A DOUBLE-BLIND, RANDOMISED, PLACEBO CONTROLLED, SEQUENTIAL ASCENDING DOSE STUDY, TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF SINGLE INTRA-ARTICULAR DOSES OF FASITIBANT IN PATIENTS WITH SYMPTOMATIC OSTEOARTHRITIS OF THE KNEE.

SUMMARY RESULTS

Study code	BKOS-03
EudraCT - Number	2011-005254-53
Investigational Medicinal Product	Fasitibant
Development phase of study	Phase Ib / IIa
Sponsor	Menarini Ricerche S.p.A.

Study centre:
The study was conducted in 4 study sites in Germany and Italy.
The Coordinating Investigator was Stefano Milleri MD; Centro Ricerche Cliniche di Verona Srl, C/O Policlinico G.B. Rossi-Italy.

Study period:
First subject in: 02 July 2012
Last subject out: 23 October 2012

Objectives:
Primary objective:
To evaluate the safety and tolerability of fasitibant up to 5mg formulated as 1 mL solution to be administered as single IA knee injection to osteoarthritic (OA) patients.
Secondary objective:

- To assess main plasma pharmacokinetic parameters, including dose-proportionality, of fasitibant monocomponent (up to 5 mg) and fasitibant 5 mg in extemporaneous combination with sodium hyaluronate in patients with knee OA.
- To evaluate changes in plasma or serum levels, as appropriate, of OA biomarkers and preliminary efficacy data in knee OA patients following IA single doses of fasitibant up to 5 mg alone and in extemporaneous combination with sodium hyaluronate.

Number of subjects (planned and analysed):
The study was completed in 60 patients as planned.

Main criteria for inclusion:
Male or female patients ≥ 30 years old and a BMI < 30 kg/m² with the following target disease:

- Symptomatic primary or secondary knee osteoarthritis since ≥ 3 months with documented Kellgren Lawrence Grade I to III of radiological severity.
- Pain score > 5 and > 12 points assigned to the index knee at WOMAC NRS 3.1- A1 (pain while walking on a flat surface) and at WOMAC NRS 3.1- A score (total pain), respectively.
- Pain in the index knee on at least 50% of the days in the month preceding the screening.
- Minimum flexion of 90 degrees in both knees.
- Ability to perform the 15-meter walk test without the support of crutches or other assistive devices.

Test product, dose and mode of administration:
Fasitibant 1 mg, 2.5 mg, and 5 mg, 1 ml solution each for one single IA knee injection.

Reference therapy, dose and mode of administration:

- Placebo 1 ml solution for one single IA knee injection (fully matching fasitibant).
- Sodium Hyaluronate 20mg/2mL for one single IA knee injection administration as locally commercially available.

Methodology:
Double-blind, randomised, placebo controlled, sequential ascending dose, pharmacokinetic study, testing single knee intra-articular (IA) doses of fasitibant versus placebo in three sequential cohorts of patients (1mg, 2.5mg, 5mg), plus an additional fourth cohort testing fasitibant 5 mg+sodium hyaluronate 20mg versus placebo + sodium hyaluronate 20mg.
In the first three cohorts, randomisation to fasitibant or placebo was in the ratio 3:1, while in the fourth cohort randomisation it was in the ratio 1:1. In each cohort, the double blind condition referred to fasitibant OR placebo administration, whereas the fasitibant dose level was open.
The individual study duration was lasting up to 5 weeks, encompassing:
Visit 1: screening (within 3 weeks prior to randomisation).
Visit 2: baseline blood sampling for pharmacodynamic (PD) biomarkers and knee OA assessment; randomisation and single IA knee administration of study treatment followed by a 10 hour on-site safety monitoring and blood sampling for pharmacokinetics (PK), with a last PK sample collected shortly before patients leaving the study site.
Visit 3: safety and PK assessment, approx. 72 hours after treatment administration.
Visit 4: safety and PK and PD assessment 1 week after treatment administration.
Visit 5: End of Study- safety and PK assessment 2 weeks after treatment administration.

Criteria for evaluation:

Safety:

Safety assessment included:

- Safety Changes in vital signs, 12-lead ECG, laboratory safety battery, physical examination up to Visit 4 versus baseline.
- Incidence and severity of AEs occurring between randomisation (Visit 2) and End of Study (Visit 5).

