to total drug appeared to be independent on cycle day, but dependent on dose level. For 400, 800 and 1200 mg dose groups, the average ratio of unbound to total drug (as %) was 0.07%, 0.11%, and 0.14%, respectively.

As to the main metabolites, DF 2243Y C_{max} and AUC_{0-8} increased in a dose proportional manner.

Within each dose group, DF 2243Y C_{max} and AUC_{0-8} both tended to be similar throughout cycle Days -3 to 21, although with a great deal of variability.

Rac_{0-8} values indicate low to moderate accumulation, although there was no consistent trend with respect to dose level or cycle day.

After adjusting for molecular weight differences, Cmax of DF2243Y was about 32% and AUC_{0-8} about 49% that of parent.

On Days -3 and 21, the mean $t_{1/2}$ ranged from 1.3 to 3.8 hours and was independent of dose over the range from 400 mg to 1200 mg.

For DF 2188Y, C_{max} and AUC_{0-8} increased with each increase in dose in a greater than dose proportional manner.

Although with a great deal of variability, within each dose group C_{max} and AUC_{0-8} both tended to be similar throughout cycle Days -3 to 21.

From Rac_{0-8} values, there was little or no accumulation at 400 mg, and low to moderate accumulation at 800 mg and 1200 mg.

After adjusting for molecular weight differences, DF 2188Y C_{max} was about 16% and AUC_{0-8} about 19% that of parent.

Mean $t_{1/2}$ of DF 2188Ywas about 1.8 hours and did not vary in relation to dose or cycle day.

Ibuprofen Cmax and AUC_{0-8} increased in a dose related manner. On Days -3 and 1, the increase was approximately proportional to dose, while on Days 8 and 21, the increase was greater than proportional to dose.

Cmax or AUC_{0-8} were approximately the same from day to day on all cycle days from Day-3 to Day 21, although with a great deal of variability.

From Rac_{0-8} values, in the 400 mg group, there was little or no accumulation. For the 800 mg and 1200 mg dose groups, there was a low to moderate level of accumulation.

After adjusting for molecular weight differences, Cmax of ibuprofen was about 3% and AUC_{0-8} about 5% that of parent.

The mean $t_{1/2}$ was about 4 hours (2 to 7 hours) and did not appear to be related to dose or cycle day over Days -3 and 21.

Paclitaxel dose was 80 mg/m2 at all reparixin dose levels, so as expected, C_{max}, AUC_{0-24} and AUC_{0-inf} were each similar for all treatment groups on both cycle days [Figure 7].

From Rac_{0-24} values, there was little or no accumulation from Day 1 to Day 8

The mean $t_{1/2}$ was about 8 hours and was independent of treatment group or cycle day. Mean CL/F and Vz/F were also independent of treatment group or cycle day. Thus, it can be concluded that orally administered reparixin over the dose range 400-1200 mg t.i.d. does not influence the pharmacokinetic of paclitaxel administered as a 1 hour i.v. infusion.

Table 46: Reparixin (DF 1681Y), DF 2243Y, DF 2188Y and ibuprofen PK parameters after oral administration of reparixin 400 mg t.i.d. Days -3, 1, 8 and 21 of Cycle 1 (PK Population)

Dose regimen			Cohort 1 400mg t.i.d.															
			Day -3				Day 1				Day 8				Day 21			
			N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
Reparixin	$C_{predose}$	(µg/mL)	4	0	0		4	1.05	0.37	35.3	4	0.79	1.05	134	3	1.25	0.76	60.8
	C_{max}	(µg/mL)	4	37.9	15.2	40.1	4	30.7	19	62.1	4	17.9	7.11	39.8	3	19.4	3.27	16.9
	T_{max}	(hr)	4	0.9	0.45	49.9	4	2.15	1.44	67	4	1.3	0.46	35.7	3	2.66	0.57	21.4
	AUC_{0-8}	(hr* µg/mL)	4	86.7	34.5	39.8	4	86.5	40.3	46.6	4	49.8	38.4	77	3	57.6	11.8	20.6
	AUC_{INF}	(hr* µg/mL)	4	90.5	34.4	38	1	124			1	101			2	68.2	8.69	12.8
	$t_{1/2}$	(hr)	4	1.65	0.51	30.6	1	1.97			1	2			2	1.89	0.57	30
	Vz/F	(L)	4	12.9	9.96	77.3	1	9.2			1	11.4			2	16.4	6.88	42
	Cl/F	(L/hr)	4	5.04	2.25	44.6	1	3.24			1	3.96			2	5.92	0.76	12.8
	Rac, $_{0-8}$						4	0.97	0.14	14.9	4	0.63	0.39	61.8	3	0.82	0.22	26.5
	Rac, 0-inf										1	0.92	0.92		2	0.74	0.09	11.8
DF 2243Y	$C_{predose}$	(µg/mL)	4	0	0		4	1.08	0.59	54.7	4	0.77	0.86	112	3	2.09	2.24	107
	C_{max}	(µg/mL)	4	5.85	1.5	25.6	4	5.79	2.84	49	4	5.07	4.32	85.2	3	7.71	1.63	21.2
	T_{max}	(hr)	4	3.3	0.87	26.4	4	3.51	0.99	28.3	4	4.29	2.94	68.6	3	3.71	0.62	16.7
	AUC_{0-8}	(hr* µg/mL)	4	25.03	4.05	16.2	4	25.8	9.2	35.7	4	21.6	18.4	85.3	3	31	7.5	24.2
	AUC_{INF}	(hr* µg/mL)	1	28.7			0				0				1	33.1		
	$t_{1/2}$	(hr)	1	1.34			0				0				1	1.97		
	Rac, $_{0-8}$						4	1.02	0.28	27	4	0.78	0.64	81	3	1.15	0.19	16.7
	Rac, 0-inf														1	1.15		
DF 2218Y	$C_{predose}$	(µg/mL)	4	0	0		4	0.24	0.11	44.9	4	0.18	0.26	142	3	0.35	0.25	71.3
	C_{max}	(µg/mL)	4	3.01	0.99	32.9	4	2.9	1.53	52.9	4	2.11	1.25	59.3	3	3.29	0.73	22.1
	T_{max}	(hr)	4	2.05	0.71	34.8	4	1.78	0.52	29.3	4	2.3	1.23	53.5	3	3.33	0.57	17.1
	AUC_{0-8}	(hr* µg/mL)	4	9.79	1.77	18.1	4	11	3.93	35.8	4	8.2	6.01	73.2	3	11	1.83	16.7
	AUC_{INF}	(hr* µg/mL)	3	10.6	2.42	22.9	1	9.96			0				2	13.6	0.03	0.2
	$t_{1/2}$	(hr)	3	1.63	0.23	14	1	1.51			0				2	1.9	0.2	10.6
	Rac, $_{0-8}$						4	1.12	0.34	30	4	0.77	0.54	69.4	3	1.04	0.09	8.5
	Rac, 0-inf						1	1.23			0				2	1.16	0.15	13.2
Ibuprofen	$C_{predose}$	(µg/mL)	4	0.04	0.04	96.4	4	0.41	0.4	97.4	4	0.12	0.13	105	3	0.3	0.36	119
	C_{max}	(µg/mL)	4	0.76	0.68	89.8	4	0.86	0.86	101	4	0.34	0.32	92.7	3	0.58	0.64	112
	T_{max}	(hr)	4	3.5	0.58	16.5	4	4.02	2.86	71	4	4.29	2.94	68.6	3	3.37	0.67	20
	AUC_{0-8}	(hr* µg/mL)	4	4.35	3.9	89.8	4	5.14	5.21	101	4	1.69	2.29	135	3	3.17	3.76	119
	AUC_{INF}	(hr* µg/mL)	2	14.3	3.65	25.5	0				0				2	1.33	0.7	52.7
	$t_{1/2}$	(hr)	2	6.76	2.04	30.2	0				0				2	3.12	0.56	17.8
	Rac, $_{0-8}$						4	1.09	0.22	19.7	4	0.65	0.61	92.9	3	1.18	0.74	62.3
	Rac, 0-inf						0				0				0			

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Table 47: Reparixin (DF 1681Y), DF 2243Y, DF 2188Y and ibuprofen PK parameters after oral administration of reparixin 800 mg t.i.d. Days -3, 1, 8 and 21 of Cycle 1 (PK Population)

Dose regimen			Cohort 2 800mg t.i.d.															
			Day -3				Day 1				Day 8				Day 21			
			N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
Reparixin	$C_{predose}$	(µg/mL)	3	0	0		3	4.24	3.39	79.8	3	2.4	1.78	74	3	6	5.56	92.7
	C_{max}	(µg/mL)	3	35.6	28.6	80.3	3	45.4	3.88	8.5	3	34.4	11.5	33.6	3	44.6	25.3	56.6
	T_{max}	(hr)	3	1.82	1.25	68.7	3	1.01	0.01	1	3	1.72	0.48	27.9	3	2.32	1.52	65.2
	AUC_{0-8}	(hr* µg/mL)	3	108	58.1	53.6	3	124	32.2	26	3	150	44	29.3	3	153	47.2	30.9
	AUC_{INF}	(hr* µg/mL)	2	143	60.4	42.1	3	131	35.5	27.1	1	260			2	190	44	23
	$t_{1/2}$	(hr)	2	1.82	0.82	45.2	3	1.73	0.09	5.42	1	3.57			2	1.97	0.55	28
	Vz/F	(L)	2	14.5	0.49	3.4	3	15.9	3.28	20.7	1	15.9			2	11.9	0.62	5.2
	Cl/F	(L/hr)	2	6.13	2.58	42.1	3	6.38	1.5	23.6	1	3.08			2	4.33	1	23
	$Rac_{, 0-8}$						3	1.3	0.53	40.7	3	1.7	0.88	51.8	3	1.61	0.63	39.4
	Rac, 0-inf										1	2.58	2.58		2	1.52	0.95	62.1
DF 2243Y	$C_{predose}$	(µg/mL)	3	0	0		3	4.68	5.28	113	3	2.79	2.64	94.8	3	3.92	3.17	80.8
	C_{max}	(µg/mL)	3	8.46	3.05	36	3	13.8	3.51	25.3	3	10.6	0.32	3.1	3	12.6	3.13	24.8
	T_{max}	(hr)	3	4.32	1.52	35.2	3	2.67	1.16	43.6	3	3.32	1.15	34.5	3	3.66	2.08	56.9
	AUC_{0-8}	(hr* µg/mL)	3	40.4	11.9	29.4	3	65	13.8	21.3	3	49.8	7.89	15.9	3	58.1	17.7	30.4
	AUC_{INF}	(hr* µg/mL)	1	68.1			0				0				2	81.6	17	20.8
	$t_{1/2}$	(hr)	1	3.79			0				0				2	2.46	0.59	24.1
	$Rac_{, 0-8}$						3	1.82	1.06	58.1	3	1.3	0.37	28.3	3	1.44	0.11	7.75
	Rac, 0-inf														1	1.38		
DF 2218Y	$C_{predose}$	(µg/mL)	3	0	0		3	1.36	1.78	131	3	0.54	0.44	82.6	3	0.91	0.76	83
	C_{max}	(µg/mL)	3	3.67	1.42	38.7	3	7.37	1.29	17.5	3	4.42	0.89	20.2	3	5.17	1.42	27.5
	T_{max}	(hr)	3	2.81	1.24	44.1	3	1.33	0.58	43.3	3	2.03	0.06	2.8	3	2.33	1.14	48.9
	AUC_{0-8}	(hr* µg/mL)	3	16	4.54	28.3	3	24.6	0.86	3.5	2	20.8	2.93	14.1	3	21.2	3.56	16.8
	AUC_{INF}	(hr* µg/mL)	2	21	7.6	36.1	2	25	0.6	2.4	0				2	24.7	3.34	13.5
	$t_{1/2}$	(hr)	2	2.53	1.53	60.4	2	1.48	0.07	4.8	0				2	1.77	0.21	12
	$Rac_{, 0-8}$						3	1.61	0.4	25.1	3	1.32	0.22	16.7	3	1.35	0.15	10.8
	Rac, 0-inf						1	1.57			0				2	1.23	0.28	23.2
Ibuprofen	$C_{predose}$	(µg/mL)	3	0.02	0.02	70.9	3	1.25	1.39	112	3	0.53	0.56	104	3	0.86	0.75	87.3
	C_{max}	(µg/mL)	3	1.02	0.59	58	3	1.98	1.46	73.6	3	1.24	0.75	60.3	3	2.16	1.74	80.3
	T_{max}	(hr)	3	3.99	1.99	49.9	3	2.66	1.16	43.6	3	3.32	1.15	34.5	3	3.99	2	50.1
	AUC_{0-8}	(hr* µg/mL)	3	5.97	4.38	73.5	3	10.93	7.55	91.1	3	6.82	4.08	59.9	3	9.96	6.75	67.8
	AUC_{INF}	(hr* µg/mL)	1	19.8			0				0				1	8.64		
	$t_{1/2}$	(hr)	1	6.15			0				0				1	3.81		
	$Rac_{, 0-8}$						3	1.99	1.38	69.3	3	1.25	0.28	22	3	1.89	0.86	45.5
	Rac, 0-inf						0				0				0			

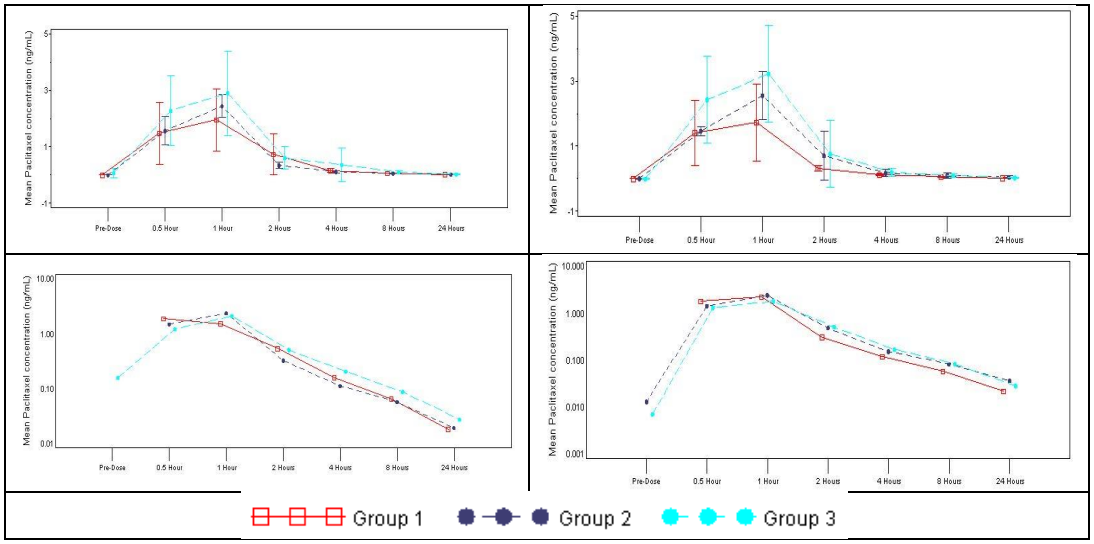
Table 48: Reparixin (DF 1681Y), DF 2243Y, DF 2188Y and ibuprofen PK parameters after oral administration of reparixin 1200 mg t.i.d. Days -3, 1, 8 and 21 of Cycle 1 (PK Population)

Dose regimen			Cohort 3 1200mg t.i.d.															
			Day -3				Day 1				Day 8				Day 21			
			N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
Reparixin	$C_{predose}$	(µg/mL)	12	0	0		12	5.74	6.77	118	10	3.55	2.05	57.9	9	4.98	4.32	86.8
	C_{max}	(µg/mL)	12	65	21.1	32.5	11	58.7	22.9	39	10	60.2	26.5	44.1	8	71.2	24.6	34.6
	T_{max}	(hr)	12	1.41	0.55	39.2	11	2.64	2.07	78.6	10	2.45	2.35	95.7	8	1.4	0.35	25.2
	AUC_{0-8}	(hr* µg/mL)	12	194	71.96	37.1	11	216	96.91	44.8	8	178	88.1	49.4	8	191	61.6	32.3
	AUC_{INF}	(hr* µg/mL)	11	209	94.3	45.1	3	260	146	56	4	202	110	55	8	203	58.6	28.9
	$t_{1/2}$	(hr)	11	1.91	0.64	33.6	3	2.14	0.45	21.2	4	1.88	0.27	14.6	8	1.95	0.56	28.8
	Vz/F	(L)	11	17.8	7.86	44.1	3	16.8	6.96	41.5	4	20.1	9.69	48.3	8	18.9	10.32	54.6
	Cl/F	(L/hr)	11	6.82	2.87	42	3	5.98	3.84	64.3	4	7.79	4.62	59.3	8	6.37	1.84	28.8
	$Rac_{, 0-8}$						11	1.21	0.41	34	10	1.01	0.3	29.5	8	1.02	0.21	20.8
	Rac, 0-inf										4	0.75	0.24	32.6	7	1.05	0.25	24
DF 2243Y	$C_{predose}$	(µg/mL)	12	0	0		12	6.52	7.25	111	10	6.44	7.04	109	9	5.98	4.03	67.4
	C_{max}	(µg/mL)	12	19.5	6.73	34.5	11	20.6	8.35	40.6	10	18.3	8.22	45	8	21.4	8.75	40.9
	T_{max}	(hr)	12	3.41	1	29.2	11	4	1.57	39.3	10	3.4	1.9	56	8	2.87	0.83	29.1
	AUC_{0-8}	(hr* µg/mL)	12	90.21	35.3	39.1	11	108.7	55.2	50.8	10	99	53.2	53.8	8	108	50.5	46.9
	AUC_{INF}	(hr* µg/mL)	7	135	78.1	57.8	0				0				6	164	106	64.7
	$t_{1/2}$	(hr)	7	2.81	1.2	42.8	0				0				6	3.35	1.63	48.6
	$Rac_{, 0-8}$						11	1.18	0.27	22.8	10	1.08	0.23	21	8	1.16	0.17	14.4
	Rac, 0-inf														5	1.23	0.14	11.5
DF 2218Y	$C_{predose}$	(µg/mL)	12	0	0		12	1.43	2.04	142	10	1.3	1.95	150	9	1.21	1.01	83
	C_{max}	(µg/mL)	12	10.5	4.85	46.3	11	13.4	7.68	57.4	10	14.5	9.3	64.1	8	12.2	5.83	47.9
	T_{max}	(hr)	12	2.44	1.08	44	11	2.45	1.04	42.4	10	2.2	1.37	62.3	8	1.77	0.96	53.9
	AUC_{0-8}	(hr* µg/mL)	12	36.3	16.8	46.2	11	57.2	39.4	68.9	10	46.5	29	62.4	8	36.6	21.6	59
	AUC_{INF}	(hr* µg/mL)	7	45.7	24.5	53.6	0				3	52.7	15.7	29.8	7	46.9	34.3	73.2
	$t_{1/2}$	(hr)	7	1.65	0.4	24	0				3	1.42	0.15	10.6	7	2.18	1.44	65.9
	$Rac_{, 0-8}$						11	1.52	0.51	33.8	10	1.22	0.27	22	8	0.95	0.15	16
	Rac, 0-inf						0				2	1.17	0.1	8.19	6	1.01	0.2	19.7
Ibuprofen	$C_{predose}$	(µg/mL)	12	0.03	0.04	146	12	1.08	0.97	90	10	1.07	0.77	71.9	9	1.21	0.9	74
	C_{max}	(µg/mL)	12	3.11	2.59	83.4	11	2.95	2.1	71.2	10	3.59	2.67	74.4	8	3.99	2.74	68.7
	T_{max}	(hr)	12	2.86	0.81	28.3	11	4	2.18	54.4	10	2.15	1.44	67.1	8	2.58	0.97	37.6
	AUC_{0-8}	(hr* µg/mL)	12	14.8	12.7	85.7	11	16.17	12.07	74.7	10	17.5	13.8	78.5	8	19.4	13.3	68.5
	AUC_{INF}	(hr* µg/mL)	6	32.1	20.2	62.8	0				2	12.2	0.51	4.2	6	27.8	21.4	77
	$t_{1/2}$	(hr)	6	3.76	0.77	20.5	0				2	2.15	0.09	4.4	6	3.15	0.53	16.8
	$Rac_{, 0-8}$						11	1.18	0.43	36.4	10	1.23	0.3	24.7	8	1.24	0.26	21.4
	Rac, 0-inf						0				0				4	1.16	0.3	25.8

Table 49: Plasma Unbound Reparixin concentration (ng/mL) on Days -3, 1, 8 and 21 of Cycle 1 (PK Population)

Reparixin (1681Y) dose	day-3/1 hr	day-3/2 hrs	day 1/1 hr	day1/2 hrs	day8/1 hr	day8/2 hrs	day21/1 hr	day21/2 hrs
400 mg t.i.d.								
n	4	4	4	4	4	3	3	3
mean (*SD*)	13.7 (*10.13*)	13.1 (*7.27*)	12.3 (*14.64*)	13.2 (*7.79*)	9.1 (*7.63*)	9.3 (*5.0*)	2.5 (*0.96*)	14.9 (*16.49*)
median (*min,max*)	13.9 (*4, 23*)	14.4 (*4,20*)	6.8 (*2, 34*)	15.3 (*2, 20*)	7.3 (*3, 19*)	11.3 (*4, 13*)	2.2 (*2, 4*)	7.9 (*3, 34*)
800 mg t.i.d.								
n	3	3	3	3	3	3	3	3
mean (*SD*)	22.4 (*19.14*)	37.2 (*26.35*)	78.9 (*38.86*)	25.0 (*11.57*)	13.0 (*13.27*)	21.6 (*8.37*)	44.9 (*61.18*)	34.1 (*21.54*)
median (*min,max*)	12.6 (*10, 44*)	41.8 (*9, 61*)	90.4 (*36, 111*)	23.0 (*15, 38*)	9.3 (*2, 28*)	22.6 (*13, 29*)	17.9 (*2, 115*)	41.7 (*10, 51*)
1200 mg t.i.d.								
n	12	12	11	11	10	10	9	9
mean (*SD*)	109.1 (*131.15*)	74.5 (*63.82*)	76.2 (*107.84*)	83.9 (*97.73*)	84.4 (*147.04*)	30.4 (*29.46*)	117.2 (*84.65*)	50.8 (*42.04*)
median (*min,max*)	54.5 (*6, 448*)	44.9 (*21, 223*)	37.1 (*1, 366*)	42.8 (*13, 346*)	20.8 (*2, 455*)	16.4 (*4, 94*)	107.6 (*10, 262*)	31.4 (*11, 146*)

Figure 7: Paclitaxel versus Time on Day 1 and Day 8 on a Linear (Upper) or Semi-Log (Lower) Axis



Antitumor activity of the combination as measured by objective response rate was observed at the time of data cut off for main analyses (when 7 patients were still actively receiving treatment) in 1 patient (complete remission) at the 400 mg t.i.d. dose level and in 6 patients (1 complete remission and 5 partial remissions) at the 1200 mg dose level. Response duration (days) at the time of data cut off for main analyses was 645+, 285+ for CR, and 280+, 169, 113+, 85+, 47 for PR. No pre- and post-treatment biopsies have been obtained from patients enrolled in this trial so that changes in tumor microenvironment including effects on CSC markers could not be studied. Circulating tumor cells decreased from baseline or remained the same in 17/20 patients for whom at least two samples were available.

In summary, the results of this study show that oral reparixin administered with paclitaxel was safe and well tolerated in patients with HER-2 negative MBC. Several objective responses were recorded; however, due to the small numbers of patients within each treatment group and considering that single agent weekly paclitaxel has established activity against MBC, the response rate should be interpreted with caution. Nonetheless, given the clear safety data and good tolerability of the combination, coupled with a sizeable response rate and some interesting long term responders, further investigation of the combination is warranted. Considerations for future studies should include the use of the highest reparixin dose (1200 mg) in combination with paclitaxel with the aim of increasing the duration of responses and progression-free survival.

6.2.4.2. A single arm, preoperative, pilot study to evaluate the safety and biological effects of orally administered reparixin in early breast cancer patients who are candidates for surgery [REP0210].

A pilot "window of opportunity" clinical study in patients with operable breast cancer is also ongoing with reparixin oral tablets as single agent in the time period between clinical diagnosis and surgery. This study aims to evaluate the effects of orally administered reparixin on CSCs in the primary tumor and the tumoral microenvironment in an early breast cancer population.

CSCs (defined as ALDEFLUOR positive - ALDH-1+ and/or CD44 high/CD24 low) will be measured in tissue samples by flow cytometry, examination of RNA transcripts by RT-PCR, and/or immunohistochemistry (IHC). In addition, epithelial-mesenchymal transition markers (Snail, Twist, Notch) and serine-threonine protein kinase (AKT), focal adhesion kinase (FAK) and CXCR1 levels will be measured in tissue samples by IHC.

Markers of inflammation will be measured in plasma and markers of angiogenesis (CD31 staining), tumor infiltrating leukocytes, autophagy (P62 and LC3 by IHC) will be measured in tumor tissue samples.

The aim of the study is to investigate if CSCs and pathway markers decrease in two early breast cancer subgroups (ER^+ and/or progesterone receptor positive/HER-2 and ER negative/progesterone receptor negative/HER-2) and to compare any differences between the two subgroups to try to better identify a target population. 20 patients will be enrolled to each subgroup at up to 10 sites in the US.

The first clinical site was activated in January 2013. To date, a total of 9 centers are activated, and 20 patients have been enrolled to study, 18 with hormone receptor positive breast cancer, and 2 with TNBC.

To date only one SAE has been reported in this study and is considered to be unrelated by the treating investigator.

6.2.5. Clinical studies not sponsored by Dompé

The two studies reported below are academic and independent clinical trials. Upon Ethics Committee approval, Dompé provided only the study drug under a Material Transfer Agreement.

6.2.5.1. Effects of the specific interleukin-8 inhibitor reparixin on endotoxin induced inflammation. *[Department of Clinical Pharmacology, Division of Immunohaematology and Department of Anesthesia and Intensive Care Medicine; Medical University of Vienna] – [EK 116/2004] – Personal communication* [7]

Reparixin antagonizes IL-8 on the level of signal transduction *in vitro*. It was hypothesized that IL-8 mediates some of the reactions occurring during acute inflammation and specifically that IL-8 may be a mediator of neutrophilia, therefore the effects of reparixin on humoral and cellular parameters in LPS-induced acute systemic inflammation have been tested.