Pharmacokinetics:

Serial blood samples for fasitibant (and its major metabolite MEN 19148) plasma concentration measurements were to be taken at pre-defined time points, from baseline up to 2 weeks after treatment administration. The following PK parameters and analyses were carried out:

- PK fasitibant parameters: C_{max}, t_{max}, C_{last}, k_e, $AUC_{(0-t)}$, $AUC_{(0-72h)}$, $AUC_{(0-\infty)}$, $\%AUC_{ex}$, $AUMC_{(0-\infty)}$, $t_{1/2}$, MRT, CL/F, Vd/F calculated/estimated on plasma concentrations by treatment including fasitibant. The ratio of metabolite over fasitibant exposure ($AUC_{(0-t)}^{MEN\ 19148}/AUC_{(0-t)}^{fasitibant}$ *100) was also to be calculated, when feasible.
- Fasitibant dose proportionality on Cmax and AUC.
- PK of fasitibant 5 mg when co-administered with sodium hyaluronate versus fasitibant single agent.
- Exploratory evaluation of fasitibant exposure-response relationship (Population PK/PD analysis).

Pharmacodynamics – biomarkers:

By using commercially available Elisa and colorimetric kit assays the following OA biomarkers indicative of inflammation of cartilage catabolism were assessed immediately prior to dosing at Visit 2 (baseline) and 1 week after treatment administration (Visit 4): COMP, keratan sulfate, aggrecan, GAGs, CTXII, HA, MMP1, MMP2, MMP3, MMP7, MMP9, TIMP-1, TIMP-2, IL-15, eotaxin-2, bradykinin, IL-6, CRP, IL17, VEGF, uric acid, IL18.

Summary of results:

Baseline characteristics of study population:

Patients were well comparable with respects to demographics and baseline characteristics.

On average, study patients were 64.5 years of age (± 9.64 years), with a mean body weight of 76.3 kg (± 13.21 kg), and mean BMI=26.6 kg/m² (± 2.86 kg/m²) without any clinically significant differences between treatment groups.

As far as the gender distribution, 48.3% of patients were male.

The 12-Lead ECGs at baseline showed abnormal values in the majority of patients, nevertheless none of these abnormalities was judged as clinically significant by investigators.

There were no significant clinically differences regarding baseline characteristics observed between all treatment groups. All patients of the ITT-population had primary or secondary symptomatic osteoarthritis of the knee according to the American College of Rheumatology (ACR) criteria for ≥ 3 months, and with X-ray documented Kellgren Lawrence Grade I to III radiological severity, and an average time from onset of OA symptoms = 8.4±7.18 years, without any relevant differences between treatment groups.

Safety results:

Treatment Emergent Signs and Symptoms (TESS):

In total, 15 patients (25.0%) experienced 18 TESS (n= 5 (55,6%) at fasitibant 1 mg group, n= 2 (22,2%) at 2.5 mg group, n= 0 (0,0 %) patients in the fasitibant 5 mg group, n= 4 (33.3%) patients in the placebo+ sodium hyaluronate 20 mg group, n= 3 (25.0%) patients in the fasitibant 5 mg group + sodium hyaluronate 20 mg group and n= 1 (11.1%) patient in the placebo group). One patient (1.7%) in the fasitibant 1 mg group reported two treatment-related TESS (flatulence and headache).

In 12 of the 15 patients reporting a TESS, the events resolved spontaneously and there was no TESS (0.0%) across all treatment groups rated as being of severe intensity.

The majority of all reported TESSs were of moderate (14 of 18 TESS [77.7%]) or mild (4 of 18 TESS [22.2%]) intensity.

The most common TESSs were reported in the "Nervous system disorders" SOC (10.0% of patients in total), the most frequent being headache that occurred especially in the lower dose group of fasitibant 1 mg. The second most common TESSs were reported in the "musculoskeletal and connective tissue disorders" SOC (8.3% of patients in total), the most frequent being arthralgia which was experienced in particular in the placebo + sodium hyaluronate group.

No patient (0.0%) across all treatment groups experienced a serious TESS. No deaths or serious adverse events (SAE) have been reported in this study.

There were no clinically relevant changes in *vital signs, ECG and laboratory parameters* and *physical examination parameters* related to study treatments.

Pharmacokinetic results:

Fasitibant was rapidly absorbed from the synovial fluid to the systemic circulation after intra-articular injection to the knee, with a median time to the peak plasma concentration (T_{max}) ranging from 1.67 to 4 hours (Table S-1). Fasitibant concentrations declined from the peak plasma concentration (C_{max}) in a biphasic manner with an apparent terminal half-life ($t_{1/2}$) of approximately 99 to 127 hours, similar across doses. Table S-2 contains point estimates and 95% confidence intervals (CIs) for the slopes from the power model used to assess dose proportionality of fasitibant $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max} over the dose range 1 to 5 mg. Systemic exposure of fasitibant increased in a dose proportional manner and the increase showed no significant deviation from linearity ($\beta \sim 1$) when the whole dose range was considered. Dose proportional increases were observed for all absorption parameters over the dose range.

Table S-1. Plasma PK parameters (standard non-compartmental analysis) of fasitibant by single intra articular doses (geometric means and 90% CI or mean and range for T_{max}).

	Fasitibant 1 mg (N=9)	**Fasitibant 2.5 mg (N=9)**	**Fasitibant 5 mg (N=9)**	**Fasitibant 5 mg + Sodium Hyaluronate 20 mg (N=12)**
C_{max} (ng/mL)	229.6 (188.8-279.3)	506.204 (424.7-603.4)	1019.7 (890.7-1167.4)	906.3 (642.8-1277.8)
T_{max} (h)	4.0 (1.0-10.0)	1.67 (0.75-2.0)	1.5 (0.75-4.0)	2.0 (0.75-6.0)
$AUC_{(0-72)}$ (ng*h/mL)	9305.9 (7933.3-10916.0)	20715.0 (18578.4-23097.4)	41219.2 (36298.9-46806.4)	38099.8 (27217.5-53333.3)
$AUC_{(0-t)}$ (ng*h/mL)	20204.6 (17486.3-23345.5)	46627.1 (41333. 6-52598.5)	97243.3 (87521.4-108045.1)	78440.0 (55719.8-110424.6)
$AUC_{(0-\infty)}$ (ng*h/mL)	22861.5 (19468.8-26845.4)	53776.1 (47134.0-61354.2)	114014.4 (102702.1-126572.8)	86794.2 (61293.3-122904.6)
AUC Extr (%)	10.1 (7.3-13.9)	12.6 (10.3-15.5)	13.9 (11.5-16.9)	9.1 (7.5-10.9)
$AUMC_{(0-\infty)}$ ($ng*h^2/mL$)	3409803 (2598865-4473782)	8608441 (7030909-10539927)	20052344 (17146253-23450984)	11879955 (8071383-17485646)
$t_{1/2}$ (h)	107.1 (89.9-127.5)	111.9 (102.1-122.6)	126.5 (112.3-142.5)	99.4 (91.7-107.8)
Vd/F (L)	6.8 (5.8-8.0)	7.5 (6.8-8.3)	8.0 (6.9-9.3)	8.3 (6.0-11.4)
CL/F (mL/h)	43.7 (37.3-51.4)	46.5 (40.8-53.1)	43.8 (39.5-48.7)	57.6 (40.7-81.6)
MRT (h)	149.3 (127.8-174.4)	160.2 (145.8-176.2)	176.0 (158.0-196.1)	136.9 (126.0-148.7)

Table S-2. Dose proportionality of fasitibant following IA injections of single doses of 1, 2.5 and 5 mg in patients with OA of the knee - N=27 [model slope (β) and 95%CI].

	Estimated Slope (β)	**95% CI**
C_{max} (ng/mL)	0.92	0.76 - 1.09
$AUC_{(0-t)}$ (ng*h/mL)	0.97	0.85 - 1.09
$AUC_{(0-\infty)}$ (ng*h/mL)	1.00	0.87 - 1.13

The apparent volume of distribution (Vd/F) and total clearance (CL/F) were very low (approximately 7-8 L and 44-58 mL/h, respectively) and similar within the range of doses, thus indicating fasitibant low distribution phase to the peripheral tissues and slow elimination from the systemic circulation by mechanisms which were not saturated under the study experimental conditions. According to the low value of the CL/F, fasitibant

metabolism is expected to be very limited *in vivo* in humans. MEN 19148, the mono-hydroxylated derivative, formed *in vitro* via oxidative metabolism by the cytochrome P450 enzyme, was not detected in human plasma samples.

The combined administration of fasitibant (5 mg) and sodium hyaluronate (20 mg) did not alter the pharmacokinetics of fasitibant. The mean concentration-time course of fasitibant 5 mg - single agent was virtually super imposable with that obtained after its extemporaneous combination with sodium hyaluronate.