The study was a randomized, double-blind, placebo-controlled parallel group trial. Twenty healthy volunteers were randomized to receive either reparixin (12) or placebo (8) by i.v. One hour after the start of reparixin L-lysine salt (4.2 mg/kg/h for 8.5 h)/placebo infusion a bolus of 2 ng/kg endotoxin was infused over 1-2min. Blood samples were obtained over 24h.

LPS-induced neutrophilia was not significantly affected by reparixin in human volunteers. Consistently, reparixin did not alter the lymphocyte or monocyte counts and had no effect on LPS-induced systemic inflammation as measured byTNF-α or IL-6 release. Regulation of IL-8 receptors CXCR1 and 2 and the degranulation marker CD11b showed the expected kinetics. Reparixin had no effect on thrombin formation as measured by prothrombin fragment (F_{1+2}).

Finally, reparixin, i.e. its metabolite ibuprofen effectively suppressed the cyclooxygenase pathway as assessed by serum thromboxane levels. In conclusion, the study showed that reparixin was safe but had no impact on endotoxin induced inflammation.

6.2.5.2. Pilot study on the effect of reparixin during cardiopulmonary bypass *[Department of Clinical Pharmacology, Division of Immunohaematology and Department of Anesthesia and Intensive Care Medicine; Medical University of Vienna]* – **[EK 231/2004]** [8,9]

This academic study was designed to investigate if reparixin attenuates/decreases CPB-induced neutrophilia and/or neutrophil migration into the alveolar space and if there is a trend towards decreased release of standard markers of myocardial injury under CXCL8 antagonism by reparixin. The study was a randomized (1:1 active:placebo), placebo-controlled, pilot trial.

Thirty-two patients (18-90 years) undergoing elective coronary artery bypass grafting with CPB were randomized to receive a bolus infusion of reparixin (4.5 mg kg-1 h-1 for 30 min), followed by a continuous infusion (2.8 mg kg-1 h-1 for 8 h).

Reparixin was well tolerated and no serious AEs were reported. No significant differences were reported in regard to patient's demographics, operating room time, duration of CPB, cross clamp time, time to extubation, the amount of transfused blood products, core temperature, hemoglobin and lactate levels, administered catecholamines as well as in additional parameters (**Errore. L'origine riferimento non è stata rovata.**).

Table 50: Laboratory tests

Study EK 231/2004	**Placebo group**		**Reparixin group**	
Parameters	**Preoperative**	**Postoperative**	**Preoperative**	**Postoperative**
Hematocrit [%]	**37** ± 4	**32** ± 3	**36** ± 3	**33** ± 3
Platelets [10^3/µL]	**192** ± 35	**129** ± 32	**209** ± 39	**153** ± 40
Fibrinogen [mg/dL]	**380** ± 96	**265** ± 104	**412** ± 106	**275** ± 68
aPTT [s]	**35** ± 4	**40** ± 0	**35** ± 5	**37** ± 4
Normotest [%]	**102** ± 26	**65** ± 13	**102** ± 28	**65** ± 15
CEPI-CT [s]	**139** ± 50	**197** ± 80	**150** ± 59	**204** ± 419
CADP-CT [s]	**131** ± 54	**150** ± 52	**102** ± 28	**167** ± 52
Creatinine [mg/dL]	**1.0** ± 0.2	**1.0** ± 0.2	**1.0** ± 0.2	**1.00** ± 0.2
C-reactive protein [mg/dL]	**0.3** ± 0.5	**17.7** ± 4.6	**0.8** ± 1.1	**16.0** ± 4.5
Urine output [mL]	**0**	**720** ± 380	**0**	**770** ± 450
Fluid balance [mL]	**0**	**3240** ± 610	**0**	**2660** ± 1270

aPTT = activated partial thromboplastin time, CEPI-CT = collagen-epinephrine closure time, CADP-CT = collagen-ADP closure time [Platelet Function Analyzer (PFA-100)]

Neutrophil count declined in both groups with the beginning of CPB with higher levels being determined in the placebo group ($p > 0.05$). After CPB, neutrophil count exceeded baseline levels in the two groups with a greater increase in the placebo group as compared to the reparixin group ($p < 0.05$). Significant group differences were also detected at 4 hours post-CPB.

The rise of the neutrophil count after CPB was less marked in the reparixin group. This may indicate that inflammatory induced ischemia-reperfusion injury is less severe in patients after surgery on CPB when IL-8 is inhibited.

6.2.6. Cumulative Adverse Drug Reactions – I.V. formulation

The following provides a comprehensive evaluation of the safety profile of reparixin as derived from Phase 1 and Phase 2 clinical studies using the I.V. formulation of reparixin.

Cumulative Adverse Drug Reactions (ADRs), i.e. treatment-emergent adverse events judged at least possibly related to reparixin, are presented in Table 51.

A total of 374 subjects have been treated in phase 1 and phase 2 clinical completed studies. Among these, 236 have been exposed to reparixin.

Phase 1 studies I.V. formulation

A total of 136 subjects, of whom 103 adult healthy volunteers (including 3 females) and 17 patients with different grades of renal impairment (including 5 females) and 16 patients undergoing cardiopulmonary bypass (including 6 females), have been treated with reparixin.

Phase 2 studies I.V. formulation

A total of 100 patients, 46 undergoing lung transplantation (23 M and 23 F), 48 patients undergoing kidney transplant (including 17 females) and 6 patients undergoing intrahepatic islet transplantation (3 M and 3 F) have been treated with reparixin.

The figures below include 14 healthy volunteers concomitantly treated with reparixin and the two probe drugs (midazolam/tolbutamide), therefore the ADRs evidenced in this group were included in this evaluation nevertheless they were reasonably related to the pharmacological properties of the two probe drugs.

Exposure included short or prolonged i.v. infusion being 10.6 mg/kg over 30 min, 133.9 mg/kg over 48 h and 465.7 mg/kg over 7 days respectively the maximum administered doses of reparixin.

Reparixin was generally well tolerated at all doses studied. Overall, 115 ADRs were reported in a total of 70 subjects out of 236 exposed to reparixin (phase 1 studies: 71 ADRs in 45 subjects out of 136 treated; in Phase 2 studies: 44 ADRs in 25 subjects out of 100 treated).

The most frequent (>10%) ADRs observed in the phase 1 and phase 2 studies were:

Nervous system disorders (about 22%), including headache, dizziness, hypoaesthesia, somnolence.

Gastrointestinal disorders (about 22%), including nausea, vomiting, abdominal pain, dyspepsia, flatulence, gastroesophageal reflux disease.

General disorders and administration site conditions (about 19%), including cannula site reaction, injection site thrombosis, infusion site oedema and peripheral oedema, fatigue, lethargy.

The relatively high frequency of the administration site reaction is due to a cluster of events occurring in cohort 1 of the 48 h-infusion study. The use of a less concentrated solution with increased administration volume, i.e. increased infusion rate, in cohort 2 and 3 reduced the incidence of infusion site reactions.

Table 51: Summary of Adverse Drug Reactions (ADRs) IV formulation

Number of Reports by Terms			Frequency
MedDRA Body System / LLT ADR term	**Non Serious**	**Serious**	
Blood and lymphatic system disorders	**3**	**2**	**4.30%**
Anaemia	2	**1**	
Coagulopathy		1	
Lymphadenopathy	1		
Cardiac Disorders	**1**	**0**	**0.9%**
Tachycardia	1		
Gastrointestinal Disorders	**44**	**3**	**21.7.%**
Abdominal pain NOS	1		
Dyarrhea	1		
Dyspepsia	1		
Flatulence	2		
Gastroesophageal reflux disease	1		
Gastrointestinal haemorrhage		1	
Nausea	11	1	
Vomiting	5	1	
General disorders and administration site conditions	**22**	**0**	**19.1%**
Cannula site reaction	13		
Fatigue	1		
Injection site thrombosis	3		
Infusion site oedema	2		
Lethargy	1		
Oedema	1		
Oedema peripheral	1		
Immune system disorders	**0**	**1**	**0.9%**
Lung transplant rejection		1	
Injury, poisoning and procedural complications	**1**	**1**	**1.7%**
Complications of transplanted kidney	1		
Drug Administration Error		1	
Investigations	**2**	**0**	**1.7%**
Blood amylase increased	1		
Liver function test abnormal	1		
Metabolic and nutrition disorders	**1**	**0**	**0.9%**
Hyperkalaemia	1		
Musculoskeletal and connective tissue disorders	**1**	**0**	**0.9%**
Arthralgia	1		
Nervous system disorders	**25**	**0**	**21.7%**
Dizziness	3		
Headache	8		
Hypoaesthesia	3		
Somnolence	11		
Psychiatric disorders	**4**	**0**	**3.5%**
Abnormal dream	1		
Restlessness	1		
Euphoric mood	2		
Renal and urinary disorders	**4**	**0**	**3.50%**
Renal failure	1		
Renal tubular necrosis	2		
Urinary retention	1		
Respiratory, thoracic and mediastinal disorders	**6**	**1**	**6.1%**
Bradypnoea	1		
Cough	2		
Nasopharyngitis	2		
Respiratory failure		1	
Sore throat	1		
Skin and subcutaneous system disorders	**9**	**0**	**7.8%**
Erythema	6		
Infusion site erythema	2		
Pruritis	1		
Vascular disorders	**3**	**3**	**5.2%**
Haemorrhage		1	
Hypotension	3		
Retroperitoneal heamorrhage		2	
TOTAL	**104**	**11**	

Table does not include ADRs reported from on-going studies

Table 52 allows an evaluation of the incidence of ADRs versus the extent of exposure.

When considering the 30min exposure, it seems that the percentage of subjects experiencing ADRs slightly increases with the administered dose. However, this finding was not confirmed by data obtained in the 48h infusion study. In this study, the percentage of subjects experiencing ADRs remains comparable in all dose groups even when ADRs other than infusion site reactions are considered (about 20%).

In the 48h infusion study, the majority of ADRs occurred after at least the first 24h of treatment suggesting that they were mainly related to the SS condition (maintenance dose). This allows also a comparison of the safety profile at different duration of exposure within the same dose. As shown in the table, in the dose range of 1-4.2 mg/kg the percentage of subjects experiencing ADRs does not increase with the length of exposure.

The safety of reparixin seems to be confirmed also in patients with different grades of renal impairment. Out of 17 such patients only 3 experienced mild ADRs.

There were no increases of the ADRs in the lung and kidney transplant studies. A similar incidence of ADRs was also seen after intermittent or continuous infusion during the kidney transplant study.

Data obtained in the pilot trial in islet transplantation further support the safety profile of the proposed dose, even after a 7 days administration, repeated twice in a few patients. Most frequent ADRs were erythema, hypotension, nausea, vomiting; great majority of these were mild to moderate in nature and none required discontinuation of the Investigational Product. Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion because the patient received a dose of reparixin 3 times as high as that foreseen in the protocol (medical error). These events were assessed as serious by the investigator and by the Sponsor.

Table 52: Incidence of Adverse Drug Reactions (ADRs) versus the extent of exposure i.v. formulation

Dose reparixin L -lysine salt	**Time of infusion**											
	30 min		**6 h**		**8.0 h**	**8.5 h**	**24 h**	**48 h**	**48 h**	**30 min (n=12)**	**12 h**	**7 days #**
	healthy vol.s	**renal failure**	**normal vol.s**	**renal failure**	**healthy vol.s**	**healthy vol.s**	**healthy vol.s**	**healthy vol.s**	**Lung transplant**	**Kidney transplant**		**Islet transplant**
1 (mg/kg)	**1** (1/4) [*25%*]	-	-	-		**-**		-				
1 (mg/kg/h)	-	-	-	-		**-**		**22** (9/9) [*100%*]				
2 (mg/kg)	**1** (1/4) [*25%*]	-						-				
2 (mg/kg/h)	**-**	**-**	**5** (4/6) [*66%*]	**3** (3/13) [*23%*]		**-**		**7** (4/12) [*33%*]				
3.4 (mg/kg)	**-**	**0** (0/4) [0%]	-	-		**-**		**-**		**7**(5/23) [*22%*]		
4 (mg/kg)	**2** (2/4) [*50%*]	-	-	-		**-**		-				
4.2 (mg/kg/h)	**-**	**-**			**13**(12/14)* [*86%*]	**0** (0/12) [0%]*	**0** (0/8) [0%]	**7** (3/9) [*33%*]	**11**(10/46) [*22%*]		**4**(4/25) [*16%*]	**22** (6/6) [*100%*]
8 (mg/kg)	**4** (2/4) [*50%*]	-	-	-		-		-				
16 (mg/kg)	**4** (2/4) [*50%*]	-	-	-		-		-				

x (y/n) [*z%*] = **number of ADR** (number of subjects with ADR/exposed subjects) [*percent of subjects with ADR*]; *independent academic study (personal communication)
* interaction study: healthy volunteers concomitantly treated with reparixin and the two probe drugs (midazolam/tolbutamide), the ADRs evidenced in this study were included in this evaluation netherless they were reasonably related to the pharmacological properties of the two probe drugs.
#: In 4 patients, reparixin was administered twice. Figure includes a patients who received reparixin at a dose 3 time higher than that foreseen (medical error).
Table does not include ADRs reported from on-going studies

6.2.7. Cumulative Adverse Drug Reactions – oral formulation (oncology studies)

The following provides an evaluation of the safety profile as derived from the first data analysis of the Phase 1b oncology study using the oral formulation of reparixin.

Cumulative Adverse Drug Reactions (ADRs), i.e. treatment-emergent adverse events judged at least possibly related to reparixin, are presented in Table 53.

REP0111 Phase 1b study of reparixin in combination with weekly paclitaxel in patients with HER-2 negative metastatic breast cancer (MBC).

A total of 33 subjects were enrolled in the phase 1b clinical study. Of these, 30 female patients with metastatic breast cancer received reparixin in combination with paclitaxel.

Reparixin was generally well tolerated at all doses studied. Overall, 207 ADRs were reported in the 30 patients in the safety population.

82% of the ADRs were grade 1 (mild), 16% were grade 2 (moderate) and 2% were grade 3 (severe).

The most frequent (>10%) ADRs observed in the study were:

Gastrointestinal disorders (39.6%), including nausea, vomiting, abdominal pain or distension, dyspepsia, flatulence, constipation.

General disorders and administration site conditions (25.1%), including fatigue and peripheral oedema.

Table 53: Summary of Adverse Drug Reactions (ADRs) Oral formulation

Number of Reports by Terms						
N=207 events*	Non-serious ADRs				Serious ADRs	Total
MedDRA Body System/ADR Preferred Term	CTCAE Grade 1 n (%)	CTCAE Grade 2 n (%)	CTCAE Grade 3 n (%)	CTCAE Grade 4 n (%)	All CTCAE grades N (%)	
Blood and Lymphatic System Disorders	**2 (0.09%)**	**3 (1.4%)**	**3 (1.4%)**	**0 (0%)**	**0 (0%)**	**8 (3.8%)**
Anaemia	1	2	1	0	0	4
Neutrophil count decreased	0	1	2	0	0	3
Thrombocytopenia	1	0	0	0	0	1
Cardiac Disorders	**1 (0.5%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**1 (0.5%)**
Palpitations	1	0	0	0	0	1
Eye Disorders	**0 (0%)**	**0 (0%)**	**1 (0.5%)**	**0 (0%)**	**0 (0%)**	**1 (0.5%)**
Ulcerative keratitis	0	0	1	0	0	1
General Disorders and Administration Site Conditions	**39 (18.8%)**	**13 (6.3%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**52 (25.1%)**
Chest discomfort	2	0	0	0	0	2
Fatigue	20	11	0	0	0	31
Asthenia	0	1	0	0	0	1
Feeling abnormal	1	0	0	0	0	1
Pain	1	0	0	0	0	1
Peripheral Oedema	11	1	0	0	0	12
Face oedema	3	0	0	0	0	3
Pyrexia	1	0	0	0	0	1
Gastrointestinal Disorders	**73 (35.3%)**	**9 (4.3%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**82 (39.6%)**
Abdominal distension	5	1	0	0	0	6
Abdominal discomfort	4	0	0	0	0	4
Abdominal pain	3	0	0	0	0	3
Constipation	5	3	0	0	0	8
Diarrhoea	2	0	0	0	0	2
Dry mouth	1	0	0	0	0	1
Dyspepsia	7	1	0	0	0	8
Dysphagia	1	0	0	0	0	1
Epigastric discomfort	1	0	0	0	0	1
Eructation	3	0	0	0	0	3
Flatulence	7	0	0	0	0	7
Gastroesopageal reflux disease	2	0	0	0	0	2
Gastrointestinal disorder	0	1	0	0	0	1
Headache	2	0	0	0	0	2
Lip pain	1	0	0	0	0	1
Nausea	16	2	0	0	0	18
Proctalgia	1	0	0	0	0	1
Stomatitis	1	1	0	0	0	2
Upper abdominal pain	1	0	0	0	0	1
Vomiting	10	0	0	0	0	10
Infections and Infestations	**0 (0%)**	**4 (1.9%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**4 (1.9%)**
Cellulitis	0	1	0	0	0	1
Herpes dermatitis	0	1	0	0	0	1
Upper Respiratory tract infection	0	1	0	0	0	1
Urinary tract infection	0	1	0	0	0	1
Injury, Poisoning, and Procedural Complications	**0 (0%)**	**1 (0.5%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**1 (0.5%)**
Infusion related reaction	0	1	0	0	0	1
Investigations	**10 (4.8%)**	**1 (0.5%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**11 (5.3%)**
Alanine aminotransferase increased	2	1	0	0	0	3
Aspartate aminotransferase increased	2	0	0	0	0	2
Blood calcium decreased	1	0	0	0	0	1
Weight decreased	3	0	0	0	0	3
White blood cell count decreased	2	0	0	0	0	2
Metabolism	**7 (3.4%)**	**3 (1.4%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**10 (4.8%)**
Hypercalcaemia	1	0	0	0	0	1
Decreased appetite	6	3	0	0	0	9

Musculoskeletal	**3 (1.4%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**3 (1.4%)**
Arthralgia	1	0	0	0	0	1
Joint swelling	1	0	0	0	0	1
Pain	1	0	0	0	0	1
Nervous System Disorders	**12 (5.8%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**12 (5.8%)**
Dizziness	2	0	0	0	0	2
Dysgeusia	2	0	0	0	0	2
Headache	4	0	0	0	0	4
Peripheral motor neuropathy	1	0	0	0	0	1
Peripheral neuropathy	2	0	0	0	0	2
Sinus headache	1	0	0	0	0	1
Reproductive System and Breast Disorders	**1 (0.5%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**1 (0.5%)**
Pelvic pain	1	0	0	0	0	1
Respiratory, Thoracic and Mediastinal disorders	**13 (6.3%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**13 (6.3%)**
Cough	4	0	0	0	0	4
Dysphonia	1	0	0	0	0	1
Dyspnoea	3	0	0	0	0	3
Epistaxis	2	0	0	0	0	2
Nasal congestion	1	0	0	0	0	1
Nasal dryness	1	0	0	0	0	1
Pneumonitis	1	0	0	0	0	1
Skin and Subcutaneous Tissue Disorders	**7 (3.4%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**7 (3.4%)**
Alopecia	2	0	0	0	0	2
Erythema	1	0	0	0	0	1
Palmar erythema	1	0	0	0	0	1
Rash	2	0	0		0	2
Skin hyperpigmentation	1	0	0	0	0	1
Surgical and medical	**1 (0.5%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**1 (0.5%)**
Sinus operation	1	0	0	0	0	1
Total	**169 (82%)**	**34 (16%)**	**4 (2%)**	**0 (0%)**	**0 (0%)**	**207**

*ADRs continuing across more than one treatment cycle without complete resolution and reported more than once in CRF to reflect changes in CTCAE grade, will be reported in this table as single ADRs with worst CTCAE grade. ADRs occurring more than once per patient but not continuing (i.e. with clear break between one occurrence and the next) will be reported in this table as separate ADRs.

Table 54 allows an evaluation of the incidence of ADRs of any grade (all non serious) versus the extent of exposure to the combination treatment of reparixin oral tablets and weekly paclitaxel in REP0111 clinical trial at the time of data cut-off for first analysis. Taking into consideration the patient population, i.e. metastatic breast cancer patient with up to three prior lines of chemotherapy for advanced disease [not including (neo)adjuvant chemotherapy] and unrestricted prior lines of hormonal therapy, the incidence of at least one ADR of any grade in the vast majority of patients is expected. Despite the limited number of patients as in most phase 1b studies, there does not appear to be a relationship between extent of exposure (measured by the number of treatment cycles completed) and incidence of adverse reactions, with patients taking the combination treatment for 8 or more 28-day cycles at the highest reparixin dose level experiencing very few or no ADRs. This observation suggests the absence of cumulative effects on the incidence of ADRs. Whether or not this observation in patients receiving treatment for 8 or more cycles also reflects, at least in part, fewer prior lines of therapy and/or lower tumor burden at baseline and/or sensitivity of the disease to the combination treatment remains to be determined on larger patient numbers. Also when considering the average number of ADRs per patient at the three dose levels, the highest dose is not associated with an increased incidence of ADRs.

Table 54: Incidence of Adverse Drug Reactions (ADRs) versus the extent of exposure to orally administered reparixin in combination with weekly paclitaxel

	Number of Treatment Cycles													
DF1681 Y dose	1*	2	3	4	5	6	7	8	9	10	11	12	13	24
400 mg t.i.d.	**0** (0/1) [*0%*]	**6** (2/2) [*100%*] 1 - 5	-	-	-	-	-	-	-	-	-	-	-	**16** (1/1) [*100%*]
800 mg t.i.d		**17** (2/2) [*100%*] 3 - 14	**17** (1/1) [*100%*]	-	-	-	-	-	-	-	-	-	-	-
1200 mg t.i.d.	**0** (0/3) [*0%*]	**48** (8/9) [*88.8%*] 0 - 12	**1** (1/1) [*100%*]	**44** (3/3) [*100%*] 9 - 22	**27** (2/2) [*100%*] 11 - 16	**5** (1/1) [*100%*]	-	**0** (0/1) [*0%*]	-	**22** (1/1) [*100%*]	-	-	**3** (1/2) [*50%*] 0 - 3	-

x (y/n) [*z%*] = **number** of ADR (number of patients with ADR/exposed patients) [*percent of patients with ADR*]; w - k = minimum and maximum number of ADRs per patient
Based upon safety population (400 mg t.i.d. n = 4; 800 mg t.i.d. n = 3; 1200 mg t.i.d. n = 23) and the number of 28-day cycles of treatment completed at the time of data cut-off for first analysis.
***** 4 patients did not complete the first cycle of treatment
Table does not include ADRs reported from ongoing studies with orally administered reparixin

6.3. MARKETING EXPERIENCE

Reparixin has not been marketed or approved in any country. Reparixin has not been denied approval/registration for marketing or been withdrawn for marketing/registration in any country.

6.4. REFERENCE SAFETY INFORMATION

ADRs reported for reparixin in completed clinical trials are detailed in sections 6.2.6 and 6.2.7.

Considering the early development phase of the conpound and the low number of total subjects exposed to reparixin, it may be judged misleading to consider expected an adverse reaction or event. Also taking into account that current clinical development addresses very different clinical indications/conditions (breast cancer and islet transplantation) where each adverse event has to be carefully evaluated, all adverse events/reactions (notwithstanding seriousness) should be considered unexpected at this time.

6.5. REFERENCES (SECTION 6)

1. Ascending dose tolerability and preliminary pharmacokinetic assessment of DF 1681B in healthy male volunteers after intravenous administration [REP0101 (ME0706); A0303]
2. The tolerability, pharmacokinetics and pharmacodynamics of a continuous infusion of reparixin L-lysine salt in healthy male volunteers [REP0102 (ME0735)]
3. Pharmacokinetic analyses of methanesulfonamide in plasma and urine of healthy volunteers treated with reparixin L-lysine salt for 48 h by continuous intravenous infusion and in plasma of end stage renal disease patients treated with reparixin L-lysine salt for 30 minutes by intravenous infusion. [M0409, ME0823/001, ME0823/002]
4. Study to assess the potential pharmacokinetic interaction between reparixin L-lysine salt and probe substrates for CYP3A4 (midazolam) and CYP2C9 (tolbutamide) in adult healthy male volunteers. [REP0103 (ME0757)]
5. The pharmacokinetics and tolerability assessment of I.V. infusion of reparixin L-lysine salt in adult patients with chronic impaired renal function. [REP0203-CM8003-ME0761]
6. Bertini R, Allegretti M, Bizzarri C., Moriconi A, Locati M, et al. A new class of non-competitive allosteric inhibitors of the inflammatory chemokine receptors CXCR1 and CXCR2: prevention of reperfusion injury. PNAS 2004; 101: 11791-6.
7. Leitner JM, Mayr FB, Firbas C, Spiel AO, Steinlechner B, Novellini R, Jilma B. Reparixin, a specific interleukin-8 inhibitor, has no effects on inflammation during endotoxemia.Int J Immunopathol PharmacolI. 2007 Jan-Mar;20(1):25-36.
8. Safety profile of reparixin- - A novel IL-8 Inhibitor in patients undergoing on-pump coronary artery bypass graft surgery" Abstract - 10th International Congress of Cardiothoracic and Vascular Anesthesia. August 27-30, 2006, Hilton Hotel Prague Czech republic.
9. Effect of reparixin on plasma neutrophil count in patients undergoing on pump coronary artery bypass surgery. B. Steinlechner, E. Base, B. Birkenberg, B. Mora, B. Jilma2, M. Dworschak. Abstract - 81st Clinical and Scientific Congress of the International Anesthesia Research Society- March 23–27, 2007, Orlando, Florida

7. SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

The following information is based upon animal studies of reparixin and the human Phase 1 and Phase 2 studies conducted so far.

7.1. DOSAGE AND ADMINISTRATION

Reparixin should be administered by i.v. and oral route to subjects strictly according to the clinical protocol.

7.2. CONTRAINDICATIONS

Reparixin is contraindicated in subjects with known hypersensitivity to ibuprofen, as pharmacokinetic and metabolism studies have shown that one of possible metabolites of reparixin is ibuprofen, a non-steroidal antiinflammatory drug. Besides, due to the limited number of patients exposed so far to reparixin, the compound should not be administered to patients with known hypersensitivity to more than one NSAIDs.

Furthermore, since methanesulfonamide is another major metabolite of reparixin, the compound should not be administered to patients with known hypersensitivity reactions to

more than one medication belonging to the class of sulfonamides, such as sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib; hypersensitivity to sulphanilamide antibiotics alone (e.g. sulfamethoxazole) does not qualify for exclusion from clinical trials.

7.3. PRECAUTIONS

The continuous i.v. infusion of reparixin into a peripheral vein at the concentration of 3.96 mg/mL or more should be avoided.

7.4. INTERACTIONS

From an *in vitro* study, reparixin was found to slightly inhibit human CYP3A4, CYP2C9 and, in minor extent, CY2C19 isoenzymes. However, the results of a clinical study, investigating the interaction of reparixin with probe drugs for CYP2C9 and CYP3A4 showed that the concomitant administration of reparixin does not have a significant effect on pharmacokinetics of drugs metabolized by the above isoenzymes.

7.5. PREGNANCY AND NURSING MOTHERS

Since available data on potential effects of reparixin on fertility in animals are still limited, no pregnant and nursing mothers should be included in the clinical trials. Moreover, all the females of childbearing potential should be recommended to use reliable forms of contraception.

7.6. SPECIAL MONITORING

Because reparixin is extensively metabolized and its metabolites are eliminated by the kidneys, hepatic and renal function should be assessed before and monitored during treatment.

7.7. ADVERSE REACTIONS

Reparixin i.v. infusion was generally well tolerated at all doses studied as derived from Phase 1 and Phase 2 clinical studies.

Overall, 115 ADRs were reported in a total of 70 subjects out of 236 exposed to reparixin (phase 1 studies: 71 ADRs in 45 subjects out of 136 treated; in Phase 2 studies: 44 ADRs in 25 subjects out of 100 treated).

The most frequent (>10%) ADRs observed in the phase 1 and phase 2 studies were:

Nervous system disorders (about 22%), including headache, dizziness, hypoaesthesia, somnolence.

Gastrointestinal disorders (about 22%), including nausea, vomiting, abdominal pain, dyspepsia, flatulence, gastroesophageal reflux disease.

General disorders and administration site conditions (about 19%), including cannula site reaction, injection site thrombosis, infusion site oedema and peripheral oedema, fatigue, lethargy.

In the pilot trial in islet transplantation most frequent ADRs were erythema, hypotension, nausea, vomiting; great majority of these were mild to moderate in nature and none required discontinuation of the Investigational Product. Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion because the patient received a dose of reparixin 3 times as high as that foreseen in the protocol (medical error).

The relatively high frequency of the administration site reaction is due to a cluster of events occurring in cohort 1 of the 48h-infusion study. The use of a less concentrated solution with increased administration volume, i.e. increased infusion rate, in cohorts 2 and 3 reduced the incidence, type and severity of infusion site reactions.

Reparixin oral tablets has been studied in a phase 1b clinical trial in combination with weekly paclitaxel in 30 women with metastatic breast cancer. The most frequent ADRs to the combination treatment were:

Gastrointestinal disorders (39.6%) including nausea, vomiting, abdominal pain or distension, dyspepsia, flatulence, constipation,

General disorders and administration site conditions (25.1%), including fatigue and peripheral oedema.

82% of the ADRs were of grade 1 (mild) and 16% were of grade 2 (moderate), with only 2% of ADRs being of grade 3 (severe).

7.8. OVERDOSAGE

One case of overdosage was reported in a female patient receiving, for medication error, a dose of reparixin 3 times as high as that foreseen in the protocol (REP0110). Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed early after the beginning of reparixin infusion; the treatment was immediately discontinued and the patient recovered.

8. APPENDICES

8.1. PHYSICAL, CHEMICAL, PHARMACEUTICAL PROPERTIES AND FORMULATION OF REPARIXIN L-LYSINE SALT

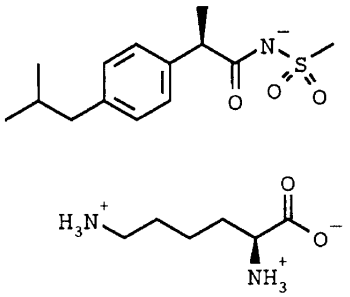
NOTE:
The study drug used in Phase 2 clinical studies is reparixin whose chemical properties and formulation are described in the § 4.
The study drug used in nonclinical and Phase 1 clinical studies described throughout this brochure was reparixin L-lysine salt whose chemical properties and Phase 1 formulations are described below.
The reparixin formulation has the same final product composition of the previous formulations; in fact, it contains an equimolar amount of L-lysine as solubilizer ingredient and the same buffering system used in the previous formulations.

Conversion factor for reparixin L-lysine salt to reparixin equivalent:
dose of reparixin L-lysine salt x 0.66 = equivalent dose of reparixin

8.1.1. Nomenclature

Recommended International Nonproprietary Name (INN):	Reparixin L-lysine salt
Former Generic Name (until December 2004):	Repertaxin L-lysine salt
Chemical Name:	[R(-)-4-Isobutyl-alpha-methylphenylacetyl-methanesulfonamide L-lysine salt]
Dompé Laboratory Code:	DF 1681B
Relative Molecular Mass:	429.58 g/mole
Molecular Formula:	$C_{20}H_{35}N_3O_5S$
CAS (Chemical Abstracts Service) Number:	266359-93-7

8.1.2. Molecular Structure



8.1.3. Physical and chemical characteristics

Reparixin L-lysine salt is a white or almost white, hygroscopic powder. The specific optical rotation value is about –23°. It is freely soluble in water, very soluble in ethanol, and practically insoluble in hexane.

8.1.4. Structural similarities to other known compounds

Reparixin is structurally related to the known anti-inflammatory drug ibuprofen. Specifically, reparixin is an acylmethanesulfonamide obtained starting from R(-) enantiomer of ibuprofen.

8.1.5. Investigational product

The investigational product "Reparixin L-lysine salt ampoules", used in Phase 1 studies was a sterile, isotonic aqueous solution for i.v. infusion administration. The solution of the drug substance was contained in clear glass Type I ampoules. Each ampoule contained 5 mL of a 6 mg/mL or 50 mg/mL reparixin L-lysine salt solution.
The single dose had to be diluted with saline to a proper concentration.
The composition of a single 5 mL unit of the investigational product used in each study is reported in the following table:

Formulation Components

NAME OF INGREDIENT	PER-UNIT FORMULA		FUNCTION OF INGREDIENT	REFERENCE TO QUALITY STANDARDS
	A	B		
Active substance				
Reparixin L-lysine salt (DF 1681B)	30 mg	250 mg	active ingredient	Internal monograph
Excipients				
Monosodium phosphate dihydrate	95.7 mg	39.2 mg	buffer agent	European Pharmacopoeia 4th edition
Sodium hydroxide	up to pH 8	up to pH 8	buffer agent	European Pharmacopoeia 4th edition
Water for injections	up to 5 mL	up to 5 mL	solvent	European Pharmacopoeia 4th edition
A = Study No.: REP0101; REP0102 (Cohort 1); B = Study No.: REP0102 (Cohort 2 and Cohort 3); REP0103; REP0203				

In phase 2 and 3 studies the Investigational product is reparixin 33 mg/mL concentrate for solution for i.v. infusion; in phase 2 trials reparixin has been provided as clear glass class I ampoules, each containing 10 mL of 33 mg/mL; in phase 3 study reparixin will be provided as vials containing 250 mL.

8.1.6. Storage and Handling

The investigational product was a photostable solution. No specific storage conditions were required. Not to be frozen.

8.1.7. References (section 8)

There are no references for this section.

Creative Regulatory Solutions, LLC

July 20, 2016

Food and Drug Administration
Center for Drug Evaluation & Research
Renata Albrecht, M.D., Director
Division of Transplant and Ophthalmology Products
Central Document Room
5901 B-Ammendale Road
Beltsville, MD 20705-1266

ATT: Judit Milstein, Chief Project Management Staff

Re: Dompé farmaceutici S.p.A
Drug Name – Reparixin
IND – 117390
Serial Submission Number 0019
Indication: Prevention of Graft Loss in Pancreatic Islet Auto-Transplantation
Submission of Revised Protocol REP0112 (Amendment 1/Site Specific)
SUBMISSION CONTAINS ELECTRONIC PYHSICAL MEDIA (CD-ROM)

Dear Dr. Albrecht,

Reference is made to Serial Submission Number 0002 dated October 30, 2013 which contained Protocol REP0112 identified as Version No. 1 dated October 11, 2013 and titled "A Phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation". Protocol REP0112 underwent a Special Protocol Assessment (SPA) and an Agreement Letter dated August 16, 2013 was received from the Division of Transplant and Ophthalmology Products. The purpose of this IND Amendment is to submit a site specific amendment for the REP0112 protocol identified as Amendment Number 1/Version #2 dated July 18, 2016. The amendment allows for up to 48 patients to be enrolled at the site of the primary investigator, Melena Bellin, MD., Schulze Diabetes Institute; University of Minnesota Medical School. This is a change from the previous REP0112 protocol which allowed up to a maximum of 40 patients to be enrolled at a single center. The need to have this protocol change reviewed as a modification to the existing SPA was discussed with Judit Milstein, Chief Project Management Staff, Division of Transplant and Ophthalmology Products. An email from Judit Milstein was received dated July 14, 2016 which stated that the SPA did not need to be modified but that a revised protocol would need to be submitted to the IND in order to ensure a complete regulatory file.

Creative Regulatory Solutions, LLC
14 Edinburgh Drive
Randolph, NJ 07869 US

Tel: 862-397-4534
Fax: 862-397-4535

Division of Division of Transplant and Ophthalmology Products
Serial Submission: #0019
REP0112 Protocol Amendment
July 20, 2016

The submission contains a protocol Amendment #1 summary document and a copy of the complete REP0112 revised protocol and has been submitted on the enclosed CD-ROM.

Should you have any questions, please feel free to contact the undersigned by telephone at (862) 397-4534 or by e mail at bmccormack@creativeregulatory.com.

Sincerely,

Robert McCormack, PhD
Regulatory Affairs Consultant
Creative Regulatory Solutions, LLC

Enclosure:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

INVESTIGATIONAL NEW DRUG APPLICATION (IND)

(Title 21, Code of Federal Regulations (CFR) Part 312)

Form Approved: OMB No. 0910-0014
Expiration Date: April 30, 2015
See PRA Statement on page 3.

NOTE: No drug/biologic may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40)

1. Name of Sponsor
Dompe farmaceutici S.p.A

2. Date of Submission *(mm/dd/yyyy)*
07/20/2016

3. Sponsor Address

Address 1 *(Street address, P.O. box, company name c/o)*
Via San Luica 6

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City
Milano

State/Province/Region

Country
Italy 20122

ZIP or Postal Code

4. Telephone Number *(Include country code if applicable and area code)*
011-3902-58383559

5. Name(s) of Drug *(Include all available names: Trade, Generic, Chemical, or Code)*
Reparixin (DF 1681Y)

Continuation Page for #5

6. IND Number *(If previously assigned)*
117390

7. (Proposed) Indication for Use
Prevention of graft loss in auto-pancreatic islet cell transplantation

Is this indication for a rare disease (prevalence <200,000 in U.S.)? ☑ Yes ☐ No

Does this product have an FDA Orphan Designation for this indication? ☑ Yes ☐ No

If yes, provide the Orphan Designation number for this indication: 12-3733

Continuation Page for #7

8. Phase(s) of Clinical Investigation to be conducted ☐ Phase 1 ☐ Phase 2 ☐ Phase 3 ☑ Other *(Specify)*: Phase 2/3

9. List numbers of all Investigational New Drug Applications (21 CFR Part 312), New Drug Applications (21 CFR Part 314) , Drug Master Files (21 CFR Part 314.420) , and Biologics License Applications (21 CFR Part 601) referred to in this application.
IND 67023 - IV reparixin in the Division of Transplant and Ophthalmology Products (CDER) for IV toxicology reports
IND 15194 - Office of Cellular, Tissue, and Gene Therapy (CBER) for drug substance and drug product information

10. IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 0000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 0001." Subsequent submissions should be numbered consecutively in the order in which they are submitted..
Serial Number: 0019

11. This submission contains the following *(Select all that apply)*

☐ Initial Investigational New Drug Application (IND)
☐ Request For Reactivation Or Reinstatement
☐ Development Safety Update Report (DSUR)
☐ Response to Clinical Hold
☐ Annual Report
☐ Other *(Specify)*:
☐ Response To FDA Request For Information
☐ General Correspondence

Protocol Amendment(s)	Information Amendment(s)	Request for	IND Safety Report(s)
☐ New Protocol	☐ Chemistry/Microbiology	☐ Meeting	☐ Initial Written Report
☑ Change in Protocol	☐ Pharmacology/Toxicology	☐ Proprietary Name Review	☐ Follow-up to a Written Report
☐ New Investigator	☐ Clinical ☐ Statistics	☐ Special Protocol Assessment	
☐ PMR/PMC Protocol	☐ Clinical Pharmacology	☐ Formal Dispute Resolution	

12. Select the following only if applicable. *(Justification statement must be submitted with application for any items selected below. Refer to the cited CFR section for further information.)*

Expanded Access Use, 21 CFR 312.300

☐ Emergency Research Exception From Informed Consent Requirements, 21 CFR 312.23 (f)
☐ Charge Request, 21 CFR 312.8
☐ Individual Patient, Non-Emergency 21 CFR 312.310
☐ Individual Patient, Emergency 21 CFR 312.310(d)
☐ Intermediate Size Patient Population, 21 CFR 312.315
☐ Treatment IND or Protocol, 21 CFR 312.320

For FDA Use Only

CBER/DCC Receipt Stamp	DDR Receipt Stamp	Division Assignment
		IND Number Assigned

FORM FDA 1571 (1/13) **Page 1 of 3** PSC Publishing Services (301) 443-6740 EF

Previous Page | Next Page

13. Contents of Application – This application contains the following items *(Select all that apply)*

- [x] 1. Form FDA 1571 *(21 CFR 312.23(a)(1))*
- [ ] 2. Table of Contents *(21 CFR 312.23(a)(2))*
- [ ] 3. Introductory statement *(21 CFR 312.23(a)(3))*
- [ ] 4. General Investigational plan *(21 CFR 312.23(a)(3))*
- [ ] 5. Investigator's brochure *(21 CFR 312.23(a)(5))*
- [x] 6. Protocol(s) *(21 CFR 312.23(a)(6))*
  - [x] a. Study protocol(s) *(21 CFR 312.23(a)(6))*
  - [ ] b. Investigator data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
  - [ ] c. Facilities data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
- 6. Protocol(s) *(Continued)*
  - [ ] d. Institutional Review Board data (21 CFR 312.23(a)(6)(iii)(b)) or completed Form(s) FDA 1572
- [ ] 7. Chemistry, manufacturing, and control data *(21 CFR 312.23(a)(7))*
  - [ ] Environmental assessment or claim for exclusion *(21 CFR 312.23(a)(7)(iv)(e))*
- [ ] 8. Pharmacology and toxicology data (21 CFR 312.23(a)(8))
- [ ] 9. Previous human experience *(21 CFR 312.23(a)(9))*
- [ ] 10. Additional information *(21 CFR 312.23(a)(10))*
- [ ] 11. Biosimilar User Fee Cover Sheet *(Form FDA 3792)*
- [ ] 12. Clinical Trials Certification of Compliance *(Form FDA 3674)*

14. Is any part of the clinical study to be conducted by a contract research organization? [x] Yes [ ] No

If Yes, will any sponsor obligations be transferred to the contract research organization? [x] Yes [ ] No

If Yes, provide a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred *(use continuation page)*. Continuation Page for #14

15. Name and Title of the person responsible for monitoring the conduct and progress of the clinical investigations

Fiona Wingrave, Manager Clinical Research, inVentiv Health Clinical, Burlington, ON L7L 6G4, Canada

16. Name(s) and Title(s) of the person(s) responsible for review and evaluation of information relevant to the safety of the drug

Andrea Vergani, MD, Associate Medical Director, Diabetes and Transplantation, Dompe Inc., New York, NY.

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold or financial hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set forth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

17. Name of Sponsor or Sponsor's Authorized Representative

Robert J. McCormack, PhD.

18. Telephone Number *(Include country code if applicable and area code)*	19. Facsimile (FAX) Number *(Include country code if applicable and area code)*
862-397-4534	862-397-4535

20. Address

Address 1 *(Street address, P.O. box, company name c/o)*: 14 Edinburgh Drive	
Address 2 *(Apartment, suite, unit, building, floor, etc.)*:	
City: Randolph	State/Province/Region: New Jersey
Country: USA	ZIP or Postal Code: 07869

21. Email Address: bmccormack@creativeregulatory.com

22. Date of Sponsor's Signature *(mm/dd/yyyy)*: 07/19/2016

23. Name of Countersigner

24. Address of Countersigner

Address 1 *(Street address, P.O. box, company name c/o)*:	
Address 2 *(Apartment, suite, unit, building, floor, etc.)*:	
City:	State/Province/Region:
Country: United States of America	ZIP or Postal Code:

WARNING : A willfully false statement is a criminal offense (U.S.C. Title 18, Sec. 1001).

25. Signature of Sponsor or Sponsor's Authorized Representative — Sign

26. Signature of Countersigner — Sign

FORM FDA 1571 (1/13) **Page 2 of 3**

AMENDMENT No. 1

To the CLINCIAL STUDY PROTOCOL version No. 1 – Final 11 October 2013

APPLICABLE TO SITE No. 01 ONLY

[Schulze Diabetes Institute; University of Minnesota Medical School]

Study Number: REP0112

IND Number: 117390

Investigational Product: Reparixin

Title: A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation.

Amendment No. 1 Version – Date Version No. 1 – final 18 July 2016

STATEMENT OF CONFIDENTIALITY

Information in this amendment and accompanying documents contains privileged or confidential information that is the property of Dompé farmaceutici s.p.a. It is understood that the information will not be used, divulged, or published without prior written consent of Dompé, except to the extent such disclosure is required by applicable laws and regulations.

NOTES:
This Amendment does not result in a change to either the CRF or the ICF.

PROTOCOL AMENDMENT APPROVAL SIGNATURES

SPONSOR:

Medical Expert

Signature: _____ Date: _____

Pier Adelchi Ruffini, MD – Development Director

CLINICAL CENTRE: PRINCIPAL INVESTIGATOR [PRIMARY INVESTIGATOR]

I have read study protocol REP0112 "A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation".

Name of Principal Investigator: Melena Bellin, MD

Signature: _____ Date: _____

3. OVERALL STUDY DESIGN AND PLAN DESCRIPTION

3.1. STUDY DESIGN

The following paragraph [protocol, page 20] has been changed from:

Recruitment will be competitive among the study sites, until the planned number of patients is enrolled. Competitive recruitment has been chosen to increase the speed of recruitment and to account for any difference in transplant rate among study sites. Each centre will enroll patients as rapidly as possible, up to a maximum of 40 patients (as per the randomization list).

To:

Recruitment will be competitive among the study sites, until the planned number of patients is enrolled. Competitive recruitment has been chosen to increase the speed of recruitment and to account for any difference in transplant rate among study sites. Each centre will enroll patients as rapidly as possible, up to a maximum of 40 patients (as per the randomization list). A maximum of 48 patients is allowed for the site of the Primary Investigator.

Reason: The study design has been changed considering current patient enrollment status. Specifically, since the first patient randomized to the trial back in February 2014, recruitment is extending considerably longer than originally expected. To date, 86 patients have received TP-IAT, out of 89 randomized. New sites in addition to those originally involved have joined the trial with Cincinnati and UCSF having been initiated early in 2016.
Despite this continuous effort, overall enrollment rate across the 9 participating sites is lower than predicted, mainly due to the very rare condition of the TP-IAT procedure, coupled with a non-homogeneous distribution of this procedure across the US states/sites. In particular, enrollment rate has significantly decrease since the site of the Primary Investigator (UMN) has reached the cap of 40 patients (out of 89 randomized to date) allowed at a single site as per protocol. The high enrollment rate of this site actually reflects the high volume of TP-IAT procedures specific for Minnesota.
Therefore, considering the importance of:

- maintaining study continuity;
- achieving a timely completion of the trial in order to avoid “dilution” over time of data obtained and potential bias on data reproducibility;
- distribution of TP-IAT procedures across different US sites;

the study design has been changed to allow enrollment at the site of the Primary Investigator up to a total of 48 patients.

9. STATISTICAL ISSUES

9.2. RANDOMIZATION

The following paragraph [protocol, page 42] has been changed from:

The randomization list will be generated with a computer procedure by the method of random permuted blocks in which treatment (in blocks of 4) will be balanced within centres. A master randomization list will be generated, randomizing an excess of patients (a maximum of 40 for each site) to allow competitive recruitment within each centre.

To:

The randomization list will be generated with a computer procedure by the method of random permuted blocks in which treatment (in blocks of 4) will be balanced within centres. A master randomization list will be generated, randomizing an excess of patients (a maximum of 40 for each site – additional 8 patients for the site of the Primary Investigator) to allow competitive recruitment within each centre.

Reason: To account for the change in study design (see section 3.1.)

CLINICAL STUDY PROTOCOL - CONFIDENTIAL

Study Number: REP0112

IND Number: 117390

Investigational Product: Reparixin

Title: A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation.

Protocol Version – Date Version No. 2 – Final 18 July 2016

APPLICABLE TO SITE No. 01 ONLY

[Schulze Diabetes Institute; University of Minnesota Medical School]

This protocol version results from revision of protocol version No. 1 (Final, 11 October 2013) according to Amendment No. 1 (final 18 July 2016).

STATEMENT OF CONFIDENTIALITY

Information in this protocol and accompanying documents contains privileged or confidential information that is the property of Dompé s.p.a. It is understood that the information will not be used, divulged, or published without prior written consent of Dompé, except to the extent such disclosure is required by applicable laws and regulations.

CONTACT INFORMATION

SPONSOR Dompé s.p.a. [Dompé]
Via Campo di Pile; 67100 L'Aquila, Italy
Milan Offices: Via S. Lucia 6, 20122 Milan, Italy
Phone: +39 02 583831; Fax: +39 02 58383324

Medical Expert Pier Adelchi Ruffini, MD - Development Director
Dompé s.p.a. - Milan Offices;
Direct line: +39 02 58383500; Mobile: +39 346 8745908
e-mail:

Clinical Trial Manager Luisa Daffonchio, PhD - Clinical Development Manager
Dompé s.p.a. - Milan Offices;
Direct line: +39 02 58383246;
e-mail: luisa.daffonchio@dompe.it

Pharmacovigilance Dr. Laura Boga, QPPV
Dompé s.p.a. - Milan Offices
Direct line: +39 02 58383462; Mobile: +39 335 219580
Fax: +39 02 36026913
e-mail:

CLINICAL RESEARCH ORGANIZATION inVentive Health Clinical [inVentive]
504 Carnegie Center; Princeton, NJ 08540, USA
Phone: +1 609 951 6800; Fax: +1 609 514 0390

Project Manager Fiona Wingrave - Manager, Clinical Research
5515 North Service Rd, Suite 200; Burlington, ON L7L 6G4, Canada
Phone: +1 289 313 3037; Fax: +1 937 637 9086
e-mail: fiona.wingrave@inventivhealth.com

INVESTIGATIONAL SITES

Primary Investigator

Melena Bellin, MD; Assistant Professor, Pediatric Endocrinology and Diabetes
Schulze Diabetes Institute; University of Minnesota Medical School
MMC 404, 420 Delaware St SE - Minneapolis, MN 55455
Phone: 612-625-4686 or 612-626-5716
Fax: 612-624-0420 (SDI), 612-626-5262 (Pediatric Endocrinology)

Full list of investigational sites will be kept in the Trial Master File. Updated versions, if any, will be filed chronologically.

CENTRALIZED LABORATORIES

List of centralized laboratories will be kept in the Trial Master File. Updated versions, if any, will be filed chronologically.

PROTOCOL APPROVAL SIGNATURES

SPONSOR:

Medical Expert

Signature: _____ Date: _____

Pier Adelchi Ruffini, MD - Development Director

Clinical Trial Manager

Signature: _____ Date: _____

Luisa Daffonchio, PhD – Clinical Development Manager

CLINICAL CENTRE: PRINCIPAL INVESTIGATOR [PRIMARY INVESTIGATOR]

I have read study protocol REP0112 "A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation".

Name of Principal Investigator: Melena Bellin, MD

Signature: _____ Date: _____

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List of Abbreviations and Definitions of Terms

AE	Adverse Event
ADR	Adverse Drug Reaction
ALT	Alanine Aminotransferase
ATG	Anti-Thymocyte Globulin
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
AUC_{0-t}	Area under the plasma concentration-time curve from time zero to time t (time of last quantifiable plasma concentration) of reparixin and metabolites
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from time zero to infinity of reparixin and metabolites
BP	Blood Pressure
ºC	Degrees Celsius
CL	Clearance of reparixin and metabolites
CLcr	Calculated Creatinine Clearance (Cockcroft - Gault formula)
$C_{initial}$	Plasma concentration of reparixin and metabolites just prior to the end of administration
Cmax	Maximum Plasma Concentration
CMED	Concomitant Medication
cmH_2O	Centimeters of Water
CPB	Cardiopulmonary Bypass
CRA	Clinical Research Associate
CRF	Case Report Form
CRP	C-reactive Protein
Css	Steady State Concentration
CXCL8	CXC ligand 8 [formerly interleukin (IL)-8]
ddPCR	Droplet Digital PCR
DMC	Data Monitoring Committee
g	Grams
GCP	Good Clinical Practice
HbA1c	Glycated hemoglobin
HR	Heart rate
kg	Kilogram
IAT	Islet Auto-Transplantation
ICH	International Conference on Harmonization
IRB	Institutional Review Board
IEQ	Islet Equivalent
IND	Investigation New Drug
INR	International Normalized Ratio
ITT	Intent to Treat
i.v.	Intravenous
LD_{50}	Lethal $Dose_{50}$
mg	Milligram
miR-375	microRNA-375
mL	Millilitre
mmHg	Millimeters of mercury
MMTT	Mixed Meal Tolerance Test
ng	Nanogram
NGSP	National Glycohemoglobin Standardization Program
NSAID	Non Steroidal Anti-Inflammatory Drug
PICD	Patient Informed Consent Document
PMN	Polymorphonuclear leukocyte
p.o.	per os (taken by mouth)
PTT	Partial Thromboplastin Time
SAE	Serious Adverse Event
s.c.	Subcutaneous
t½	Elimination half life
tmax	Time of maximum plasma concentration of reparixin and metabolites
TPN	Total Parenteral Nutrition
U-CRA	Unblinded-CRA
ULN	Upper Limit of Normal
Vz	Volume of distribution of reparixin
XDP	Fibrin Degradation Products
µg	Microgram
λ_z	Terminal phase rate constant reparixin and metabolites

1. STUDY SYNOPSIS AND OVERALL DESIGN

Study title

A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation.

Study Number REP0112 [**US IND #** 117390]

Study period Projected starting date (first-patient-in): December 2013
Projected completion of patient accrual (last-patient-in): March 2015
Projected study end date (last-patient-last-visit): March 2016

Study design

The study will be a phase 2/3, multicenter, double-blind, parallel assignment study. It will involve 100 adult recipients of an intra-hepatic pancreatic Islet Auto-Transplantation (IAT).

Patients will be randomly (1:1) assigned to receive either reparixin treatment (continuous i.v. infusion for 7 days – treatment group) or placebo (control group). The two groups will be balanced within centres. Recruitment will be competitive among the study sites, until the planned number of patients is enrolled.

Objectives/endpoints

The objective of this clinical trial is to assess whether reparixin leads to improved transplant outcome as measured by the proportion of insulin-independent patients following IAT. The safety of reparixin in the specific clinical setting will be also evaluated.

Efficacy endpoints will be:

- The proportion of insulin-independent patients following IAT [**Primary endpoint**. time frame: day 365±14 after the transplant].
- Area Under the Curve (AUC) for the serum C-peptide level during the first 4 hours of an MMTT, normalized by the number of Islet Equivalent (IEQ)/kg [Time frame: day 75±14 and 365±14 after the transplant].
- Average daily insulin requirements [time frame: day 75±14 and 365±14 after the transplant].
- Basal (2 basal samples in the range between -20 to 0) to 240 min time course of glucose, C-peptide and insulin derived from the MMTT [time frame: day 75±14 and 365±14 after the transplant].
- β-cell function as assessed by β-score [time frame: day 75±14 and 365±14 after the transplant].
- The proportion of patients with an HbA1c ≤6.5% [time frame: day 365±14 after the transplant].
- Cumulative number of severe hypoglycemic events [time frame: from day 75±14 to day 365±14 after the transplant].
- The proportion of patients with an HbA1c ≤6.5% at day 365±14 **AND** are free of severe hypoglycemic events from day 75±14 to day 365±14 inclusive [time frame: day 365±14 and from day 75±14 to day 365±14 after the transplant].

Safety endpoints will be:

- Incidence and severity of Adverse Events and Serious Adverse Events [time frame: throughout the study up to day 365±14 after the transplant].

- Standard laboratory tests including hematology (hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count), clinical chemistry (sodium, potassium, serum creatinine, blood urea nitrogen, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and coagulation (International Normalized Ratio (INR), partial thromboplastin time (PTT)) [time frame: pre-transplant hospital admission and post-transplant hospital discharge].
- Vital signs, i.e. blood pressure (BP) and heart rate (HR) [time frame: pre-transplant hospital admission and post-transplant hospital discharge].
- ALT/AST, INR/PTT, fibrin degradation products (XDPs), C-reactive protein (CRP) [time frame: all daily from day 1 up to day 7 after the transplant; ALT/AST also on day 75±14 after the transplant].
- Weight loss from pre-transplant value [time frame: day 75±14 and 365±14 after the transplant].
- Serum level of albumin and pre-albumin (absolute and change from pre-transplant value) [time frame: day 75±14 and 365±14 after the transplant].
- Severity of steatorrhea [time frame: day 75±14 and 365±14 after the transplant].
- Malnutrition risk (poor prognosis, significant risk, increased risk, normal) according to pre-albumin level [time frame: day 75±14 and 365±14 after the transplant].
- Cumulative number of episodes of documented hypoglycemia (documented symptomatic; asymptomatic) [time frame: from day 75±14 to day 365±14 after the transplant].
- Cumulative number of diabetic ketoacidosis-related events [time frame: from day 75±14 to day 365±14 after the transplant].

Exploratory endpoints will be:

- Time course of inflammatory chemokines/cytokines as assessed by serum level of CXCL8, CCL2 (MCP-1), CCL3, CCL4, CXCL10 (IP-10), CXCL9 (MIG), IL-6, IL-10, INF-γ, TNF-α, and IL-1β [time frame: pre-infusion hospital admission (2 basal samples collected 6 to 24 hrs apart; both samples will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion].
- Proportion of patients who are randomized but do not receive IAT [time frame: transplant day].
- Time course of serum microRNA-375 (miR-375) [time frame: pre-infusion hospital admission (sample will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion].

Pharmacokinetic endpoints will be:

- Plasma levels of reparixin (total and unbound) and relevant metabolites (DF2243Y and ibuprofen) [time frame: post-operative day 1, 3, and 5 (steady state) in all patients; just prior to, and then at 1, 3, 5, 6, 8, and 12 hrs after termination of Investigational Product administration in a subset of at least 20-24 patients].

Number of patients

The goal of this study is to reach a total of 100 patients who are randomized and receive IAT after total or completion pancreatectomy.

Inclusion/exclusion criteria

Patients aged ≥ 18 years given written informed consent who are eligible for an IAT following total (or completion) pancreatectomy will be included in this study.

Recipients of a previous IAT (if completion pancreatectomy) as well as patients undergoing total pancreatectomy due to pancreatic cancer or pancreatic benign diseases (e.g. pancreatic pseudocystis, insulinomas, etc.) will be excluded.

Patients who have inadequate renal reserve (calculated creatinine clearance < 60 mL/min according to the Cockcroft-Gault formula), hepatic dysfunction (increased ALT/AST > 3 x upper limit of normal **or** increased total bilirubin above upper limit of normal at local laboratory, except patients with Gilbert's syndrome), a preoperative International Normalized Ratio (INR) > 1.5 or any known coagulopathy, hypersensitivity to ibuprofen or to more than one non steroidal anti-inflammatory drug or to more than one medication belonging to the class of sulfonamides (e.g. sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib - hypersensitivity to sulphanilamide antibiotics alone, e.g. sulfamethoxazole, does not qualify for exclusion) will be also excluded.

Also, patients will be excluded from study participation if they have concurrent sepsis (as per positive blood culture(s) and/or fever associated with other signs of systemic sepsis syndrome), have received a treatment with systemic steroids in the 2 weeks prior to enrolment (except for the use for physiological replacement only), or any investigational agent (including any anti-cytokine/chemokine agents) or any immune modulators in the 4 weeks prior to enrolment, if they have pre-existing diabetes or evidence of impaired β-cells function (pre-operative fasting blood glucose >115 mg/dL and/or a HbA1c > 6.5%) or require treatment with any anti-diabetic medication (e.g. insulin, metformin, etc) within the 2 weeks prior to enrollment, if they have past or current history of alcohol abuse, if they have evidence of pre-operative portal hypertension (clinical history **and** abdominal/liver imaging by ultrasound techniques).

Pregnant or breast-feeding women or patients unwilling to use effective contraceptive measures (females and males) will not participate in the study.

Investigational Product

The investigational drug will be reparixin or matching placebo. Reparixin will be administered as a continuous i.v. infusion into a (high flow) central vein at a dose of 2.772 mg/kg body weight/hour for 7 days (168hrs), starting approximately 12hrs (6-18 hrs) before pancreatic islet infusion. Placebo (0.9% w/v sodium chloride solution) will be infused at matching volume/rate.

Procedures

Each patient will be involved in the study for a 7 day hospital stay during pancreatectomy followed by islet transplantation, for all required measurements up to hospital discharge and for 2 post-transplant visits scheduled at day 75±14 and 365±14 after the transplant.

Statistics

All patient data collected on the CRF and on the Diary will be listed by patient, treatment group and centre. The data will be presented in the Clinical Study Report.

Appropriate descriptive statistics will be produced, according to the variable.

For the primary efficacy endpoint, the proportion of insulin-independent patients following IAT over the time frame of day 365±14 after the transplant, the differences in proportions will be assessed using the Pearson Chi-square statistic, stratified by the three groups at baseline: < 2500 IEQ/kg vs. 2500-5000 IEQ/kg vs. > 5000 IEQ/kg.

A Statistical Analysis Plan will be issued describing details of all the statistical methods and analysis to be applied to trial results. Any deviations from the original statistical plan will be described in the Clinical Study Report.

2. BACKGROUND INFORMATION

Reparixin is a specific inhibitor of CXC ligand 8 [CXCL8; formerly interleukin (IL)-8] biological activity, stemming from a program of drug design of molecules intended to modulate chemokine action.

Reparixin is the first low molecular weight blocker of CXCL8 biological activity in clinical development. Relevant pre-clinical, toxicological and phase 1 and 2 clinical data are summarized below. Please also refer to the Investigator's Brochure for more detailed information.

Reparixin (formerly repertaxin) was granted orphan drug designation for the "prevention of delayed graft function after (solid) organ transplantations" by the European Commission of Orphan Medicinal Products in September 2001 and by the Food and Drug Administration in January 2003. More recently orphan drug designation has been granted in EU (Septemebr 2011) for the "prevention of graft loss in pancreatic islet transplantation" and in the US (September 2012) for the "prevention of graft loss in islet cell transplantation".

2.1. RELEVANT NON-CLINICAL PHARMACOLOGY

Reparixin is *in vitro* a potent and specific inhibitor of CXCL8 biological activity. *In vitro* chemotaxis experiments have shown that reparixin inhibits CXCL8-induced chemotaxis of human polymorphonuclear leukocytes (PMN) in the low nanomolar range. Studies to elucidate the mechanism of action have shown that reparixin is a non-competitive allosteric inhibitor of the CXCL8 receptors CXCR1 and CXCR2. Interaction of reparixin with CXCL8 receptors inhibits the intracellular signal transduction events activated by binding of CXCL8 to CXCR1 and CXCR2 [*Bertini, 2004; Allegretti, 2005*].

In vivo, reparixin prevented PMN infiltration into the transplanted kidney and reduced creatinin levels in a rat model of kidney transplantation. Similarly, in a rat model of lung transplantation, reparixin improved isolated graft oxygenation, decreased pulmonary oedema, and significantly reduced neutrophil infiltration into transplanted lungs. Moreover, reparixin prevented PMN infiltration and tissue damage in other animal models of ischemia/reperfusion injury of liver, brain, intestine, heart and spinal cord. In these models, *in vivo* inhibition of PMN recruitment ranged from 40 to 90%, and inhibition of tissue damage ranged from 50 to 80%. Efficacy was seen in all models at reparixin dose of 9.90 mg/kg [*Bertini, 2004; Cugini, 2005; Souza, 2004; Cavalieri, 2005; Garau, 2005; Villa, 2007; Gorio, 2007*].

More recently, reparixin lysine salt was evaluated in different models of intrahepatic pancreatic islet transplantation in mice, which include syngeneic and allogeneic settings. Reparixin was administered by s.c. continuous infusion for 7 or 14 days starting from day -1 of islet transplantation. A dose of 5.28 mg/kg/hour was administered in all experiments. Reparixin was able to significantly improve islet engraftment, as demonstrated by its ability to increase the likelihood of and to reduce the time to gain non-fasting blood glucose levels less than 200 mg/dl (normo-glycaemia) in marginal mass syngeneic islet transplantation model. In the fully mismatched allogeneic model, reparixin not only protected islets from early graft failure, but was also able to increase the time to rejection, as shown by post-transplant prolongation of normo-glycaemia. Graft function was indefinitely prolonged in 20/30% of mice treated with reparixin and rapamycin, suggesting possible tolerance induction. In parallel, reparixin treatment reduced intrahepatic infiltration of PMNs, macrophages, T helper and dendritic cells [*Citro, 2012a*].

Reparixin also delayed the onset of diabetes induced in mice by multiple low doses of streptozotocin. Also, mice treated with reparixin retained a better glycemic control (lower glycemic levels) even after diabetes development [*Citro, 2012b*].

2.2. A SUMMARY OF TOXICOLOGY DATA

Reparixin was tested for toxicity in rodent and non-rodent animal species after single and repeated i.v. doses. The repeated dose administration studies were conducted by i.v. continuous infusion, according to the intended human administration route.

The general toxicological profile of i.v. reparixin, in the studies conducted to date, is characterized by a low toxicity after single or repeated dose administrations in rats (LD_{50} = 229.68 mg/kg i.v.; 660.00 mg/kg/day as No Observed Adverse Effect Level from 4 weeks studies) and mice (401.94 mg/kg i.v.). Continuous i.v. administration to dogs for 2 weeks resulted in a safe dose of 39.60 mg/kg/day.

Continuous i.v. infusion of reparixin to the male and female rat at dose levels of up to 660.00 mg/kg/day did not have any significant adverse effects on mating performance and fertility.

Reparixin poses no genotoxic hazard for humans.

Reparixin lysine salt, at doses in excess of those intended to be used in humans, has a safe pharmacology profile in the renal, cardiovascular and respiratory systems of rats and dogs.

The local tolerability of reparixin lysine salt was assayed in the rabbit ear lateral vein. The compound was well tolerated in concentrations up to 4.95 mg/mL (1 mL/kg) infused over a minute.

In order to provide evidence of the safety of DF2243Y, the main metabolite of reparixin excreted in urine in humans, safety pharmacology and toxicity studies have been performed at doses 2 to 3 times higher than those reached in man, as may occur during the treatment of patients receiving kidney transplantation.

2.3. PHARMACOKINETICS AND PRODUCT METABOLISM

PK studies by i.v. injection revealed that reparixin is very rapidly eliminated in rats and humans ($t_{1/2}$ 0.5-3hrs and 1.0-1.5hrs, respectively) whereas elimination is slower in dogs (12-28hrs). The PK of reparixin was linear in rats and in dogs but linearity was less evident in humans.

Reparixin undergoes complete metabolism (oxidation + conjugation) in all the species tested. The *in vitro* human hepatic, phase I metabolism of reparixin is catalysed by CYP2C9 and to a lesser extent by CYP2C19. DF2243Y, DF2188Y, methanesulfonamide and ibuprofen are the metabolites detected in human plasma and urine, with DF2243Y being the major metabolite. Exposure to ibuprofen after administration of reparixin 2.77 mg/kg/h for 48hrs (the highest dose tested in humans) was similar or lower than that obtained after a standard therapeutic single dose of ibuprofen (300mg).

Due to extensive metabolism, unchanged reparixin was poorly or not excreted into the urine of rat, dogs and humans so that the PK profile of reparixin is not influenced by renal impairment.

In vitro protein binding of [^{14}C]-reparixin showed that reparixin is highly bound (approximately 99%) to plasma proteins in rats, dogs, rabbits, cynomolgus monkeys and humans. Albumin is likely to be the major binding protein in plasma in all species, accounting for 99.2% in humans.

Reparixin has some potential *in vitro* for a non-competitive inhibition of the human hepatic enzyme CYP3A4 that is involved in the metabolism of cyclosporine A, tacrolimus and rapamycin. However, since inhibition is evident at concentration far higher than the free plasma concentration of reparixin at steady state in humans, it is predicted that the clinical relevance of such inhibition is remote. Indeed, reparixin does not affect to a clinically relevant extent the activity of CYP3A4 and CYP2C9 (enzyme involved in reparixin metabolism), as revealed by an interaction study where the PK of midazolam and tolbutamide (probe substrates for these enzymes) was evaluated in healthy subjects receiving single oral doses of the probes alone or in combination with reparixin.

2.4. A SUMMARY OF CLINICAL DATA

Clinical development includes phase 1 pharmacokinetics, tolerability and pharmacodynamic studies and 2 phase 2 studies conducted in lung and kidney transplant patients. An independent study was also carried out in cardiopulmonary bypass (CPB) patients.

To date, a total of 363 subjects were involved in completed clinical studies, of whom 230 were exposed to reparixin.

A pilot phase 2 study is ongoing in islet transplantation and is addressed below.

2.4.1. Pharmacokinetics and product metabolism in humans

Three phase 1 PK/safety studies were performed which included 30min to 48h i.v. infusion of ascending doses in healthy male subjects and 6h i.v. infusion of a selected dose in male and female subjects with normal to severely impaired renal function [*REP0101; REP0102; REP0203*]. An additional interaction study was also conducted in healthy subjects [*REP0103*]. PK results are discussed in paragraph 2.3.

2.4.2. Efficacy

In the first US-Canada phase 2 study [*REP0104*], 101 patients (46 on reparixin, 55 on placebo) undergoing single or bilateral lung transplant were included in the intent to treat (ITT) and safety population out of 114 enrolled. The patients were randomized to receive 48h i.v. continuous infusion (loading: 4.488 mg/kg/h for 30min, maintenance: 2.772 mg/kg/h for 47.5hrs) of either reparixin or placebo starting a few hours before the transplant. The study failed to show any statistically significant difference between the reparixin and placebo groups in the primary efficacy variable (PaO_2/FiO_2 ratio) and in any of the secondary efficacy variables assessed up to month 12 post-transplant, except survival. Indeed, there was a statistically significant difference in patient survival at Month 12 post-transplant between the placebo (7 deaths) and reparixin (no deaths) groups (p-value = 0.0111 [Log-Rank]).

The second phase 2 study [*REP0204*] was conducted in US, Italy, Spain and France and involved patients undergoing kidney transplantation at increased risk of developing Delayed Graft Function. This was a pilot study designed to explore the efficacy and safety of two different dosing schedules selected to minimize the impact of possible post-transplant renal dysfunction on reparixin PK. Out of 80 patients randomized, 74 patients (25 reparixin continuous infusion; 23 reparixin intermittent infusion; 26 pooled placebo) and 73 patients (24 reparixin continuous infusion; 23 reparixin intermittent infusion; 26 pooled placebo) were included in the safety and ITT analysis, respectively. The patients were randomized to receive reparixin or placebo as continuous infusion of 2.772 mg/kg/h for 12hrs or 12 intermittent i.v. infusions of 2.244 mg/kg for 30min with 1.5 hour intervals over a total period of 22.5hrs or matched placebo. Overall, the study failed to show a significant difference between the treatment groups in the primary efficacy variable (early CLcr) and in any of the secondary efficacy variables assessed up to month 12 post-transplant. However, a trend for a better graft function, measured by early (1-3 and 10-12hrs after transplant) CLcr and day 1 to 7 serum Cr was observed in the reparixin intermittent infusion group, which however did not reach a statistical significance. Also a higher, even not significant, percentage of patients in this group had immediate graft function, with only one patient experiencing graft failure.

The Medical University of Vienna conducted an additional independent placebo-controlled, randomized pilot trial in 32 patients undergoing elective coronary artery bypass grafting with CPB. Reparixin was infused at a loading dose of 4.488 mg/kg/h for 30min followed by a maintenance dose of 2.772 mg/kg/h for 8h. The rise in neutrophil count after CPB was less marked in the reparixin than in the placebo group ($p < 0.05$). Significant group differences were also detected at 4 hours post-CPB.

A phase 2 multicenter, randomized, open-label, parallel-assignment, pilot study is ongoing and is assessing the efficacy and safety of reparixin following a single-infusion islet allo-transplantation [REP0110; NCT01220856]. Patients are on an immunosuppression regimen consisting of induction with ATG followed by maintenance immunosuppression. Patients are randomly (1:1) assigned to receive either no additional experimental intervention (control group) or reparixin treatment (2.772 mg/kg body weight/hour i.v. continuous infusion for 7 days) – treatment group). Inclusion criteria restrict enrolment to patients who are expected to receive an islet mass (4000 to 7000 islet equivalent (IEQ)/kg body weight) in the lower range of the currently accepted transplantable islet amount.

Due to these preliminary results obtained in 7 patients, protocol REP0110 was amended to allow randomization to the reparixin treatment group only. Also, patients already treated with reparixin with a functioning graft were allowed to receive a 2nd islet infusion. Follow-up was re-scheduled to provide measurements up to one year after the 2nd islet infusion [*Citro, 2012a*].

A total of 9 patients have been enrolled: 6 have been randomized to reparixin treatment and 3 to the control group. Preliminary data for the measurements at month 1 post-1st infusion are summarized in the table below.

REP0110 – Summary preliminary data (demographics and month 1 measurements)

	Reparixin (n=6)	**Control** (n=3)
Gender (F/M) - Age (mean±SD)	3/3 - 46.2±8.8	2/1 - 48.0±7.0
Total IEQ/kg (mean±SD)	4911±897	4528±398
Peak C-peptide ng/mL (mean±SD)	1.47±1.37	0.20±0
C-peptide AUC (MMTT) normalized by IEQ/kg (meanx10^{-4}±SD)*	1.92±1.62	0.44±0.04
Insulin requirement IU/kg/day [percent decrease] (mean±SD)	0.42±0.32 [-41.87±39.89]	0.62±0.34 [0±0]
HbA1c % [percent decrease] (mean±SD)	7.60±1.07 [16.72±4.46]	7.03±1.02 [11.17±5.40]
β-score (mean±SD)	1.83±1.17	1.33±1.53
Transplant Estimated Function (mean±SD) (*Caumo, 2008*)	111.93±64.09	13.73±49.39
Number of patients with graft loss	2	3

* AUC has been calculated on the -10 to 120 min time course of C-peptide during an MMTT. Where the C-peptide value was below the detection limit (0.2 ng/mL), a value of 0.2 ng/mL has been used for calculation.

None of the patients in the control group expressed a β-cell function at month 1 post-transplant (no decrease in insulin requirement coupled with C-peptide levels < 0.3 ng/mL) and all were withdrawn due to graft loss. On the other hand, all but two of the patients treated with reparixin experienced improved transplant outcome as measured by glycemic control, decreased insulin requirement and appearance of detectable levels of C-peptide above 0.3 ng/mL. Out of 4 patients who have received a 2nd infusion to date, 3 have achieved insulin-independence, with two patients retaining insulin-independence 1 year post last (2nd) transplant.

The development of reparixin in islet allo-transplantation is being progressed and a phase 3 clinical trial is ongoing in EU and US [REP0211; NCT01817959].

2.4.3. Safety

A total of 230 subjects have been exposed to reparixin in the clinical studies completed to date which include 103 adult healthy subjects (100M/3F), 17 patients with different grades of renal impairment (12M/5F), 16 patients undergoing cardiopulmonary by pass (10M/6F), 46 patients undergoing lung transplantation (23M/23F) and 48 patients undergoing kidney transplant (31M/17F). Exposure included short or prolonged i.v. infusion up to 10.6 mg/kg over 30min or 133 mg/kg over 48h.

Overall, reparixin was safe and well tolerated in both healthy subjects and critically ill patients.

In phase 1 studies, no deaths, Serious Adverse Events (SAEs) or Adverse Event (AE)-related withdrawals were reported. The majority of AEs reported were of mild intensity. All subjects had recovered completely or had ongoing adverse events of mild intensity when they were discharged. The safety of reparixin was confirmed also in patients with different grades of renal impairment. In the interaction study no safety concerns were raised during co-administration of midazolam/tolbutamide with reparixin.

During phase 2 studies, AE and SAE profile was similar for both placebo and reparixin groups and no particular safety concerns were raised.

In the lung transplant study there were no AE-related withdrawals or deaths for either placebo or reparixin groups. A total of 28 patients experienced SAEs. Death occurred in 7 patients, all in the placebo group. Death was due to infections in 4 patients leading to sepsis/bacteraemia (3 patients) or pneumonia (1 patient). One patient died due to a cardiovascular accident. Coronary artery disease or unknown origin was the cause of death reported for the other 2 patients for whom minimal information was available.

In the kidney transplant study there was 1 AE-related withdrawal in the reparixin continuous infusion group. AEs leading to discontinuation of study drug were reported for 3 patients in the reparixin continuous infusion group and for 1 patient in the placebo group. One patient in the placebo group had an AE (septic shock, pneumonia) leading to death. SAEs were reported in 21 patients. Death occurred in 2 patients, 1 in the placebo group (septic shock, pneumonia) and 1 in the reparixin intermittent infusion group (no information available).

No SAEs were reported from the CPB study.

As to Adverse Drug Reactions (ADRs), infusion site reactions, mainly erythema or aseptic thrombophlebitis, were one of the most common ADRs. The relatively high frequency was due to a cluster of events occurring in cohort 1 of the phase 1 48h-infusion study. Local toxicity was clearly related to drug concentration since the use of a more diluted solution at a higher infusion rate markedly reduced the incidence, type and severity of infusion site reactions.

Cumulative summary tabulations of ADRs are reported in **Appendix 14.1**.

The most frequent (>10%) ADRs observed in the phase 1 and phase 2 studies were:

Nervous system disorders (about 27%), including headache, dizziness, hypoaesthesia, somnolence.

General disorders and administration site conditions (about 23%), including cannula site reaction, injection site thrombosis, infusion site oedema and peripheral oedema, fatigue, lethargy.

Gastrointestinal disorders (about 14%), including nausea, vomiting, abdominal pain, dyspepsia, flatulence, gastroesophageal reflux disease.

Overall, there was no correlation between the incidence of ADRs and the extent of exposure. Moreover, the incidence of ADRs was variable among studies regardless of the clinical condition of the population exposed to reparixin, i.e. patients under critical care (lung/kidney transplant or CPB patients) did not experience increased frequency of adverse events attributable to reparixin.

Preliminary data obtained in the ongoing pilot trial in islet allo-transplantation further support the safety of reparixin in this clinical setting. In patients treated with a 7 day course of reparixin a few AEs were reported which were judged to be at least possibly related to reparixin treatment. Most frequent ADRs were erythema, hypotension, nausea, vomiting; great majority of these were mild to moderate in nature and none required discontinuation if the Investigational Product. A total of 5 SAEs were reported to date in 4 patients. Two patients (one randomized to the control group, one to the reparixin group) experienced intraperitoneal bleeding which was judged to depend on the islet infusion procedure (not-related to the study treatment) in both cases. One patient who had received reparixin during the first islet transplant was hospitalized for diabetic ketoacidosis (due to graft failure) and diarrhea (possibly due to MMF administration). Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion; upon case evaluation, a medical error was evidenced, i.e. the patient received a dose of reparixn 3 times as high as that foreseen in the protocol; infusion of reparixin was immediately discontinued and the patient recovered. About 2 months later, this same patient required hospitalization for diarrhea and worsening of kidney function which were due to immunosupressive medications (MMF, tacrolimus).

2.5. DISEASE REVIEW AND STUDY RATIONALE

Total pancreatectomy is a surgical procedure to treat end-stage pancreatic diseases, including, but not limited to, chronic pancreatitis. Despite improvement in surgical techniques, total pancreatectomy is associated with long term complications which are mostly accounted for by endocrine insufficiency. While exocrine insufficiency is considered easier to overcome, the ensuing surgical brittle diabetes presents with a high risk of hypoglycemic unawareness that is a leading cause of patient death [*Sarr, 1993; Berney, 2000; Gruessner, 2004; Garcea, 2009*].

After total pancreatectomy, the absence of endogenous insulin is coupled with the lack of other regulatory hormones, such as glucagon, resulting in the well known poor post-operative diabetes control [*Farley, 1995; Linhean, 1988*]. Segmental pancreatic or islet auto-transplantation (IAT) has therefore been considered since the 1970s as feasible adjunctive procedures to prevent severe post-surgical diabetes in patients undergoing total pancreatectomy for chronic pancreatitis. IAT offers advantages over heterotopic pancreas auto-transplantation as intraportal islet infusion is a smaller procedure and should be favoured whenever the patient has lost pancreatic exocrine function [*Sutherland, 1978; Hogle, 1978; Gruessner, 2004; Dong, 2011*].

Despite β-cell mass, as estimated by Islet Equivalent (IEQ)/kg body weight, is less than for islet allografts, long term successful auto-graft function can be achieved in most patients, with a rate of insulin independence as high as >80% of recipients, even if lower figures have been reported, according to the total amount of IEQ infused [*Sutherland, 2012; Pollard, 2011; Dong, 2011*].

In addition to end-stage chronic pancreatitis, IAT post pancreatectomy has been utilized for an expanded series of pancreatic benign diseases including, pancreatic pseudocystis, insulinomas, etc. More recently, patients bearing pancreatic cancer were admitted to IAT with no evidence of hepatic recurrence of pancreatic disease up to almost one year follow-up, offering some neoplastic patients a chance for post-pancreatectomy glycemic control [*Dong, 2011; Balzano, 2011*].

In contrast to allo-transplantation in type 1 diabetes patients, where immunological mechanisms involving allo- and auto-antibodies affect long-term graft function, outcome in IAT is independent from immunological processes and does not require immunosuppression management. On the other hand, early inflammatory events intrinsic to the intra-portal islet infusion have been demonstrated to impact islet engraftment. Among possible mechanisms, PMNs have been found to be the predominant cell types infiltrating the liver in a syngeneic model of islet transplantation in mice. Also, the intra-hepatic mRNA for CXCL1/KC, the murine counterpart of human CXCL8, was strongly induced immediately after islet infusion. Consistent with this results, transplanted CXCR2 knock out mice had a significant improvement in islet function [*Citro, 2011*]. Thus, CXCL8 might represent a relevant therapeutic target to prevent early graft failure after IAT.

Data obtained in experimental models of islet transplantation in mice demonstrate a clear effect of reparixin in improving graft survival and function. Protection from the loss and/or deterioration of transplanted islets was evident regardless of the immunological mechanisms involved in islet damage, suggesting that the ability of reparixin to modulate early inflammatory responses readily impact graft outcome. Results obtained with reparixin in the experimental model of multiple low doses of streptozotocin further emphasize the ability of reparixin to protect β-cells from an inflammatory damage which appears to impact subsequent graft outcome.

Thus, the use of reparixin may emerge as a potential useful medication in the control of non specific inflammatory events surrounding the early phases of IAT.

Preliminary data obtained in transplanted patients recruited in the ongoing pilot trial coupled with the safety shown in human phase 1 and 2 studies provide a sound rationale for further development of reparixin also in IAT and prompt the conduct of this phase 2/3 clinical study aimed at assessing the efficacy and safety of reparixin in improving graft outcome in patients undergoing total (or completion) pancreatectomy followed by IAT.

2.5.1. Selection of dose and treatment schedule in the study

The dose of reparixin proposed for this trial (2.772 mg/kg body weight/hour) is the same being administered in the ongoing clinical trials in islet allo-tranplantation. It was originally derived from the effective reparixin concentrations found both in "in vitro" inhibition of CXCL8-induced chemotaxis of human PMN and in experiments in mouse models of syngeneic and allogeneic transplantation. Such a dose was found safe in previous phase 1 and 2 studies. Also, preliminary data obtained in the ongoing pilot trial in islet allo-transplantation confirm the efficacy and the safety of such a dose.

Consistent with experimental data in animals and preliminary data in the ongoing pilot trial in islet allo-transplantation, the proposed schedule of administration (continuous infusion for 7 days) is intended to expose the patient to reparixin throughout the time-window of relevant inflammatory post-transplant events after both allo-transplantation and IAT [*Pollard, 2011; Citro, 2011*].

2.5.2. Alternative treatments

There are no specific pharmacologic treatments addressed to the prevention of graft failure after IAT. A trial is currently ongoing to test the potential effect of sitagliptin in IAT (NCT01186562). Because of this, patients not willing to participate in the study may either be offered to participate in another trial of experimental treatment or will not be offered any specific alternative treatment.

All patients, regardless of study participation, will receive the standard of optimal care for the transplanted organ (procurement, storage, isolation, infusion) and the recipient.

2.5.3. Risk - benefit evaluation

2.5.3.1. General risks

Transplantation of islets is associated with several potential risks. These are intrinsic to the procedure and may result from the intra-hepatic islet infusion as well as testing involved in the care and evaluation of transplanted subjects. All patients receiving IAT are at risk of complication regardless of study participation.

2.5.3.2. Risk related to the Investigational Product

Results from preclinical studies support the level of drug exposure planned in this study. Also, past clinical experience with the same dose as planned in this study provides a clear cut evidence of reparixin safety. Very few SAEs were reported with this dose even from clinical trials conducted in severe clinical conditions such as patients undergoing lung or kidney transplantation.

Preliminary data obtained in the ongoing pilot trial in islet allo-transplantation further support the safety of reparixin in this clinical setting. In patients treated with a 7 day course of reparixin a few AEs were reported which were judged to be at least possibly related to reparixin treatment. Most frequent ADRs were erythema, hypotension, nausea, vomiting; great majority of these were mild to moderate in nature and none required discontinuation if the Investigational Product. A total of 5 SAEs were reported to date in 4 patients. Two patients (one randomized to the control group, one to the reparixin group) experienced intraperitoneal bleeding which was judged to depend on the islet infusion procedure (not-related to the study treatment) in both cases. One patient who had received reparixin during the first islet transplant was hospitalized for diabetic ketoacidosis (due to graft failure) and diarrhea (possibly due to MMF administration). Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion; upon case evaluation, a medical error was evidenced, i.e. the patient received a dose of reparixn 3 times as high as that foreseen in the protocol; infusion of reparixin was immediately discontinued and the patient recovered. About 2 months later, this same patient required hospitalization for diarrhea and worsening of kidney function which were due to immunosupressive medications (MMF, tacrolimus).

The very short half life of reparixin ($t_{1/2}$ 1.0-1.5hrs) represents an important safety factor as plasma levels decline rapidly after drug discontinuation. This PK profile, coupled with the mechanism of reparixin action (reversible inhibition), makes readily effective appropriate stopping rules.

Administration via a high flow central vein, which was proven to be safe in lung and kidney recipients, as well as in patients undergoing islet transplant, is proposed for the study to minimize the risk of infusion-site toxicity seen in a cohort of volunteers receiving i.v. infusion via a peripheral vein.

2.5.3.3. Central line

The study drug will be administered as a continuous i.v. infusion over 7 days with a catheter inserted in a (high flow) central vein. The risks of a central line are not considered study-related risks since a central line is routinely placed in patients undergoing post-pancreatectomy IAT for their routine clinical care. This required access will be maintained for a period of time that might be slightly longer than that routinely used. Nevertheless, central line care as routinely practiced by the participating sites will minimize the risk of thrombosis and infections.

2.5.3.4. Blood sampling

In addition to the routine blood sampling, participation in the study will require an amount of blood slightly higher than routine sampling at some time points (not exceeding 5mL at each time point), to allow study specific measurements and centralized assay of C-peptide, HbA1c, inflammatory chemokines/cytokines, miR-375 and PK samples.

2.5.3.5. Other study related procedures

No other procedures specifically associated to the study are required. Type, timing and frequency of some measurements, e.g. stimulation test by a Mixed Meal Tolerance Test (MMTT), might be guided by the protocol, but such measurements are basically part of standard patient care and would be performed regardless of study participation.

2.5.3.6. Potential benefit

To the patients: According to preliminary data obtained in islet allo-transplantation, patients receiving reparixin are likely to benefit with improved auto-graft function, but this is to be confirmed. Patients assigned to the control arm are expected to obtain no additional benefit other than that established in IAT recipients.

To society: This study may identify a useful medication that will make post-pancreatectomy IAT more effective for future recipients.

3. OVERALL STUDY DESIGN AND PLAN DESCRIPTION

3.1. STUDY DESIGN

The study will be a phase 2/3, multicenter (US), randomized, double-blind, placebo-controlled, parallel assignment pilot study.

The goal of this study is to reach a total of 100 adult patients who are randomized and receive IAT after total or completion pancreatectomy. Patients will be randomly (1:1) assigned to receive either reparixin [continuous i.v. infusion for 7 days (168hrs)], or matched placebo (control group),starting approximately 12hrs before islet infusion. The two groups will be balanced within each centre. All patients who are randomized and receive the Investigational Product (either reparixin or placebo) will be inlcuded in the ITT analysis. Patients will be in the ITT analysis whether or not they receive IAT, because exclusions cannot be made for events occurring after randomization that could be influenced by the randomized assignment.

Recruitment will be competitive among the study sites, until the planned number of patients is enrolled. Competitive recruitment has been chosen to increase the speed of recruitment and to account for any difference in transplant rate among study sites. Each centre will enroll patients as rapidly as possible, up to a maximum of 40 patients (as per the randomization list). A maximum of 48 patients is allowed for the site of the Primary Investigator. Each patient will be involved in the study for 7 day hospital stay during pancreatectomy followed by islet transplantation, for all required measurements up to hospital discharge and for 2 post-transplant visits scheduled @ day 75$\pm$14 and 365$\pm$14 after the transplant.

3.2. STUDY TIME TABLE

The planned patients are expected to be recruited in a 12 month period. A follow-up of 12 months is planned for each patient. Overall study timelines are reported below.

- Projected starting date (first-patient-in): December 2013
- Projected completion of patient accrual (last-patient-in): March 2015
- Projected study end date (last-patient-last-visit): March 2016

3.3. END OF STUDY

For the purpose of this trial, the end of study is defined as the date of the last visit of the last patient.

4. OBJECTIVES AND ENDPOINTS

4.1. STUDY OBJECTIVES

The objective of this clinical trial is to assess whether reparixin leads to improved transplant outcome as measured by the proportion of insulin-independent patients following IAT. The safety of reparixin in the specific clinical setting will be also evaluated

4.2. STUDY ENDPOINTS

4.2.1. Primary efficacy endpoint

- The proportion of insulin-independent patients following IAT [time frame: day 365±14 after the transplant].

  For the purpose of this protocol, insulin-independence is defined as freedom from the need to take exogenous insulin for 14 or more consecutive days, with adequate glycemic control, as defined by:
  - a glycated hemoglobin (HbA1c) level ≤6.5%;
  - fingerstick fasting blood glucose not exceeding 126 mg/dL more than three times in the past week (based on a minimum of one daily measurement;
  - a 2 hour post-prandial blood glucose not exceeding 180 mg/dL more than four times in the past week (based on a minimum of one daily measurement);
  - a laboratory fasting glucose in the non-diabetic range (<126 mg/dL).

4.2.2. Secondary efficacy endpoints

- Area Under the Curve (AUC) for the serum C-peptide level during the first 4 hours of an MMTT, normalized by the number of Islet Equivalent (IEQ)/kg [Time frame: day 75±14 and 365±14 after the transplant].
- Average daily insulin requirements [time frame: day 75±14 and 365±14 after the transplant].

  For the purpose of this protocol, daily insulin is reported as IU/kg and intake averaged over the previous week.
- Basal (2 basal samples in the range between -20 to 0) to 240 min time course of glucose, C-peptide and insulin derived from the MMTT [time frame: day 75±14 and 365±14 after the transplant].
- β-cell function as assessed by β-score [time frame: day 75±14 and 365±14 after the transplant].
- The proportion of patients with an HbA1c ≤6.5% [time frame: day 365±14 after the transplant].
- Cumulative number of severe hypoglycemic events [time frame: from day 75±14 to day 365±14 after the transplant].

  For the purpose of this protocol, a severe hypoglycemic event is defined as an event with one of the following symptoms: "memory loss, confusion, uncontrollable behavior, irrationale behavior, unusual difficulty in awakening, suspected seizure, seizure, loss of consciousness, or visual symptoms", in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54mg/dL or prompt recovery after oral carbohydrate, i.v. glucose, or glucagon administration.
- The proportion of patients with an HbA1c ≤6.5% at day 365±14 **AND** are free of severe hypoglycemic events from day 75±14 to day 365±14 inclusive [time frame: day 365±14 and from day 75±14 to day 365±14 after the transplant].

4.2.3. Safety endpoints

- Incidence and severity of Adverse Events and Serious Adverse Events [time frame: throughout the study up to day 365±14 after the transplant].

- Standard laboratory tests including hematology (hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count), clinical chemistry (sodium, potassium, serum creatinine, blood urea nitrogen, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and coagulation (International Normalized Ratio (INR), partial thromboplastin time (PTT)) [time frame: pre-transplant hospital admission and post-transplant hospital discharge].
- Vital signs, i.e. blood pressure (BP) and heart rate (HR) [time frame: pre-transplant hospital admission and post-transplant hospital discharge].
- ALT/AST, INR/PTT, fibrin degradation products (XDPs), C-reactive protein (CRP) [time frame: all daily from day 1 up to day 7 after the transplant; ALT/AST also on day 75±5 after the transplant].
- Weight loss from pre-transplant value [time frame: day 75±14 and 365±14 after the transplant].
- Serum level of albumin and pre-albumin (absolute and change from pre-transplant value) [time frame: day 75±14 and 365±14 after the transplant].
- Proportion of patients falling into one of the following levels of steatorrhea severity [time frame: day 75±14 and 365±14 after the transplant].

  For the purpose of this protocol, levels of steatorrhea severity (evaluated in the 4 weeks prior to day 75±14 and 365±14), are defined as:

  - No steatorrhea;
  - Steatorrhea few times per week;
  - Steatorrhea daily;
  - Stool incontinence.
- Proportion of patients falling into one of the following malnutrition risk levels (poor prognosis, significant risk, increased risk, normal) according to pre-albumin level [time frame: day 75±14 and 365±14 after the transplant].

  For the purpose of this protocol, malnutrition risk levels are defined as [adapted from *Bernstein, 1995*]:

  - Poor prognosis = pre-albumin level <5.0 mg/dL
  - Significant risk = pre-albumin level 5.0 to 10.9 mg/dL
  - Increased risk = pre-albumin level 11.0 to 15.0 mg/dL
  - Normal = pre-albumin level > 15.0 (up to 35.0) mg/dL
- Cmulative number of episodes of documented hypoglycemia (documented symptomatic; asymptomatic) [time frame: from day 75±14 to day 365±14 after the transplant].

  For the purpose of this protocol, the following definition applies [*Diabetes Care, 2005*]:

  - Documented symptomatic hypoglycemia = An event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration ≤70mg/dL.
  - Asymptomatic hypoglycemia = An event not accompanied by typical symptoms of hypoglycemia, but with a measured plasma glucose concentration ≤70mg/dL.
- Cumulative number of diabetic ketoacidosis-related events [time frame: from day 75±14 to day 365±14 after the transplant].

  For the purpose of this protocol, a diabetic ketoacidosis event is defined as the presence of [*Pediatrics, 2004*]:

  - hyperglycemia (blood glucose >200 mg/dL);
  - pH <7.3 or HCO3 <15;
  - ketones positive in the serum or urine.

4.2.4. Exploratory endpoints

- Time course of inflammatory chemokines/cytokines as assessed by serum level of CXCL8, CCL2 (MCP-1), CCL3, CCL4, CXCL10 (IP-10), CXCL9 (MIG), IL-6, IL-10, INF-γ, TNF-α, and IL-1β [time frame: pre-infusion hospital admission (2 basal samples collected 6 to 24 hrs apart;

both samples will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion].

- Proportion of patients who are randomized but do not receive IAT [time frame: transplant day].
- Time course of serum microRNA-375 (miR-375) [time frame: pre-infusion hospital admission (sample will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion].

4.2.5. Pharmacokinetic endpoints

- Plasma levels of reparixin (total and unbound) and relevant metabolites (DF2243Y and ibuprofen) [time frame: post-operative day 1, 3, and 5 (steady state) in all patients; just prior to, and then at 1, 3, 5, 6, 8, and 12 hrs after termination of Investigational Product administration in a subset of at least 20-24 patients]. This number of patients should guarantee post-treatment sampling in at least 10-12 patients randomized to reparixin (base on a 1:1 randomization). These patients will be enrolled at 2 of the participating sites.

5. STUDY POPULATION

The goal of this study is to reach a total of 100 patients who are randomized and receive IAT after total (or completion) pancreatectomy, each being randomised (1:1) to receive either reparixin or placebo.

Patients will be selected from those eligible for IAT following total or completion pancreatectomy. In addition to locally accepted eligibility criteria for the IAT (see below), each prospective patient will be randomized provided that (s)he fully meets all of the study-specific Inclusion Criteria and none of the study-specific Exclusion Criteria described in Sections 5.1. and 5.2. below.

5.1. INCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must fulfil ALL of the following inclusion criteria.

1. Patients eligible for an IAT following total (or completion) pancreatectomy.

Eligibility to both total (or completion) pancreatectomy and IAT will be based on locally accepted criteria and guidelines (see also exclusion below). The most restrictive criteria will be applied.

2. Ages ≥ 18 years.

3. Patients willing and able to comply with the protocol procedures for the duration of the study, including scheduled follow-up visits and examinations.

4. Patients who have given written informed consent, prior to any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to their future medical care.

5.2. EXCLUSION CRITERIA

Patients who meet any of the following criteria are NOT eligible for participation in the study.

1. Recipients of a previous IAT (if completion pancreatectomy).

2. Patients undergoing total pancreatectomy due to either pancreatic cancer or pancreatic benign diseases other than chronic pancreatitis, including insulinomas, etc.

3. Patients with inadequate renal reserve as per calculated creatinine clearance (CLcr) < 60 mL/min according to the Cockcroft-Gault formula (*1976*).

4. Patients with hepatic dysfunction as defined by increased ALT/AST > 3 x upper limit of normal (ULN) **or** increased total bilirubin above the upper limit at local laboratory). Patients with Gilbert's syndrome (elevated unconjugated bilirubin levels in the absence of any evidence of hepatic or biliary tract disease) are not excluded.

5. Patients with a preoperative International Normalized Ratio (INR) > 1.5 or any known coagulopathy.

6. Hypersensitivity to:

a) ibuprofen or to more than one non steroidal anti-inflammatory drug (NSAID).

b) more than one medication belonging to the class of sulfonamides, such as sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib; hypersensitivity to sulphanilamide antibiotics alone (e.g. sulfamethoxazole) does not qualify for exclusion.

7. Concurrent sepsis (as per positive blood culture(s) and/or fever associated with other signs of systemic sepsis syndrome).

8. Treatment with systemic steroids in the 2 weeks prior to enrolment (except for the use of ≤5mg prednisone daily or equivalent dose of hydrocortisone, for physiological replacement only) or with any immune modulators in the 4 weeks prior to enrolment.

9. Patients with pre-existing diabetes or evidence of impaired β-cells function, based on pre-operative fasting blood glucose >115 mg/dL and/or a HbA1c > 6.5%, or requiring treatment with any anti-diabetic medication (e.g. insulin, metformin, etc) within the 2 weeks prior to enrolment.

10. Use of any investigational agent in the 4 weeks prior to enrolment, including any anti-cytokine/chemokine agents.

11. Pregnant or breast-feeding women; unwillingness to use effective contraceptive measures (females and males). (NB: pregnancy should be avoided in patients or partners during the first month after completing the treatment with the Investigational Product; no other specific warnings are described, considering the treatment course of the Investigational Product, its PK profile, and the lack of significant adverse effects on mating performance and fertility in animal studies).

12. Patients with past or current history of alcohol abuse based on clinical history and/or past treatment for alcohol addiction.

13. Patients with evidence of pre-operative portal hypertension as per clinical history **and** abdominal/liver imaging by ultrasound techniques.

Sites will comply with any additional or more restrictive exclusion criteria locally accepted, as per centre practice.

5.3. ASSIGNMENT OF PATIENT NUMBER

Patient number will be assigned in a sequential manner as a patient is found to be eligible for entry into the study and is randomized. It will consist of the site number, and the patient number e.g. 0102, where first 2 digits represent site number, last 2 digits patient number. If a patient is dropped from the study for any reason, the patient's number will not be reassigned.

6. INVESTIGATIONAL PRODUCT

The Investigational Product will be either reparixin [R(-)-4-Isobutyl-alpha-methylphenylacetyl-methanesulfonamide] or matched placebo.

6.1. PRESENTATION, STORAGE, PACKAGING AND LABELING

6.1.1. Presentation of Investigational Products

Reparixin will be provided as concentrate for solution for i.v. infusion packaged into 250 mL clear Glass Vials with the following composition per single (250 mL) unit:

Reparixin composition - 33mg/mL

NAME OF INGREDIENT	PER-UNIT FORMULA	FUNCTION OF INGREDIENT	REFERENCE TO QUALITY STANDARDS
Reparixin (DF1681Y)	8.25 g	Drug substance	Manufacturer monograph and specification
Sodium Dihydrogen Phosphate Dihydrate	1.96 g	Buffer	European Pharmacopoeia - current edition
L-lysine monohydrate	4.78 g	Solubilizer	German Pharmacopoeia - current edition
Sodium hydroxide	qs to pH 8.0	Buffer	European Pharmacopoeia - current edition
Water for injections	qs to 250 mL	Solvent	European Pharmacopoeia - current edition

Placebo will be physiologic salt solution (0.9% w/v sodium chloride solution) provided as commercially available 250 mL clear Glass Vials.

Batch release certificate will be provided together with the Investigational Products.

6.1.2. Manufacturing, Packaging and Labelling of Investigational Product

Reparixin will be manufactured by Patheon (Italy). Both Investigational products will be packaged and labelled by an authorized facility which will be identified before trial commencement.

Reparixin and placebo vials will be supplied along with sterile empty Infusion Bags and corresponding Infusion Bag labels.

All the materials will have trial-specific labels. Details of packaging and labelling are reported in **Appendix 14.2**.

6.1.3. Supply, Storage and Handling of Investigational Product

An appropriate number of packages will be initially sent to the site as soon as all essential documents and FDA/ethics approvals have been obtained. Additional supplies (reparixin, placebo, Infusion Bags) will be sent on demand, according to enrolment rate.

The Investigational Product must be kept at a temperature not exceeding 30°C and must not be frozen.

A temperature probe will accompany the drug on shipment. Temperature range reached during shipment will be verified on receipt, so that potential stability concerns during shipment can be investigated and appropriate action taken.

Once received at the site, the Pharmacist (or designee) will check the package for accurate delivery and acknowledge receipt; any deviations from expected package content (inconsistency, damages) should be immediately reported to Dompé (or designee) and the use of the drug suspended until authorization for its continued use has been given by Dompé (or designee).

The Investigational Product must be stored in a secure location, in a temperature controlled room. Temperature records must be available for the Unblinded-CRA (U-CRA) to review at monitoring visits; any deviations from the recommended storage conditions should be immediately reported to Dompé (or designee) and the use of the drug suspended until authorization for its continued use has been given by Dompé (or designee).

The Investigational Product will be dispensed only by the Pharmacist (or designee). The Investigator will ensure that study treatment is only administered by designated staff within the centre.

Pharmacists will be provided with the 'Instructions to the Pharmacy', a booklet detailing instructions for Investigational Product handling and preparation of the dosing solution.

6.1.4. Preparation of the Dosing Solution

The dosing solution for infusion will be prepared at the designated Pharmacy or authorized location within each centre according to local guidelines for sterile re-consitution of i.v. injectable solutions.

For each 750 mL dosing solution volume, the content of a Vial (250 mL) will be diluted with 500 mL of 0.9% sterile saline to dispense reparixin as 11.00 mg/mL solution. The dosing solution will be placed in a 1000 mL sterile empty Infusion Bag. Dosing solutions will be prepared and used within 72 hours from preparation, unless the site has more restrictive rules.

A double-tear off label will be attached to each prepared bag, reporting patient number and relevant blinded information. Infusion Bags for the same patient/administration course will be numbered in a sequential manner, starting from No. 1. Labels for the Investigation product administration during the 2nd islet infusion will be clearly identified as such.

The Pharmacist (or designee) will make the Infusion Bags available to the hospital ward at the requested time.

6.1.5. Blinding

Reparixin solution in the vial (33mg/mL) has a slightly different appearance (pale yellow colour) as compared with placebo. Also, a transient foamy layer occasionally results from the preparation of the reparixin dosing solution. However, once the foamy layer, if any, had subsided, the dosing solution of reparixin in the Infusion Bag will be indistinguishable from that of placebo.

Individual treatment codes will be provided in tamper-resistant system (either sealed envelopes or scretch cards) to the Pharmacist (or designee). They must be kept in a secure location accessible only to designated staff in order to prevent dissemination of the treatment to personnel involved in study conduct who must remain blind.

The Pharmacist (or designee) will break the treatment code only after the patient-specific "IP Preparation Order Form" is received from the Investigator. The individual code matching the patient randomization number will be opened to prepare the dosing solution matching the patient randomization number.

The Investigators can request to an independent facility (either by telephone or web based system) an individual code in the event of an emergency only, where knowledge of the blinded treatment for that patient could influence further patient care. Any potential unauthorized code break and the reason

behind it will be recorded.

6.2. DOSE, ROUTE AND SCHEDULE OF INVESTIGATIONAL PRODUCT ADMINISTRATION

Patients will receive either reparixin at a dose of 2.772 mg/kg body weight/hour for 7 days (168 hrs) or matched (flow rate/length of infusion) placebo, according to the randomization number.

The dosing solution (if reparixin, 11mg/mL) will be administered as a continuous i.v. infusion into a (high flow) central vein, by an infusion pump adequate to provide reliable infusion rates (see below), as per treatment schedule.

Infusion of the Investigational Product will start approximately 12hrs (allowed range 6-18hrs) before the anticipated time when islet infusion is started. The Investigator will identify the time to start study drug administration.

The pump rate will be adjusted to provide an infusion rate of approximately 0.25mL/kg/hour. Actual infusion rate (mL/hour), adjusted to body weight, is tabulated in **Appendix 14.3**. Figures in the appendix represent mathematical rounding of original infusion rates derived from the following formula:

$$\text{Infusion rate (mL/hour)} = \frac{\text{dose (mg/kg/hour) x body weight (kg)}}{\text{11.00 mg/mL}}$$

Such a rounding affects actual administered dose/kg/hour by less than 1%.

6.3. CRITERIA FOR SCHEDULE ADJUSTMENT/DOSE-MODIFICATION/ DISCONTINUATION OF INVESTIGATIONAL PRODUCT

6.3.1. Criteria for schedule adjustment or dose modification

No schedule adjustment and/or dose modification is foreseen, except for discontinuation of drug as detailed below.

6.3.2. Criteria for discontinuation of Investigational Product

Phase 1 studies in patients with end stage renal disease have shown that renal function has a profound effect on plasma concentrations of a major, marginally active metabolite, carboxy-reparixin (DF2243Y), which was found to accumulate over time along with the increased elimination half-life. Even if toxicological results to date suggest that DF2243Y does not raise any safety concern, limited experience in humans recommends discontinuation of the drug in case of renal impairment as it would not be possible to predict the risk associated with elevated plasma levels.

Because reparixin undergoes extensive hepatic metabolism, hepatic dysfunction should also be monitored to avoid an increase in plasma levels. An asymptomatic, self-limited transaminitis is consistently seen after islet transplantation into the liver. This is characterized by elevation in AST/ALT which peaks at about 7 days post transplant and spontaneously normalizes within 4 weeks. Bilirubin and other liver function tests remained within the normal range, suggesting no major hepatic dysfunction that would impact reparixin metabolism. [*Rafael, 2003; Barshes, 2005*].

Therefore, the Investigational Product should be immediately discontinued in case the patient develops renal or hepatic dysfunction defined as:

Renal dysfunction: the occurrence of both CLcr < 60 mL/min **and** urine output < 0.5 mL/kg/hour on two consecutive samplings. Transient alterations of serum creatinine or urine output, other than defined above, do not indicate a renal dysfunction that would cause impaired excretion of DF2243Y.

Hepatic dysfunction: the occurrence of ALT/AST > 3 x ULN **and** total bilirubin > 3mg/dL [> 51.3 μmol/L].

Renal and hepatic function will be evaluated daily from post-operative day 1 during Investigational Product infusion. Samples will be processed immediately and results made available as soon as possible to the Investigator.

Additionally, the Investigational Product should be immediately discontinued in the event of any other possibly drug related occurrences that the Investigator believes might compromise patient safety.

Lastly, the Investigational Product will be immediately discontinued in the case the patient withdraw his/her consent or has not received the transplant.

Patients who discontinue the treatment with the Investigational Product will not be withdrawn from the study by default, but will complete observations as per the protocol, unless otherwise they withdraw their consent.

If the Investigational Product therapy is prematurely discontinued the primary reason for discontinuation must be recorded in the CRF.

6.4. ACCOUNTABILITY

All supplies will be maintained under adequate security by the responsible member of the Pharmacy staff. The Investigator will ensure that study treatment is only administered by those named as sub-investigators on the FDA form 1572 and designated staff.

When the Investigational Product is received by the Pharmacist (or designee), (s)he will check for accurate delivery and acknowledge receipt by signing and dating the documentation provided by Dompé (or designee) and returning it to Dompé (or designee). A copy will be retained for the Investigator/Pharmacy file.

The dispensing of the Investigational Product will be carefully recorded on appropriate drug accountability forms provided by Dompé (or designee) and an accurate accounting will be available for verification by an U-CRA at each monitoring visit.

Drug accountability records will include:

- confirmation of receipt of the Investigational Product at the trial site,
- the inventory at the site (vials),
- details of each bag preparation,
- the use by each patient,
- disposition of unused vial(s),
- account of any Investigational Product accidentally or deliberately destroyed.

They should include dates, quantities, batch numbers, use by date information, and any unique code numbers assigned to the Investigational Product and/or patients.

Records should be maintained to adequately document that:

- the patients were provided the doses specified by the protocol/amendment(s),
- all Investigational Product provided was fully reconciled.

The U-CRA will review the drug accountability forms for consistency with drug administraiton (as per CRF) and remaining unused Investigational Product vials.

Investigational Product which has been dispensed to a patient and returned unused will not be re-dispensed to a different patient.

Unused Investigational Product must not be discarded or used for any purpose other than the present study. Any remaining test material at the end of the trial will be returned to Dompé (or designee) or disposed of, as determined by Dompé (or designee).

6.4.1. Assessment of compliance

Compliance will be assured by the person(s) within the centre in charge of Investigational Product administration.

Immediately before the use of an Infusion Bag is started, the removable label on the bag will be detached and attached to the relevant page of the CRF. Actual date and time of infusion start and end for each container will be recorded in the CRF, as well as the infusion rate(s). Temporary interruption of > 30 min duration (cumulative within each 24 hours) during drug administration should also be reported.

Compliance with the study product dosing schedule will be verified by a CRA during on-site monitoring visits, as per records in the CRF, versus accountability records.

6.5. CONCOMITANT THERAPY

Any medications required for the patient's welfare are permitted and will be given at the discretion of the Investigator, except for drugs described in paragraphs below.

Administration of all prior (4 weeks prior to enrolment) and concomitant medications (CMEDs), apart from the agents listed below, during hospital stay will be reported in the appropriate section of the CRF. Only medications used for SAE, oral hypoglycemic drugs as well as medications that should not be used (see Section 6.5.1 below) will be reported after hospital discharge.

All the details as per the CRF fields (sequential number, drug name, indication, starting dose, start/stop date, route of administration) will be recorded. Change in dose will not be tracked.

The following agents do not need to be recorded:

Saline and other hydration solutions (including additional electrolytes)
Total parenteral nutrition and enteral feeds
Homeopatic medications
Elective vitamins and minerals
Topical agents with no or negligible systemic absorption
Osmotic laxatives and locally acting antacids

6.5.1. Other treatments

Drugs required during surgery will be administered as per centre practice.

The following prophylactic anticoagulation is suggested to be used in patients enrolled in this study

- Heparin will be administered at a dose of 70 U/kg, divided equally between the islet bags, followed by 3 U/kg/hour infused i.v. for the next 4 hours. From the 5th to the 48th hour post-transplant, heparin will be titrated to achieve and maintain PTT in the range 50-60 seconds. Comparable marker other than PTT will be used as per site experience.

- Enoxaparin will be administered at a dose of 30 mg subcutabeously from day 2 to 7 post-transplant.

Prophylactic anticoagulation management (choice of medication and/or doses) might be adjusted on the basis of clinical requirements (e.g. occurrence of bleeding) and/or centre practice. Low Molecular Weight Sulfate Dextran cannot be used in this trial.

Glycemic control in the early post-transplant period will be achieved by insulin administration to target glucose levels in the range 80-180mg/dL. Patients will be tested for blood sugar by fingerstick a minimum of 4 times per day. Insulin will be titrated to maintain fasting and pre-meal blood sugars in the range 80-125 mg/dL, and 2 hour post-prandial glucoses <180 mg/dL. For patients on continuous enteral or parental feeds, insulin will be titrated to maintain glucose in the range 80-140 mg/dL.

The following medications **should not be used** in this study:

- Systemic steroids (i.e. intravenous, intramuscular, oral; intra-articular injection allowed): from 2 weeks prior to enrolment up to the end of the study; use of ≤5mg prednisone daily or equivalent dose of hydrocortisone, for physiological replacement only is allowed.
- Any investigational agents: from 4 weeks prior to enrolment up to the end of the study;
- Any anti-cytokine/chemokine medications (e.g. anti-TNFα): from 4 weeks prior to enrolment up to the end of the study;
- Any immune modulators: from 4 weeks prior to enrolment up to the end of the study;
- Low Molecular Weight Sulfate Dextran;
- Oral hypoglycemic agents and any drugs that influence insulin sensitivity.

Since exposure to ibuprofen after administration of reparixin 2.772 mg/kg/h for 48hrs is in the range of that obtained after a standard therapeutic single dose of ibuprofen (300mg), concomitant administration of NSAIDs, including cyclo-oxygenase-2 specific inhibitors, should be considered with caution during infusion with the Investigational Product, as this may increase the risk of cyclo-oxygenase-dependent adverse affects.

7. STUDY PROCEDURE AND ASSESSMENTS

A schedule for the tests and evaluations to be conducted in this study is found in the flow chart in **Appendix 14.4**. A list of acceptable assessment/procedure time windows is detailed in **Appendix 14.5**. Methodological details are reported in **Appendix 14.6**.

7.1. HOSPITAL STAY

7.1.1. Screening and randomization

Potential study patients will be identified among those referring to the investigational site and found eligible as per site standard criteria for total (or completion) pancreatectomy followed by IAT. Consented patients will undergo the following screening evaluation, within 72 hours prior to randomization.

BP, HR, body weight (kg) and height (m) will be measured.

A baseline blood sample will be taken prior to any intervention for safety laboratory tests (assay as per centre practice). These include hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, potassium, serum creatinine, blood urea nitrogen, total bilirubin, ALT, AST, INR, PTT. Albumin and pre-albumin will be also measured.

Pregnancy test (urine dipstick or blood test) will be performed, if appropriate.

Renal reserve will be assessed by CLcr (*Cockcroft-Gault; 1976*; see details in **Appendix 14.6.2**). Hepatic function will be assessed by ALT/AST and bilirubin.

Concurrent sepsis will be diagnosed by blood culture(s) and/or fever associated with other signs of systemic sepsis syndrome.

Pre-operative portal hypertension will be diagnosed as per clinical history **and** abdominal/liver imaging (e.g. presence/absence of varices, splenomegaly, Doppler waveforms and flow direction) by ultrasound techniques (e.g. duplex ultrasonography, spectral Doppler imaging and Color Doppler imaging, power Doppler imaging).

Two basal serum samples collected 6 to 24 hrs apart will be stored for centralized assay of pre-transplant chemokines/cytokines; similarly, a basal sample will be stored for centralized assay of pre-transplant miR-375 (see **Appendix 14.6.3**). All basal samples will be obtained before surgery and before Investigational Product administration is started.

Compliance with inclusion/exclusion criteria will be verified vs demographic, laboratory test results and clinical history/information available as per local standard clinical practice.

Eligible patients will be randomized (randomization number assigned) by an Investigator (or designee) before starting the infusion of the Investigational Product.

A screening form will be completed for all patients who signed the Patient Informed Consent Document, regardless of subsequent entry into the study. Patients will be identified by their age and sex; patient number if enrolled or reasons for exclusion from the study will be recorded.

7.1.2. Start of infusion with the Investigational Product

Infusion of the Investigational Product will start approximately 12hrs (6-18hrs) before the anticipated time of islet infusion. The Investigator will identify the time to start study drug infusion. Infusion may be halted during pancreatic surgery if required, provided that interruption is not longer than 6 hours.

The infusion pump will be set to provide the infusion rate corresponding to the planned dose. Infusion rate (mL/hour) corresponding to each unit increment (kg) of body weight, is reported in **Appendix 14.3**.

Immediately before the start of infusion, the removable label on the Infusion Bag No. 1 will be detached and attached to the relevant page of the CRF. Starting time of drug administration and infusion rate will be recorded in the CRF.

7.1.3. Islet isolation and transplantation

Techniques for pancreas removal will be used, as per centre practice, including requirement for concomitant splenectomy.

Islet preparation: Site will process islets by enzymatic digestion using an FDA approved collagenase and neutral protease as per site specific standards. The pancreas will be distended by intraductal infusion of collagenase/neutral protease; if duct disruption prohibits intraductal infusion, enzyme will be manually injected into the pancreatic parenchyme. Pancreatic digestion will be performed using the semi-automated method of Ricordi. Purification will be performed when necessary to reduce tissue volume, dependent on site specific standards for islet manufacturing, using density centrifugation with COBE 2991. Samples will be taken from the pancreatic preservation solution and final islet product for sterility testing by gram stain and culture.

Islet mass will be assessed by manual counts as detailed in **Appendix 14.6.1** and quantified as total IEQ and IEQ/kg body weight. The final product is suspended in a 200 mL suspension of CMRL 1066 Transplant Media or equivalent solution. The final product is supplied in one to three 200 mL infusion bags, containing a minimum dose of >10,000 IEQ total.

Islet infusion: At all sites, islets will be transplanted on the same day as the total pancreatectomy procedure.

The islet mixture will be delivered slowly by gravity drainage into the portal vein or a tributary of the portal vein. Access to the portal vein may be obtained by direct cannulization during laparotomy or by percutaneous access by fluoroscopic, ultrasonographic, or real-time CT guidance.

At minimum, portal venous pressure will be monitored (direct venous measurement) before initiation, mid-way, and after completion of islet infusion. In case the portal pressure reaches >35 cmH_2O, islet infusion will be put on hold, and restarted when portal pressure has dropped. In the rare event that portal pressure does not lower (within a 30 min interval), infusion is definitely stopped and any remaining tissue either discarded or placed in another tissue (e.g. peritoneal cavity, duodenal wall, underneath the kidney capsule, etc.).

The following isolation and operative details will be recorded in the CRF, as per documentations provided by the local Islet Processing Facility and centre information:

- Date and time of isolation (procedure completed).
- Date and time of infusion (start and end of infusion);
- IEQ/kg infused. Islet will be counted as detailed in **Appendix 14.6.1**;
- Total islet infusion volume;
- Pre-, mid-way, and post- infusion portal pressure.

7.1.4. Drug administration during hospital stay

Administration of the Investigational Product will continue during hospital stay.

Infusion Bags will be used in a sequential manner (No. 1, No. 2, No. 3 etc.) and infusion of the Investigational Product will progress non-stop up to a total of 7 day (168hrs) administration.

The removable label on each new Infusion Bag will be detached and attached to the relevant page of the CRF. Start date/time and stop date/time for each Bag will be recorded in the CRF, along with accidental interruptions of study drug infusion for > 30min (cumulative within each 24 hours).

7.1.5. Post-operative assessments

The following will be assessed during hospital stay and recorded in the CRF. Measurement will be performed as per centre practice, unless otherwise specified.

- Renal function (CLcr, urine output): Renal function will be assessed daily from post-operative day 1 up to the end of study drug infusion. Details of Clcr calculation are reported in **Appendix 14.6.2**. There is no need to have a urine catheter in place, if this is not required by standard of care. Urine volume will be measured at urination (measuring cylinder); corresponding collection time will be calculated from the previous urination. Depending from feasibility and patient compliance, ideally more than one measurement should be done over a 24-hour period to get the best estimate of urine output.

- Total bilirubin: Total bilirubin will be assessed daily from post-operative day 1 up to the end of study drug infusion as part of hepatic function evaluation.

- XDP, ALT/AST, INR/PTT, C-reactive protein (CRP): These will be measured daily from day 1 up to day 7 post-transplant.

- Chemokines/cytokines [CXCL8, CCL2 (MCP-1), CCL3, CCL4, CXCL10 (IP-10), CXCL9 (MIG), IL-6, IL-10, INF-γ, TNF-α, and IL-1β]: A blood sample will be obtained @ 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion. Samples will be handled and stored for the centralized assay, as detailed in **Appendix 14.6.3**.

- miR-375: A blood sample will be obtained @ 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion. Samples will be handled and stored for the centralized assay, as detailed in **Appendix 14.6.3**.

- Pharmacokinetics [plasma levels of reparixin (total and unbound) and relevant metabolites (DF2243Y and ibuprofen)]: A blood sample will be obtained in all patients randomized and treated with the Investigational product on post-operative day 1, 3 and 5, ideally in the morning. In a subset of at least 20-24 patients, treated at 2 selected sites, a blood sample will be also obtained just prior to, and then at 1, 3, 5, 6, 8, and 12 hrs after termination of Investigational Product administration. Samples will be handled and stored for centralized assay, as detailed in **Appendix 14.6.3.**

- Safety laboratory tests: Laboratory tests will be performed before hospital discharge.

- Vital signs, i.e. blood pressure (BP) and heart rate (HR) will be measured before hospital discharge.

During hospital stay, all AEs (see details in Sections 8.2.) and CMEDs (see details in Sections 6.5.) will be recorded.

For all measurements above, the actual date and time of assessment, including date of sampling, will be recorded in the CRF.

Apart from compliance with protocol procedures up to day 7 post-transplant, hospital discharge will be primarily based on criteria related to total pancreatectomy (the major surgical procedure in total pancreatectomy followed by IAT) and will comply with each site's hospital standards. However, at all sites, at least the following criteria will be complied with: "A patient will be discharged if he/she at least is ambulating, has adequate nutrition (oral, enteral, or Total Parenteral Nutrition (TPN), is not requiring any intravenous medications which require inpatient monitoring, pain is adequately treated, and he/she has a stable plan for glycemic control (management of blood glucose testing and insulin administration at home)".

7.2. FOLLOW-UP PROCEDURES AND ASSESSMENTS

Since hospital discharge, patients will self monitor glucose levels and insulin intake according to standard clinical instructions at the site. Insulin will be administered to target fasting and pre-meal glucose levels in the range 80-125 mg/dL, and 2 hour post-prandial glucoses <180 mg/dL. For patients on continuous enteral or parental feeds, insulin will be titrated to maintain glucose in the range 80-140 mg/dL.

The Investigator will identify if- and the date when- it is appropriate for the patient to stop insulin intake, according to standard of centre practice. Regardless the patient is on or off insulin, he/she will report on a Diary Card (see **Appendix 14.7**) his/her self monitored glucose levels for 14 consecutive days prior to the follow-up visits on day 75±14 and 365±14 after the transplant: glucose levels will be measured at least two times a day: 1) after an overnight fast (or anyway before breakfast if the patient is still on a tube feed and TPN); 2) within 2 hours post-prandial. Also, patients still on insulin will report on the Diary Card their daily insulin intake.

Patients will attend the centre for study assessments on 2 follow-up visits scheduled on day 75±14 and 365±14 after the transplant.

At each **visit**, the following will be evaluated/measured in all patients as per centre practice, unless otherwise specified. Measurements, including the actual date and time of assessment, or the date of sampling, will be recorded in the CRF.

- Patient weight.
- Retrospective self-measured glucose and insulin requirement data in the 2 weeks prior to the visit will be reviewed and average (previous week) insulin requirement at each time point as well as insulin-independence / insulin-dependence status will be assessed.
- Retrospective episodes of severe hypoglycaemia in the interval will be assessed as per centre practice. Events of documented hypoglycemia in the interval will be obtained from review of the patient's home glucometer download from between visits and/or patient glucose logs.
- Retrospective severity of steatorrhea in the 4 weeks prior to the visit will be evaluated.
- A blood sample will be taken prior to the meal administration to measure HbA1c, albumin and pre-albumin. ALT/AST will be also measured on day 75±5.
- A MMTT test will be performed as detailed in **Appendix 14.6.4**. Blood samples will be drawn @ 0 (2 basal samples in the range between -20 to 0 prior to the meal) and 15, 30, 60, 90, 120, 180, 240 min after the meal for glucose, C-peptide and insulin measurement.
- β-score will be calculated according to *Ryan (2005)* (see **Appendix 14.6.5** for details).

C-peptide and HbA1c will be measured by a centralized laboratory with CLIA certification and National Glycohemoglobin Standardization Program (NGSP) certified assay. Details of handling and storage of samples as well as data handling are reported in **Appendix 14.6.3**.

Any SAEs that occurred after hospital discharge will be recorded at the visits, as well as CMEDs, as appropriate.

Patients should be strongly motivated to attend the centre for the planned follow-up visits. However, if a patient cannot refer to the site for protocol assessments, the Investigator will try to obtain any relevant information from the patients, including documents/lab results available from local medical care.

7.3. EARLY PATIENT WITHDRAWAL

7.3.1. Criteria for withdrawal from the study

Patients will be informed that they have the right at any time to withdraw from further participation in the study (*withdrawal of consent*), without prejudice to their medical care, and without being obliged to state their reasons. However, during the consent process, patients will be also educated about the scientific relevance of their continued participation as well as the deleterious effect that missing data will have on trial integrity.

If a patient fails to return to the centre for a scheduled visit, all reasonable attempts should be made to contact the patient to ensure that the reason for not returning is not a SAE. Likewise if a patient declares his/her wish to discontinue from the study e.g. for personal reasons, an attempt should be made to establish that the true reason is not a SAE (bearing in mind the patient is not obliged to state his/her reasons). The term *withdrawal of consent* should be used only when the patient no longer wishes to participate in the trial and no longer authorizes the Investigators to make efforts to continue to obtain his/her outcome data. Ideally, if patients withdraw their consent, it should be done in writing.

Patients who have discontinued the study treatment should be anyway followed to ensure that primary and secondary outcome measures are assessed, unless they formally have withdrawn consent.

It is important that any randomized patient remains in the study and is followed for both efficacy and safety outcomes, regardless he/she has completed or discontinued the study treatment. Investigators will be trained about the importance of patient retention and full data capture. Also, all reasonable attempts should be made by the Investigators to emphasize continued patient's participation for the full duration of the trial.

Any withdrawals must be fully documented in the CRF.

7.3.2. Replacement policy

No patient who has been randomized and withdraws from the study for any reason will be replaced.

8. ADVERSE EVENTS

8.1. DEFINITIONS

8.1.1. Definition of an Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product [*Clinical safety data management: Definitions and Standards for Expedited Reporting*].

8.1.2. Definition of a Serious Adverse Event

A Serious Adverse Event (SAE) is defined as any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening (i.e. the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is an important medical event that based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

All AEs should be followed-up to determine the outcome of the reaction. The Investigator should follow up the event until resolution or stabilization of the condition. It is the Investigator's responsibility to assure that the subjects experiencing an AE receive definite treatment for any AE, if required.

8.2. EMERGENCY PROCEDURES

The treatment allocation for each patient will be provided in individual tamper-resistant decoding systems (sealed envelopes or scratch cards) to the Dompé Pharmacovigilance department.

The Investigator can request treatment allocation for a patient to an independent facility (either by telephone or web based system) in case of emergency where knowledge of the double-blind treatment may influence the further care of the patient.

If a code is opened for any reason, the reason behind it will be recorded .

8.3. RECORDING

AE data should be obtained through observation of the patient, from any information volunteered by the patient, or through patient questioning. Specifically, patients will be asked about any episode of documented hypoglycemia and ketoacidosis-related events occurring from the transplant day up to day 75±14 post-transplant. Any episodes will be reported as an AE or SAE, as appropriate. In addition, the Investigator will capture and report episodes of non-documented hypoglycemia throughout study participation.

All AEs (serious and non-serious) encountered during the clinical study will be recorded in the appropriate section of the CRF as per the recording period defined in paragraph 8.3.1. It is important that this includes the duration of the AE (onset/resolution dates), the relationship to the drug, the severity, the outcome and relevant concomitant treatments dispensed (or other action taken) (see Sections 8.3.2. and 8.3.3. below).

8.3.1. AE recording period

All AEs (serious and non-serious) which occur during each hospital stay will be recorded in the CRF. In addition, SAEs and relevant untoward events that occur subsequent to each hospital discharge will be recorded in the CRF.

8.3.2. Relationship of AEs to the Investigational Product

The Investigator will assess the possible relationship between the AE and the investigational medication, according to the criteria in the **Table** below:

Relationship of the Adverse Event to the Investigational Product

None (Intercurrent Event)	An event that is not and cannot be related to the Investigational Product, e.g. a surgical intervention for nevus removal 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 more 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

An **Adverse Drug Reaction (ADR)** is defined as an adverse experience which is a reasonably likely to have been caused by the drug. Any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR.

8.3.3. Severity of AEs

The Investigator will grade the severity of any AE using the definitions in the **Table** below. For each episode, the highest severity grade attained should be reported.

Severity of the Adverse Event

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

8.4. SERIOUS ADVERSE EVENT REPORTING

8.4.1. Reporting Procedure for Investigators to Dompé (or designee)

The Investigator must report all SAEs, regardless of presumed causal relationship, to Dompé (or designee) by fax or e-mail within 24 hours of learning of the event. Contact details for SAE reporting are provided in the section "Contact Information".

Information on SAEs will be recorded on a specific Non-Carbon Repeat SAE form. Both electronic and blank paper copies will be included in the Investigator's Site File. Follow-up reports (as many as required) should be completed and faxed/e-mailed following the same procedure above.

Whenever more than one SAE is observed, the Investigator should identify which is the primary adverse event, i.e. the most relevant one. If other events are listed in the same report, the Investigator, along with their relatedness to the Investigational Product, should identify which adverse events are serious and which are non-serious. In any case, the Investigator is requested to record his/her opinion about the relatedness of the observed event(s) with the investigational medication.

8.4.2. Reporting Procedure for Investigators to IRB

In addition to reporting the SAE to Dompé, the Investigator must also comply with the requirements related to the reporting of SAEs to the IRB which approved the study.

The requirements of IRB varies from one state and indeed one IRB to another; however, as a minimum requirement, the Investigators must promptly report all serious unexpected* ADRs, life-threatening problems or deaths to their IRB.

Dompé (or designee) will inform Investigators of all serious unexpected ADRs which are reported to Dompé from other Investigators. These SAEs should also be reported promptly to the IRB in compliance with the local regulations.

Copies of all correspondence relating to reporting of any SAEs to the IRB should be maintained in the Investigator's Files.

* *For the purpose of this study, all ADRs are assumed to be unexpected.*

8.4.3. Reporting Procedures to the FDA

During the course of the clinical trial, Dompé (or designee) shall inform the FDA of any serious unexpected ADR* as soon as possible and in no event later than:

(*a*) seven calendar days after becoming aware of the information if the event is fatal or life threatening; and

(*b*) fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

Dompé (or designee) shall, within 8 days after having informed the FDA under paragraph (*a*), submit a complete report in respect of that information that includes an assessment of the importance and implication of any findings made.

Furthermore, Dompé (or designee) shall follow up safety information and shall report final findings in a written safety report as soon as the relevant information is available.

If the results of an investigation show that an adverse drug reaction not initially determined to be reportable is reclassified as reportable, Dompé (or designee) shall report such reaction in a written safety report as soon as possible, but in no event later than 7/15 calendar days after the determination is made.

Dompé (or designee) will inform the FDA of all serious ADRs which are reported from other Investigators.

8.5. ADVERSE EVENT EXEMPTION

The following events will neither require recording nor reporting, as they are considered routinely associated to the pancreatectomy or to the transplant procedures:

- Delayed Gastric Emptying requiring insertion of a central line for Total Parenteral Nutrition or insertion of a nasojejunal tube for Enteral Nutrition;
- Increased ALT/AST levels up to 5 x ULN, inclusive, within 2 weeks post transplant;
- Abnormal PTT and INR values during prophylactic anticoagulation.

9. STATISTICAL ISSUES

9.1. SAMPLE SIZE

The primary efficacy endpoint of the clinical trial will be the proportion of insulin-independent patients following IAT over the time frame: day 365±14 after the transplant.

In the following considerations, statistical significance for this endpoint will be achieved if the Pearson Chi-Square Statistic yields a two sided p-value less than 0.05. The corresponding false positive error rate is (one-sided) 0.025. In addition, 95% confidence interval for the difference of success (failure) rates between the reparixin and placebo groups will be provided, based on the normal distribution approximation to the standardized difference in estimated success rates.

Based on published data from a retrospective series (*Sutherland, 2012*), it appears that the likelihood of achieving insulin independence at 12 month follow-up in the placebo control arm depends upon the number of IEQ/kg, with patients categorized in three groups:

- < 2500 IEQ/kg: 13% insulin independence
- 2500-5000 IEQ/kg: 23% insulin independence
- > 5000 IEQ/kg: 55% insulin independence

In ther overall series, the yields were <2500 IEQ/kg in 36%, 2500-5000 IEQ/kg in 39% and > 5000 IEQ/kg in 24% of patients. In the more recent series of 217 patients receiving IAT from 2006 to 2011, corresponding yields were <2500 IEQ/kg in 34%, 2500-5000 IEQ/kg in 42% and > 5000 IEQ/kg in 24%:

We initially focus on the cohort that is in the range 2500-5000 IEQ/kg, since these participants will be the largest fraction of enrollees. It is anticipated that the percentage of such patients achieving insulin independence will be increased from 23% in the placebo control arm to 55% in the reparixin arm. Using standard statistical formulas based on the approximation of the binomial distribution by the normal distribution, the sample size of 44 patients per arm provides 90% power to detect a 23% vs. 55% true difference when using a Pearson Chi-Square Statistic having the traditional (one-sided) 2.5% false positive error rate. Even accounting for the discreteness, the power should be in the 87%-90% range. Furthermore, with 44 patients per arm, statistical significance (i.e., one-sided p=0.025) will be obtained if the estimated difference is 22.7% (10/44) vs. 43.2% (19/44), which is an estimated 20.5% increase in patients achieving insulin independence. Hence, with 44 patients per arm, statistical significance is achieved with an estimated effect size that is clinically meaningful.

Even though the largest percentage of patients will be in the cohort receiving IEQ/kg in the range 2500-5000 IEQ/kg, the trial will also enroll participants in two additional strata: <2500 IEQ/kg and >5000 IEQ/kg. As noted above, we are presuming the middle strata would have an increase from 23% to 55% in percentage of patients achieving insulin independence. This is an odds ratio higher than 4. If we have an odds ratio of approximately 4.3 to 4.5 in the other 2 strata and if approximately ½ of the patients would be from the 2500-5000 IEQ/kg stratum, then only a modest increase of about 12 additional patients beyond the 88 patient sample size would be needed to maintain the statistical power of the trial. An increase in success rate from 13% to 40% in the <2500 IEQ/kg stratum would be an odd ration of 4.4 and an increase in success rate from 55% to 84% in the >5000 IEQ/kg stratum would be an odd ration of 4.3.

Based on these considerations, it is planned to enroll 100 patients in this trial who receive IAT, with the expectation that there will be approximately 5 others who do not receive IAT. Under such a plan, the trial would not be overpowered even if all participants were in the 2500-5000 IEQ/kg stratum since statistical significance (i.e., one-sided p=0.025) would be obtained with 52 patients per arm if the

estimated difference is 23.1% (12/52) vs. 42.3% (22/52), which is an estimated clinically significant 19.2% increase in percentage of patients achieving insulin independence.

The sample size of 100 to 105 patients also provides high statistical power for the key secondary endpoint, C-peptide AUC (MMTT) normalized by IEQ/kg. Based on preliminary data reported from the ongoing pilot trial (see table on page 14), month 1 post transplant means and standard deviations for this endpoint are 1.92±1.62 in the reparixin group and 0.44±0.04 in the control group. With 52 patients in each treatment group, the trial will have greater than 95% power to detect a difference in means of at least 1.48 (the difference between a treated mean of 1.92 and a control mean of 0.44) assuming that the common standard deviation is 1.37 and using an 0.0025 two-sided significance level.

9.2. RANDOMIZATION

Patient will be randomized in a 1:1 fashion to either reparixin or placebo.

The randomization list will be generated with a computer procedure by the method of random permuted blocks in which treatment (in blocks of 4) will be balanced within centres. A master randomization list will be generated, randomizing an excess of patients (a maximum of 40 for each site – additional 8 patients for the site of the Primary Investigator) to allow competitive recruitment within each centre.

The randomization list will be prepared by and independent statistician and provided to Dompé in a sealed envelope to prevent unblinding.

Similarly, individual treatment codes will be provided as a tamper-resistant system (either a sealed envelope or a scratch card). Such system will definite allow to recognize if the code has been broken and guarantees detection of any code misuse. Individual treatment codes will be provided to:

- the Pharmacist (or designee) within each participating site for Investigational Product preparation;
- the Dompé Pharmacovigilance department for safety procedures.

The Investigators can request to an independent facility (either by telephone or web based system) an individual code in the event of an emergency only, where knowledge of the blinded treatment for that patient could influence further patient care. Any potential unauthorized code break and the reason behind it will be recorded.

The randomization codes will also be accessible to an Independent Statistician (liaison between the CRO database and DMC Biostatistician) who will generate the reports for the Data Monitoring Committee (DMC) evaluation (see Section 12.5).

The randomization code will be broken at study completion, i.e. when the last patient has completed his/her last follow-up visit (planned 365 days after islet infusion), and once the database has been locked.

9.3. ANALYSIS POPULATION

The Intent to Treat (ITT) population will consist of all patients who are randomized and receive the Investigational Product (either reparixin or placebo); it will be based on the treatment randomized, regardless of the treatment actually received. Patients will be in the ITT analysis whether or not they receive IAT, because exclusions cannot be made for events occurring after randomization that could be influenced by the randomized assignment. The primary and secondary efficacy analyses will be presented primarily for the ITT Population.

The Safety population will consist of all randomized patients and will be based on the treatment actually received. The Safety population will be used to present the demographic and baseline data, and all safety data.

9.4. STATISTICAL METHODOLOGY

All patient data collected on the CRF and on the Diary will be listed by patient, treatment group and centre.

Appropriate descriptive statistics will be produced, according to the variable. For continuous variables, the data will be presented according to a clinically relevant discretization. For categorical data, frequencies and percentages will be presented. If appropriate, confidence intervals around the mean or the proportions will be presented.

All the AUC analyses will be based on actual rather than scheduled timings and will be calculated using the trapezoidal rule. If the actual time is not recorded, the scheduled time will be used instead. For ease of interpretation, the AUC value obtained will be divided by the total time the scale is assessed for reporting purposes.

The data will be presented in the Clinical Study Report. A Statistical Analysis Plan will be issued describing details of all the statistical methods and analyses to be applied to trial results, including alpha spending and testing for multiple endpoints. Any deviations from the original statistical plan will be described in the Clinical Study Report.

All reasonable efforts will be made to prevent missing data. Thoughtful methods for imputation of missing data will be presented in the Statistical Analysis Plan for the clinical trial

9.4.1. Demographic and baseline characteristics

Demographic and baseline characteristics will be summarized for all patients in the Safety population, by treatment group.

9.4.2. Primary and Secondary Endpoint analysis

For the primary efficacy endpoint, the proportion of insulin-independent patients over the time frame of day 365±14 after the transplant, the differences in proportions will be assessed using the Pearson Chi-square statistic, stratified by the three groups at baseline: < 2500 IEQ/kg vs. 2500-5000 IEQ/kg vs. > 5000 IEQ/kg. The significance level used for statistical testing will be 2.5% and one-sided test will be used. The 95% confidence interval for treatment proportions and for the difference in treatment proportion between reparixin and placebo will be produced. The primary analysis will be performed using both ITT population with PP analysis serving as a sensitivity analysis.

For secondary endpoints that will be tested both at 75±14 and 365±14 days after the transplant, only the day 365±14 time point will be tested for superiority in the fixed sequence testing procedure.

The C-peptide AUC after the MMTT normalized by IEQ/kg will be analyzed at the two time points by a repeated measurements model using PROC MIXED within SAS®, including terms for treatment, time point and center. The treatment effect within each time point will be compared using a two-sided test at the 5% level. The estimated treatment difference between reparixin and placebo at each time point will be presented together with the corresponding 95% confidence interval. The confidence interval will be generated using α=0.05.

The mean in average daily insulin requirements at the two time points will be analyzed using a repeated measurements model using PROC MIXED within SAS®. The model will include fixed effect

terms for center, time point and treatment. Time point will be specified as a repeated measurement. The treatment effect within each time point will be compared using a two sided test at the 5% level.

The proportion of patients with an HbA1c $\leq$6.5% will be analyzed at day 365$\pm$14. Additionally, the proportion of patients with an HbA1c $\leq$6.5% at day 365$\pm$14 will be analyzed again, but patients are included in this second analysis if they are also free of severe hypoglycemic events from day 75$\pm$14 to day 365$\pm$14 inclusive. Treatment effect will be analyzed using Pearson Chi-square.

The effect of treatment on the rate of recurrent episodes of severe hypoglycaemia will be evaluated using an Andersen-Gill analysis with robust sandwich-type variance estimate.

The other secondary efficacy endpoints will be analysed using appropriate parametric and non-parametric tests and appropriate 95% CI will be presented.

9.4.3. Safety analysis

Safety variables will be presented for the Safety population, by treatment group.

All AEs will be coded using the most up-to-date version at the time of database lock of the MedDRA and will be presented by primary system organ class and preferred term. AEs will be presented in terms of the incidence, severity and relationship to the study drug, overall and by body system and preferred term. SAEs will be presented in the same way.

Results for laboratory test at screening and hospital discharge will be assessed as being within the normal range or outside the normal range and clinically-significant outside results. The following parameters will be also presented at different time points: AST/ALT, INR/PTT, fibrin degradation products (XDPs), C-reactive protein (CRP) will be presented for all daily from day 1 up to day 7 after transplant and on day 75$\pm$5 after transplant for AST/ALT. Serum level of albumin and pre-albumin will be summarized as absolute values and change from pre-transplant value at day 75$\pm$14 and 365$\pm$14 after transplant.

Vital signs at each time point and the change in vital signs from pre-transplant value, and weight loss will be presented using descriptive statistics.

Steatorrhea and malnutrition risk will be graded as described in 4.2.3. Analysis will be performed separately at day 75$\pm$14 and 365$\pm$14 after transplant. 2x4 contingency table will be performed at each time point. 95% confidence intervals of proportion will be also done.

Analysis of documented hypoglycemia episodes and diabetic ketoacidosis-related events, recorded as cumulative number of episodes per patient, will be performed from day 75$\pm$14 to 365$\pm$14 after islet infusion. Mean, median, SD, min, max will be calculated for the interval

9.4.4. Analysis of exploratory endpoints

Chemokines/cytokines will be presented using appropriate descriptive statistics, by treatment group.

The relationship between inflammatory chemokines/cytokines response and exposure to reparixin will be explored.

Serum level of miR-375 will be presented using appropriate descriptive statistics, by treatment group.

The comparison will be made between the reparixin and the placebo control arms regarding the rates of patients who are randomized but do not receive IAT.

9.4.5. Interim analysis

Safety and efficacy data will be reviewed on an ongoing basis by a DMC. Full details of the activities and responsibilities of the DMC are provided in the study DMC Charter.

Primary endpoint data will not be available until each patient reaches one year post randomization. Hence, it is expected that the majority of enrolment and treatment will be completed before meaningful evidence is available regarding treatment effect on the primary endpoint. Thus, traditional group sequential methods would have more limited utility in this setting.

The DMC will give careful consideration to the appropriateness of trial continuation if there is emerging evidence that Reparixin is harmful. One component of this assessment will be the rate of unexpected deaths. Based on historical experiences from the published Minnesota series for 1977-2011 that includes 356 adults, 217 underwent pancreatectomy and IAT from 2006-2011. There was 1 unexpected death within 12 months of transplant. This translates to an estimated 0.5% rate.

The DMC will consider the following guidelines regarding early termination based on the occurrence of unexpected deaths. Early termination will be considered if:

- there are at least 2 unexpected deaths in the first 33 reparixin patients (an outcome with 1.2% chance if the true event rate is 0.5%);
- there are at least 3 unexpected deaths in reparixin patients at any time in the trial (an outcome with 0.2% chance in 50 reparixin patients if the true event rate is 0.5%).

In making any recommendations about termination, the totality of data will be considered, including the number of unexpected deaths in the control group, and the available evidence about efficacy and the overall safety profile.

The DMC will also give particular attention to clinically significant coagulation abnormalities, including but not limited to intra-abdominal or gastrointestinal hemorrhage. Early termination would be considered if the rate of clinically significant post-surgical complications that require reoperation reliably exceeds the rate expected currently in standard practice settings. Insights about that rate in standard practice settings will be provided by a literature review, and will include the publication for the Minnesota series, where intra-abdominal as well as gastrointestinal hemorrhage have been reported to be the most common reasons for reoperation (*Sutherland, 2012*).

The DMC will also consider early termination if the quality of conduct of the trial is such that the trial will not be able to provide a timely and reliable answer to the questions it was designed to address.

9.4.6. Missing data

All reasonable efforts will be made to reduce the rate of missing data, since any method used for imputation for missing observations would be based on untestable assumptions that likely would be invalid.

Investigators will be trained about the importance of patient retention and full data capture. Also, any reasonable attempts should be made by the Investigators to emphasize continued patient's participation for the full duration of the trial (see details in Section 7.3.1 and 10.2). However, in order to minimize missing data, if a patient cannot refer to the site for a planned follow-up visit, the Investigator will try to obtain any relevant information from the patients, including documents/laboratory results available from local medical care.

Subjects who die before the primary endpoint assessment at 12 months will be treated as failures in the efficacy analysis. Additionally, for the primary analysis, patients who are lost to follow-up by 12

months will be considered as failures, with the recognition that the procedures described in this section have been developed to ensure the number of patients who are lost to follow-up will be very small.

9.4.7. Methods for multiplicity correction in analyses

If the primary analysis of the primary endpoint leads to rejection of the null hypothesis, the null hypotheses for the secondary endpoints will be tested in a conditional sequential manner. A null hypothesiswill be rejected if and only if the primary analysis of that endpoint and all primary analyses of preceding primary and secondary endpoints result in a rejection of the respective null hypotheses. This procedure protects the family-wise false positive error rate at the overall one-sided 0.025 level.

It is noted that for secondary endpoints that will be tested both at 75±14 and 365±14 days after the transplant, only the day 365±14 time point will be tested for superiority in the fixed sequence testing procedure.

10. ETHICAL CONSIDERATIONS

10.1. INSTITUTE REVIEW BOARD (IRB)

It is the responsibility of the Investigator to obtain approval of the trial protocol/amendments from the appropriate IRB.

Prior to the initiation of the study, the followings will be submitted to the IRB for approval:

- the study protocol,
- the Patient Informed Consent Document,
- the current version of the Investigator's Brochure,
- Investigator's current curriculum vitae,
- any other requested document(s).

A copy of the IRB approval will be sent to Dompé along with **all** other correspondence with the IRB, including the submission documents. The Investigator should file copies of all correspondence with the IRB in the Investigator Site File.

The study will not be started until full written approval has been obtained from the appropriate IRB. The letter of approval should be dated, and should specify the type (e.g. protocol number) and the date of the documents which were reviewed and approved.

The Investigator will submit any future amendment to the protocol to the IRB which granted the original approval. Any amendment will be implemented only when full approval has been obtained from the appropriate IRB, except for those amendments which involve only logistical or administrative aspects of the study.

The Investigator will send to the IRB any updated Investigator's Brochure received from Dompé (or designee).

The Investigator will submit required progress reports to the IRB which approved the protocol at least annually, as well as report any serious ADRs, life-threatening problems or deaths.

The Investigator will also inform the IRB of reports of serious ADRs occurred at other sites participating to this clinical trial and/or in other clinical studies conducted with reparixin.

The Investigator must inform the IRB of the termination of the study.

10.2. INFORMED CONSENT

No study-related procedures (including non-invasive and diagnostic procedures) will be undertaken prior to completion of the consenting process.

Each potentially eligible patient will be informed of the study's objectives and overall requirements. The Investigator will explain the study fully to him/her using the Patient Informed Consent Document (PICD). Although patients will be informed that they can withdraw consent at any time, the Investigator will also emphasize that missing data diminish the scientific value of all patients' contributions. If the patient is willing to participate in the study, (s)he will be requested to give written informed consent after being given sufficient time to consider his/her participation and the opportunity to ask for further details.

The PICD will be signed and personally dated by **both** the patient and the Investigator. Although nursing staff may be involved in describing the trial to a patient, the Principal Investigator (or a Sub-Investigator) must participate in discussions with the patient **and sign** and personally date the PICD.

A copy of the signed form will be provided to the patient, and the original signed PICD will be retained and filed in the Investigator Site File. Patient consent will be documented in the hospital records.

Individual (i.e. site specific) PICD will be based on a master document provided by Dompé and must be approved by Dompé prior to submission to the IRB. Any changes requested by the IRB must be approved by Dompé prior to the documents being used. Translation into a language different from English will be provided, if required.

10.3. CONFIDENTIALITY

All information obtained during the conduct of the study will be regarded as confidential. An agreement for disclosure will be obtained in writing by the patient and will be included in the PICD. Patient's data collected during the study will be handled in accordance with applicable data protection laws and regulations. The patient's privacy associated with the use and disclosure of the patient's protected health information will be safeguarded under applicable country laws (e.g. HIPAA).

On the CRFs or Diary Cards, patients will be identified ONLY by the assigned patient number. If patient names are included on copies of documents submitted to Dompé (or to the CRO appointed by Dompé), the names will be obliterated or masked and the assigned patient number added to the document.

The Investigator should keep a separate log (Patient Master List) of patient's codes, names and addresses.

10.4. COMPENSATION FOR MEDICINE-INDUCED INJURY AND INDEMNIFICATION

Before the trial formally starts, Dompé will take out a study-specific insurance contract covering the amount requested by the respective national laws for patients/Investigators/Institutions participating in the clinical trial.

In case of questions about medical care, cost for medical care or insurance, patients can talk to their Investigator. Contact details will be given in the PICD.

Insurance and any updates will be provided to the Investigator before trial commencement for filing into the Investigator Site File.

11. DATA HANDLING AND RECORD KEEPING

11.1. CASE REPORT FORMS COMPLETION

CRFs will be supplied by the CRO appointed by Dompé. CRFs are the sole property of Dompé and should not be made available in any form to third parties, except for authorized Dompé'designee or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

A CRF is required and should be completed for each included patient. The Investigator will be responsible for the accuracy of the data entered in the CRFs. All entries must be written in ***ENGLISH*** in black ink. Source documents should be available to support all the data recorded in the CRF.

The CRF must be available for review/collection to designated Dompé's representatives at each scheduled monitoring visit.

11.2. DIARY CARD

Diary Cards will be supplied by the CRO appointed by Dompé. They are the sole property of Dompé and should not be made available in any form to third parties, except for authorized Dompé'designee or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

A sample Diary Cards is provided in **Appendix 14.7**. Translation into a language different from English, will be provided, if required.

Diary Cards are required and should be completed for each patient. It is responsibility of the Investigator to explain to each patient how to enter the data in the Diary Card.

11.3. DATA MANAGEMENT

Data management of the CRFs and Diary Cards will be performed by the CRO appointed by Dompé.

The CRF and Diary Card pages for all patients will be data-entered (double data entry) into the study database, and the data will be verified for missing data, inconsistencies, and for any necessary medical clarifications. Queries arising from these checks will be sent to the Investigator for response and signature.

Once all data queries have been resolved, the study will be declared to be "clean", and the study data will be locked ready for analysis.

After the database lock has been achieved, the Investigator may archive the copies of the CRFs and Diary Card retained at the centre. The original CRF and Diary Card collected by the CRO appointed by Dompé will be transferred to Dompé for archiving.

11.4. DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE STUDY

In addition to the documents mentioned in Sections 10.1 and 12.1, the following will be required from the Investigator prior to the initiation visit:

- Current, signed and dated Curriculum Vitae, GCP qualification, and Financial Disclosure Statement of Principal Investigator and any Sub-Investigators. Updates should be provided at lest every two years.

- Normal ranges of all laboratory tests to be performed at the study site and a recent certification or accreditation of established quality control (or other documentation of established quality control or external quality assessment or other validation). Updates should be provided as soon as any reference value has changed.
- A signed page of the final protocol and any amendments.
- A signed original of the study Financial Agreement/Clinical Study Agreement with Dompé, including Pharmacy, laboratory etc (i.e. all study specific costs).
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).
- Form 1572 and financial disclosure form 3455 from all the persons listed on the 1572.

11.5. ESSENTIAL DOCUMENT RETENTION

The Investigator will retain copies of all the essential documents (as defined by ICH-GCP) until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the Investigational Product. These documents should be retained for a longer period however if required by the applicable regulatory requirements. The Investigator should take measures to prevent accidental or premature destruction of these documents.

The essential documents include at least: the signed protocol, copies of the completed CRFs, and Diary Cards, signed Patient Informed Consent Forms from all patients who consented, hospital records and other source documents, and all other documentation included in the Investigator Site File and Pharmacy/Dispensing File.

The Investigator will inform Dompé of the storage location of these essential documents and must contact Dompé before disposing of any. If the Investigator wishes to assign the files to someone else or to remove them to another location, he/she should consult with Dompé about this change.

Dompé will inform the Investigator in writing when these documents no longer need to be retained.

12. STUDY MANAGEMENT

The study will be performed in accordance with the protocol, the Declaration of Helsinki (59th WMA General Assembly, Seoul, October 2008) and ICH Harmonised Tripartite Guideline for Good Clinical Practice (*ICH-GCP*) and any local regulations.

12.1. REGULATORY BODY APPROVAL

The study will not be started until review from FDA has been positively completed.

12.2. STAFF INFORMATION & RESPONSIBILITIES

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

The Investigator will provide a list of delegated responsibility to the CRO appointed by Dompé detailing the various study tasks to be performed by each member of his/her study staff. Each staff member should sign in agreement to their performing each of the tasks delegated to them on the list.

12.3. MONITORING

Monitoring will be carried out by CRAs of the CRO appointed by Dompé.

Prior to study start, the Investigator will be informed of the anticipated frequency of the monitoring visits. (S)He will also receive a notification prior to each monitoring visit during the course of the study. It is expected that the Investigator and/or his/her sub-Investigator(s) and other appropriate staff will be available on the day of the visit to discuss study conduct and to cooperate with the monitor to ensure that any problems detected during the course of these monitoring visits are resolved.

The purpose of the monitoring visit is to verify that the rights and the wellbeing of the patient are protected, that the reported data are accurate, complete and verifiable from source documents and that the conduct of the trial complies with the currently approved protocol and any amendments, with ICH GCP, and with regulatory requirements.

Monitoring at the Pharmacy will be carried out by the U-CRA in order to maintain study blind with all other CRO/site staff involved.

12.3.1. Access to records

The Investigator will allow designated Dompé representatives and regulatory bodies to have direct access to the source documents to verify the data reported in the CRFs. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. Source documents should be available to support all the data recorded in the CRF. Location of source data, including those for which the CRF might be accepted as being the sole source document, will be specified and listed at the centre Initiation Visit.

12.4. AUDIT AND INSPECTION

Audit activities will be performed by the Quality Assurance of the CRO appointed by Dompé, except for audit to Protocol/Amendments, Patient Informed Consent Document (template) and CRF that will be done by the Dompé Quality Assurance Unit.

On one or more occasions the study site may be audited by the CRO appointed by Dompé. The Investigator will be informed in advance of such a visit.

Additionally the study site may be inspected by a regulatory agency on one or more occasions.

12.5. DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be established and will be responsible for safeguarding the interests of trial participants, and for enhancing the integrity and credibility of the trial. The DMC will assess the safety and efficacy of the interventions during the trial, and will monitor the overall conduct of the clinical trial. The DMC will provide recommendations to Dompé about stopping or continuing the trial. To contribute to enhancing the integrity of the trial, the DMC may also formulate recommendations to Dompé relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol-specified regimens and retention of participants, and the procedures for data management and quality control.

The DMC will operate independently of Dompé, and its members will not have connections to Dompé with the exception of the compensation to DMC members related to their activities.

The DMC will comprise three members. They will be a multidisciplinary group that will include:

- Two surgeons/physicians with extensive experience in islet transplantation and critical care medicine (e.g. a. Medical Director of a Transplant Program);
- A Biostatistician with substantial experience in the DMC process.

The DMC:

- Will review unblinded data. To this purpose, an Independent Statistician will liaise with the CRO statistician and will have access to those components of the database necessary to generate the reports to the DMC.

- Will be responsible for the ongoing (at least every 4 months) review of safety data throughout the trial. Primary among the safety data that will be reviewed are Serious AEs. In particular, the DMC will monitor the number of deaths for both arms of the study to assess whether mortality is consistent with historical data. The DMC also will give attention to post-surgical reoperation and clinically significant coagulation abnormalities (intra-abdominal as well as gastrointestinal hemorrhage have been reported to be the most common reasons for reoperation).

- Will review efficacy data in an ongoing manner to enable the assessment of the acceptability of safety in the context of emerging evidence about efficacy, i.e. measures of metabolic control and graft function.

- Will be advisory to Dompé and make recommendations to Dompé regarding the continuation of the trial and potential modifications to the design and conduct of the trial. These recommendations will be made in a manner to maintain confidentiality of emerging information about efficacy and safety, unless access to certain data is needed to enable Dompé to make decisions about the DMC recommendations. Dompé will be responsible for promptly reviewing the DMC recommendations, to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in the study conduct are required

All details of the conduct and responsibilities of the DMC will comply with Guidance for Clinical Trial Sponsors: Establishment and Operation of Clinical Trial Data Monitoring Committees and will be described in the 'DMC Charter' to be finalized during the set-up phase of the study and prior to the initiation of enrollment.

12.6. PROTOCOL DEVIATIONS/AMENDMENTS

Changes to the Protocol will be implemented only when written amendments have been signed by all individuals who signed the protocol.

Any amendment will be sent to the appropriate IRB. No deviations from or changes to the protocol will be implemented without documented approval of an amendment from the IRB which granted the original approval, except where necessary to eliminate an immediate hazard(s) to trial patient, or when the change(s) involves only logistical or administrative aspects of the trial. The deviations from or changes to the protocol implemented to eliminate an immediate hazard to the trial patient and the proposed amendment, if appropriate, should be submitted to the IRB for review and approval as soon as possible.

Any other deviation from the protocol that has not been approved by Dompé and the IRB could result in a discontinuation from the study at the centre involved.

Any written amendment will be sent to all recipients of the protocol and to the Competent Authorities.

12.7. DISCONTINUATION OF THE STUDY

Dompé reserves the right to stop the study at any time on the basis of new information regarding safety or efficacy, or if study progress is unsatisfactory, or for other valid administrative reasons.

After such a decision is made, the Investigator must inform all relevant persons e.g. study staff, potential patients etc. within 2 weeks. All delivered study materials must be collected and all CRFs completed to the extent possible.

Study discontinuation will be notified to the FDA within 5 days from decision.

12.8. PUBLICATIONS

As this study is part of a multicentre trial, publications derived from this study will be planned and agreed with the participating Investigators. Publications will include input from the Investigators, his/her colleagues, other investigators in this trial and Dompé personnel. Such input will be reflected in publication authorship. Criteria for selection of authors will be agreed. Subsequent to the multicentre publication or one year after completion of the study, whichever occurs first, an Investigator and/or his/her colleagues may publish the results of Investigator's part of the study independently.

Any manuscript, abstract or other publication or presentation of results or information arising in connection with the study must be prepared in conjunction with Dompé and must be submitted to the Dompé for review and comment at least 45 days prior to submission for publication or presentation. If such draft contains confidential patentable information, the Investigator will refrain from publishing any such information for a period not exceeding 180 days, to enable Dompé to file for the protection of any intellectual or proprietary property interest.

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14. APPENDICES

14.1. APPENDIX 1 – CUMULATIVE SUMMARY OF ADVERSE DRUG REACTIONS[1]

Adverse Drug Reactions - Number of Reports by Terms			Frequency
MedDRA Body System / LLT	**Non Serious**	**Serious**	
Blood and lymphatic system disorders	**3**	**1**	**4.30%**
Anaemia	2		
Coagulopathy		1	
Lymphadenopaty	1		
Cardiac Disorders	**1**	**0**	**1.08%**
Tachycardia	1		
Gastrointestinal Disorders	**13**	**0**	**13.98%**
Abdominal pain NOS	1		
Dyspepsia	1		
Flatulence	2		
Gastroesophageal reflux disease	1		
Nausea	6		
Vomiting	2		
General disorders and administration site conditions	**21**	**0**	**22.58%**
Cannula site reaction	13		
Fatigue	1		
Injection site thombosis	3		
Infusion site oedema	2		
Lethargy	1		
Oedema peripheral	1		
Immune system disorders	**0**	**1**	**1.08%**
Lung transplant rejection		1	
Injury, poisoning and procedural complications	**1**	**0**	**1.08%**
Complications of transplanted kidney	1		
Investigations	**2**	**0**	**2.15%**
Blood amylase increased	1		
Liver function test abnormal	1		
Metabolic and nutrition disorders	**1**	**0**	**1.08%**
Hyperkalaemia	1		
Musculoskeletal and connective tissue disorders	**1**	**0**	**1.08%**
Arthralgia	1		
Nervous system disorders	**25**	**0**	**26.88%**
Dizziness	3		
Headache	8		
Hypoaesthesia	3		
Somnolence	11		
Psychiatric disorders	**4**	**0**	**4.30%**

Adverse Drug Reactions - Number of Reports by Terms			Frequency
MedDRA Body System / LLT	**Non Serious**	**Serious**	
Abnormal dream	1		
Restlessness	1		
Euphoric mood	2		
Renal and urinary disorders	**4**	**0**	**4.30%**
Renal failure	1		
Renal tubular necrosis	2		
Urinary retention	1		
Respiratory, thoracic and mediastinal disorders	**6**	**1**	**7.53%**
Bradypnoea	1		
Cough	2		
Nasopharyngitis	2		
Respiratory failure		1	
Sore throat	1		
Skin and subcutaneous system disorders	**4**	**0**	**4.30%**
Erythema	1		
Infusion site erythema	2		
Pruritis	1		
Vascular disorders	**1**	**3**	**4.30%**
Haemorrhage		1	
Hypotension	1		
Retroperitoneal heamorrhage		2	
TOTAL	**87**	**6**	

1 = table does not include ADRs reported from the ongoing pilot trial in islet allo-transplant patients

The most frequent (>10%) ADRs observed in the phase 1 and phase 2 studies were:

Nervous system disorders (about 27%), including headache, dizziness, hypoaesthesia, somnolence.

General disorders and administration site conditions (about 23%), including cannula site reaction, injection site thrombosis, infusion site oedema and peripheral oedema, fatigue, lethargy.

Gastrointestinal disorders (about 14%), including nausea, vomiting, abdominal pain, dyspepsia, flatulence, gastroesophageal reflux disease.

In patients undergoing islet allo-transplantation and treated with a 7 day course of reparixin most frequent ADRs were erythema, hypotension, nausea, vomiting; great majority of these events were mild to moderate in nature and none required discontinuation of the Investigational Product.

Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion; upon case evaluation, a medical error was evidenced, i.e. the patient received a dose of reparixn 3 times as high as that foreseen in the protocol; infusion of reparixin was immediately discontinued and the patient recovered.

14.2. APPENDIX 2 - PACKAGING AND LABELING DETAILS

The template of label specimens is provided below.

Specimen label for the REPARIXIN Vial

STUDY REP0112

Sponsor Dompé s.p.a.; Via Campo di Pile, L'Aquila – Italy
Phone: + 39 346 8745908

REPARIXIN

INVESTIGATOR:

CONTAINS: 250 mL REPARIXIN (33 mg/mL) CONCENTRATE FOR SOLUTION FOR I.V. INFUSION

BATCH No. EXPIRY DATE mm/yyyy **DO NOT STORE AT >30°C (86°F)**

DIRECTIONS: Transfer into an INFUSION BAG the content of the vial + 500 mL of 0.9% NaCl, according to procedures detailed in the "Instructions to the Pharmacy".

Caution: New Drug-Limited by Federal law to investigational use.

Specimen label for the PLACEBO Vial

STUDY REP0112

Sponsor Dompé s.p.a.; Via Campo di Pile, L'Aquila – Italy
Phone: + 39 346 8745908

PLACEBO

INVESTIGATOR:

CONTAINS: 250 mL Physiologic salt solution (0.9% w/v sodium chloride)

BATCH No. EXPIRY DATE mm/yyyy **DO NOT STORE AT >30°C (86°F)**

DIRECTIONS: Transfer into an INFUSION BAG the content of the vial + 500 mL of 0.9% NaCl, according to procedures detailed in the "Instructions to the Pharmacy".

Caution: New Drug-Limited by Federal law to investigational use.

Specimen label for Infusion Bag - "double tear off" label

Each of the 2 sections of the double label will have identical information as per specimem below.

STUDY REP0112

Sponsor Dompé s.p.a.; Via Campo di Pile, L'Aquila – Italy
Phone: + 39 346 8745908

PATIENT No. ☐☐☐☐ **INFUSION BAG No.** ☐

INVESTIGATOR:

CONTAINS: 750 mL OF REPARIXIN (11.00 mg/mL) or PLACEBO I.V. INJECTABLE DOSING SOLUTION

PREPARED ON |__|__|/|__|__|/|__|__|__|__| |__|__| : |__|__|
dd / mm / yyyy h : min

DO NOT STORE AT >30°C (86°F)

USE BY |__|__|/|__|__|/|__|__|__|__| |__|__| : |__|__|
dd / mm / yyyy h : min

DIRECTIONS: Administer as a continuous i.v. infusion into a (high flow) central vein, by an infusion pump. Infusion will start approximately 12 hours (6 to 18) before islet infusion. Pump rate will be adjusted according to patient body weight as per Appendix 3 of Study Protocol.

Caution: New Drug-Limited by Federal law to investigational use.

NOTE: Shadowed field are those to be completed by the pharmacist at the time of preparation.

Patient No. Report the four digit number derived from the "IP Preparation Order Form" received from the Investigator.

Infusion Bag No. Report the sequential bag number within the series for that patient.

Preparation date/time Report the date and time when the preparation of the dosing solution has started.

Use by date/time Calculate the use by date and time as date/time of preparation + maximum 72 hours (expiry date/time of the dosing solution), unless the site has more restrictive rules for reconstituted solutions (e.g. 24 hours).

14.3. APPENDIX 3 - STUDY DRUG INFUSION RATE BY BODY WEIGHT

Reparixin dose = 2.772 mg/kg/hour for 7 days (168 hours)

Dosing solution = 11 mg/mL

BODY WEIGHT(kg) each figure is from 0.00 to 0.99	**INFUSION RATE (mL/hour)**	**24 hours INFUSION VOLUME (mL)**
45	**11.3**	**271.2**
46	**11.6**	**278.4**
47	**11.8**	**283.2**
48	**12.1**	**290.4**
49	**12.3**	**295.2**
50	**12.6**	**302.4**
51	**12.9**	**309.6**
52	**13.1**	**314.4**
53	**13.4**	**321.6**
54	**13.6**	**326.4**
55	**13.9**	**333.6**
56	**14.1**	**338.4**
57	**14.4**	**345.6**
58	**14.6**	**350.4**
59	**14.9**	**357.6**
60	**15.1**	**362.4**
61	**15.4**	**369.6**
62	**15.6**	**374.4**
63	**15.9**	**381.6**
64	**16.1**	**386.4**
65	**16.4**	**393.6**
66	**16.6**	**398.4**
67	**16.9**	**405.6**
68	**17.1**	**410.4**
69	**17.4**	**417.6**
70	**17.6**	**422.4**
71	**17.9**	**429.6**
72	**18.1**	**434.4**
73	**18.4**	**441.6**

BODY WEIGHT(kg) each figure is from 0.00 to 0.99	**INFUSION RATE (mL/hour)**	**24 hours INFUSION VOLUME (mL)**
74	**18.6**	**446.4**
75	**18.9**	**453.6**
76	**19.2**	**460.8**
77	**19.4**	**465.6**
78	**19.7**	**472.8**
79	**19.9**	**477.6**
80	**20.2**	**484.8**
81	**20.4**	**489.6**
82	**20.7**	**496.8**
83	**20.9**	**501.6**
84	**21.2**	**508.8**
85	**21.4**	**513.6**
86	**21.7**	**520.8**
87	**21.9**	**525.6**
88	**22.2**	**532.8**
89	**22.4**	**537.6**
90	**22.7**	**544.8**
91	**22.9**	**549.6**
92	**23.2**	**556.8**
93	**23.4**	**561.6**
94	**23.7**	**568.8**
95	**23.9**	**573.6**
96	**24.2**	**580.8**
97	**24.4**	**585.6**
98	**24.7**	**592.8**
99	**24.9**	**597.6**
100	**25.2**	**604.8**

14.4. APPENDIX 4 - STUDY FLOW CHART

Test/Examination	HOSPITAL STAY											FOLLOW-UP	
	Screening	day -1	day 0 Tx day	day 1	day 2	day 3	day 4	day 5	day 6	day 7	Hospital discharge	day 75±14 post transplant	day 365±14 post transplant
Informed consent	X												
Inclusion/exclusion criteria	X												
BP, HR, weight, height[1]	X										X[1]	X[1]	X[1]
Safety laboratory tests	X										X		
Pregnancy test	X												
Renal reserve/function[2] & Total bilirubin	X			X	X	X	X	X	X				
Albumin and pre-albumin	X											X	X
Concurrent sepsis	X												
Pre-operative portal hypertension	X												
miR-375; Inflammatory chemokines/cytokines[3]	XX[3]		XXX[3]	X[3]		X[3]		X[3]		X[3]			
Study drug administration[4]		X----------								X			
PK sampling[4]				X		X		X	X[4]				
ALT/AST				X	X	X	X	X	X	X		X	
XDP, INR/PTT, CRP				X	X	X	X	X	X	X			
Average daily insulin; HbA1c												X	X
Severe, Documented Symptomatic, Asymptomatic hypergycemia events												X----------	X
MMTT[5]: Glucose, C-peptide, insulin												X	X
β-score – TEF calculation												X	X
Steatorrhea[6] and malnutrituion risk[6]												X	X
Adverse Events		X----------									Record **all** AEs up to each hospital discharge. Then, only SAEs	----------	X
Concomitant Medication	X-------Record **all** CMEDs up to each hospital discharge. Then, only drugs used for SAEs, oral hypoglycemic drugs and forbidden medications -------X												

1 = Measure only BP and HR at hospital discharge. Measure only weight at follow-up visits.
2 = Assess Creatinine clearance on pre-transplant. Assess creatinine clearance AND urine output from day 1 to day 6 post-transplant.
3 = Store a serum sample for centralized assay as detailed in **Appendix 14.6.3**. Take the 2 chemokines/cytokines pre-transplant samples 6 to 24 hours apart; obtain all basal samples before surgery and before the Investigational Product administration is started. Take post-transplant samples @ 6, 12, 24, 72, 120 and 168hrs after islet infusion.
4 = Start study drug infusion approximately 12 (6-18) hours before islet infusion. Obtain PK ideally in the morning (day 1, 3, 5 post-transplant). Obtain (if you are one of the 2 selected sites) a sample just prior to, and then 1, 3, 5, 6, 8, and 12 hours after the end of study drug administration.
5= Mixed Meal Tolerance Test: measure glucose, C-peptide and insulin prior to (2 basal samples in the range between -20 to 0 prior to the meal) AND @ 15, 30, 60, 90, 120, 180 and 240 min after the meal, as detailed in **Appendix 14.6.4**. Store a blood sample for centralized assay of C-peptide and HbA1c as detailed in **Appendix 14.6.3**.
6= Evaluate steatorrhea in the 4 weeks preceeding the follow-up visit. Evaluate malnutrition risk level as per pre-albumin value.

14.5. APPENDIX 5 - ACCEPTABLE TIME WINDOWS FOR ASSESSMENTS/ PROCEDURES

Assessment/Procedure	Window
Investigational Product administration	
Start of Investigational Product infusion @ 12hrs prior to islet transplant.	6-18hrs prior to start of islet infusion
End of Infusion @ 7 days (168hrs).	± 12hrs
Follow-up visits	
Visit @ day 75 and 365after the transplant	± 14 days
Mixed Meal Tolerance Test	
Sampling @ 15, 30, 60, 90, 120, 180, 240 mins after the meal	± 5 mins (15 min and 30 min) or ± 10 mins (60 to 240 min)
Chemokine/cytokines; miR-375	
Sampling @ 6, 12, 24, 72, 120 and 168hrs after islet infusion	± 10 mins (6 to 24 hrs) or ± 30 mins (72 to 168hrs)
PK sampling	
Sampling on post-operative day 1, 3 and 5, ideally in the morning	Between 5.30 a.m. and 12.30 p.m.
Sampling just prior to termination of Investigational Product administration	Within 6 hrs before the end of Investigational Product administration
Sampling @ 1, 3, 5, 6, 8, and 12 hrs after termination of Investigational Product administration	± 10 mins

14.6. APPENDIX 6 - METHODOLOGICAL DETAILS

14.6.1. Islet determination

The following method will be used to quantify isolated and infused IEQ.

After staining dithizone, islets will be counted by phase contrast microscope and grouped in classes according to their size (islet diameter; μm). IEQ will be derived from the islet number in each class integrated by a conversion factor, as detailed in the table below, where "n" is the number of islet in each class.

Class [islet diameter range (μm)]	Conversion into IEQ [islets of 150 μm diameter]
50-100	n / 6
101-150	n / 1.5
151-200	n x 1.7
201-250	n x 3.5
251-300	n x 6.3
301-350	n x 10.4
>350	n x 15.8

14.6.2. Calculation of creatinine clearance

Renal function will be evaluated by creatinine clearance (CLcr), calculated by the following formula (*Cockcroft-Gault, 1976)*:

$$\text{Male: CLcr} = \frac{[140 - \text{age (years)}] \bullet \text{Weight (kg)}}{\text{Serum Creatinine (mmol / L)} \bullet 815}$$

$$\text{Female: CLcr} = \frac{[140 - \text{age (years)}] \bullet \text{Weight (kg)}}{\text{Serum Creatinine (mmol / L)} \bullet 815} \bullet 0.85$$

14.6.3. Handling of samples for centralized assays and assay methods

Detailed instructions will be provided for preparation, storage and shipment of samples. Tubes and labels for storage will be provided by the CRO appointed by Dompé, along with storage and shipment tracking forms.

Samples will be shipped in appropriate package in dry ice (solid CO_2) to maintain frozen conditions. All samples will be shipped on an ongoing basis during the trial, according to logistics. However, samples from the last patient visits will be shipped as soon as possible to ensure timely availability of results.

Arrangements will be made with each centralized laboratory to provide appropriate procedures for receipt and analysis of the samples and, apart from PK analysis (see section "PK samples below"), back-communication of the results to the sending site. All steps will be tracked to ensure correct data reporting.

Once received from the centralized laboratory, each site will enter values pertaining to its patients in the corresponding CRF. All samples will be destroyed after final study report has been issued or after the patient has withdrawn his/her consent.

The centralized laboratory for the assay of C-Peptide, HbA1c and Inflammatory chemokines/cytokines will be:
Northwest Lipid Metabolism and Diabetes Research Laboratories (Santica Marcovina, Ph.D., D.Sc.); University of Washington - 401 Queen Anne Ave North; Seattle, WA 98109 - US. Phone: +001 (206) 685 3331; smm@u.washington.edu. The laboratory is certified by CLIA and all the analyses are performed under strict quality control. Also, the laboratory participates in the National Glycohemoglobin Standardization Program (NGSP) and the method is yearly certified at the level of Laboratory 1 Certification to ensure that the HbA1c values are traceable to the DCCT values.

The centralized laboratory for the assay of miR-375 will be:
Diabetes Research Institute, β-cell Biology Unit (Lorenzo Piemonti, MD); IRCCS Ospedale San Raffaele; 1st floor, Lot Q L33; Via Olgettina, 60; 20132 Milan, Italy.

The centralized laboratory for the assay of PK samples will be:
CDMO Department (Gaetano D'Anniballe BSc), Quality Control Laboratory; Dompé s.p.a. - Via Campo di Pile; 67100 L'Aquila - Italy. Phone: +39 0862 338361; e-mail: gaetano.danniballe@dompe.it

- C-peptide

Blood samples will be kept at room temperature for at least 30 min to allow clotting. They will then be centrifuged at 700 × g within one hour from sampling. Cell free serum samples for each time-point will be aliquoted (at least 2 x 0.5mL aliquots) and stored between -70°C and -80°C until shipment to the centralized laboratory. Determination of C-peptide levels will be performed by a two site immuno-enzymometeric assay using a Tosoh 2000 auto-analyzer (TOSOH, Biosciences, Inc., South San Francisco, CA).

- HbA1c

EDTA whole blood samples will be collected and at least 2 x 1 mL aliquots will be stored between -70°C and -80°C until shipment to the centralized laboratory. Measurement of the relative proportion of hemoglobin subclasses and calculation of the HbA1c levels are performed by a dedicated analyzer (TOSOH, Biosciences, Inc) using non-porous ion exchange high performance chromatography to achieve rapid and precise separation of stable HbA1c from other hemoglobin fractions.

- Inflammatory chemokines/cytokines

Blood samples, obtained before (2 basal samples collected 6 to 24 hrs apart; both samples will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion, will be immediately kept at 4°C, protected from the light and centrifuged within 2 hrs at 700 × g at 4°C. Cell free serum will be aliquoted (4 x 100µL aliquots) and stored between -70°C and -80°C until shipment to the centralized laboratory and analysis.

Starting from 25µL of thawed serum the following chemokines/cytokines will be determined by a multiplex technology that uses magnetic bead sets (BioRad BioPlex instrument using a Human Cytokine Panel; Millipore Inc., HCYTOMAG-60K): CXCL8, CCL2 (MCP-1), CCL3, CCL4, CXCL10 (IP-10), IL-6, IL-10, INF-γ, TNF-α, and IL-1β. Due to cross-reactivity with the Human Cytokine Panel (HCYTOMAG-60K), analysis of CXCL-9 (MIG) is performed on a BioRad BioPlex system using MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel III (Millipore, Inc., HCYP3MAG-63K).

- miR-375

Blood samples, obtained before (one basal sample collected before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion, will be immediately kept at 4°C, protected from the light and centrifuged within 2 hrs at 700 × g at 4°C. Cell free

serum samples for each time-point will be aliquoted (at least 2 x 300µL aliquots) and stored between -70°C and -80°C until shipment to the centralized laboratory and analysis.

Circulating miR-375 levels will be measured by droplet digital PCR (ddPCR); serum samples will be extracted with commercially available kits and the recovered miRNA fraction will be first reverse transcribed and then partitioned into approximately 20000 droplets using the droplet generator of the Bio-Rad QX100 droplet digital PCR system. After PCR amplification in a conventional thermal cycler, each individual droplet from each sample will be read and its fluorescent status assessed using the QX100 droplet reader. The output of the QX100 reader will be analyzed using the QuantaLife 1.3 software.

- PK samples

Blood samples will be taken into lithium heparin monovettes and centrifuged ideally within 15 minutes at 2500 x g at 4°C for 10 minutes. If that is not possible then samples can be stored at room temperature for not more than 2 hours or at 4°C for not more than 24 hours before centrifugation. Plasma will be aliquoted (2 x 1.5mL aliquots) and stored at a temperature in the range -20°C to -80°C into stopped tubes (glass or plastic), until shipment to the centralized analytical laboratory.

Analaysis will be carried out within one year from sampling. i.e. before the study blind is broken. Therefore, all samples obtained during the conduct of the trial will be sent to the centralized laboratory and analyzed in order to maintain the study blind. Appropriate procedures will be implemented by the centralized laboratory to guarantee the blinding; also, results will be released only after study blind has been broken.

Analysis will be performed according to standardized and validated methods as per Good Laboratory Practices. In particular, the following PK parameters will be determined:

$C_{initial}$	Plasma concentration of reparixin and metabolites (DF 2243Y and ibuprofen) just prior to the end of infusion;
C_{max}	Maximum plasma concentration of reparixin and metabolites;
t_{max}	Time of maximum plasma concentration reparixin and metabolites;
λ_z	Terminal phase rate constant of reparixin and metabolites;
$t_{½}$	Terminal half life of reparixin and metabolites;
AUC_{0-t}	Area under the plasma concentration-time curve from time zero ($C_{initial}$) to time t (time of last quantifiable plasma concentration) of reparixin and metabolites;
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from time zero ($C_{initial}$) to infinity of reparixin and metabolites
V_z	Volume of distribution of reparixin
CL	Clearance of reparixin

Data will be summarised in a separate report that will be integrated into the final study report.

14.6.4. Mixed Meal Tolerance Test

The MMTT will be performed on all patients possibly after an overnight fast, according to Greenbaum (*2008*). The test will be initiated before 10 a.m. The Boost High Protein will be used for the MMTT.

Patients will be given 6mL/kg of Boost preparation up to a maximum of 360mL, to be drunk within 5 min. Blood samples for glucose, C-peptide and insulin measurement will be withdrawn in basal condition (two samples in the range between -20 to 0 prior to the meal) and then @ 15, 30, 60, 90, 120, 180 and 240 min after the meal.

14.6.5. β-score calculation

β-score will be calculated on a 0-8 scoring system (*Ryan, 2005*) which gives 0-2 points each for glucose, HbA1c, stimulated C-peptide and insulin requirement, as per the table below.

Components	Score 2	Score 1	Score 0
Fasting (or before breakfast) plasma glucose (mg/dL OR mmol/L)	≤99 OR ≤5.5	100 – 124 OR 5.6-6.9	≥125 OR >7.0
HbA1c (%)	≤6.1	6.2 – 6.9	≥7.0
Daily average (previous week) insulin (IU/kg/day)	---	0.01 – 0.24	≥0.25
Stimulated C-peptide (ng/mL)[1]	≥0.9*	0.3 – 0.89	<0.3#

1: C-peptide level in the blood sample taken 90 min after drinking the mixed meal. *: If fasting C-peptide is ≥0.9 ng/mL, then the stimulated C-peptide level is assumed to be ≥0.9 ng/mL. #: If stimulated C-peptide is <0. 3 ng/mL, then an overall score of 0 is awarded

14.7. APPENDIX 7 – SAMPLE DIARY CARD

Clinical Trial REP0112

PATIENT DIARY CARD

PATIENT No. ☐☐☐☐

Sponsor: Dompé s.p.a.

Please report in the table below your blood glucose (fingerstick) for 14 days before the planned follow-up visit. You are required to report at least one value taken after an overnight fast (or anyway before breakfast) and one value taken within 2 hours after one of the two meals. Also, please report the total amount of insulin you have taken each day.
Please add the date as day/month/year (example 25/07/2012 for 25 July 2012) and the time as 24 hours clock (example 9.35 in the morning, 21.35 in the afternoon). Please enter the information requested with indelible ink.

Day	**Breakfast glucose**		**Lunch glucose**		**Dinner glucose**		**Daily Insulin**
	Time	Value Before	Time	Value After	Time	Value After	
Monday - date:							
Tuesday - date:							
Wednesday - date:							
Thursday - date:							
Friday - date:							
Saturday - date:							
Sunday - date:							

Investigator's signature: _____ date: _____