All the PK parameters were also very similar between the two treatments. The ANCOVA analysis confirmed that no statistically significant differences ($p>0.05$) for the main fasitibant PK parameters (Cmax, AUC, Tmax and t1/2) were detectable between the single agent and the sodium hyaluronate combined treatments.

Therefore, no clear evidence was found that the combined injection with sodium hyaluronate altered fasitibant PK or prolonged its residence time in the knee delaying the drug onset in the systemic circulation.

Pharmacodynamic results:

The model fit for biomarkers change as dependent variable, considering treatment and basal concentration of biomarkers as covariates, was significant for BK, COMP, CRP, HA, MMP-2, MMP-3, MMP-9, TIMP- 1, TIMP-2, eotaxin VEGF, and uric acid ($p<0.05$). On the other hand, basal biomarker concentrations or treatment, significantly accounted for the changes of BK, COMP, CRP, eotaxin, HA, MMP-1, MMP-2, MMP-3, MMP-9, TIMP-1, TIMP-2, VEGF and uric acid levels. MMP-2 was statistically significant lower in the OA group treated with 1 mg ($p=0.0533$) and 2.5 mg ($p=0.0079$) of fasitibant versus placebo; TIMP-2 approached only the statistical significance due the α level adjustment ($p=0.0209$ for 1 mg and $p=0.0651$ for 2.5 mg).

ANOVA models on pain score in function of treatment (with basal pain score, biomarkers change (%) and their interaction with the treatment as covariates) selected the following biomarkers as the most influent in the explanation of pain clinical outcome: CTX-II ($p=0.0150$), TIMP-1 ($p=0.0076$), TIMP-2 ($p=0.0170$), basal BK ($p= 0.0386$ and $p=0.0466$), MMP-9, HA, and eotaxin (nearly significant).

The factor analysis applied on this set of biomarkers determined two different groups of biomarkers: Factor 1, i.e. CTX-II, HA, TIMP-2, BK; Factor 2, i.e. MMP-9, eotaxin-2, TIMP-2. TIMP-1 was excluded from the analysis due to the high correlation with TIMP-2; Furthermore TIMP-2 is present twice because it has a similar influence in both factors. In Addition a factor analyses was performed also for demographics variables (BMI, age, sex) and OA related variables (onset time, effusion, knee circumference) obtaining the following groups: Factor 1, i.e. BMI and the three knee circumference parameters; Factor 2, i.e. the three effusion parameters (as dummy 1 to 3); Factor 3, i.e. age, time of OA onset, gender.

In the stepwise procedure for WOMAC A, B, none of the covariated factors had a significant influence on the explanation of variance in the fasitibant versus placebo treated groups, Nevertheless the correction by biomarkers Factor 1 and OA and demographic Factor 1 contributed to render statistically significant the difference in WOMAC B-stiffness favouring fasitibant 2.5 mg over placebo. Statistically significant results were, instead, obtained when WOMAC C-function score or overall index were considered as dependent variables. Both demographic, OA characteristics and differences in changes of MMP-9, eotaxin, and TMP-2 reduced significantly the residual variance of the model. Moreover, higher difference, and consequently higher statistical significance, favoring fasitibant 1 mg and 2.5 mg over placebo occurred.

Table S-3 Comparison of differences in the fasitibant treatment arms: ANOVA model considering or not biomarkers and OA and demographic variables as covariates

	ANOVA	
	Not corrected	**Corrected for biomarkers and OA and demographic variables**
	p-value	**p-value**
WOMAC A-pain		
fasitibant 1 mg versus placebo	0.968	0.8188
fasitibant 2.5 mg versus placebo	0.238	0.2822
fasitibant 5 mg versus placebo	0.624	0.097
WOMAC B-stiffness		
fasitibant 1 mg versus placebo	0.86	0.0554
fasitibant 2.5 mg versus placebo	0.21	**0.0233**
fasitibant 5 mg versus placebo	0.677	0.922
WOMAC C-function		
fasitibant 1 mg versus placebo	0.934	**0.0252**
fasitibant 2.5 mg versus placebo	**0.049**	**0.0015**
fasitibant 5 mg versus placebo	0.943	0.8225
WOMAC Overall index		
fasitibant 1 mg versus placebo	0.897	**0.0341**
fasitibant 2.5 mg versus placebo	**0.049**	**0.0026**
fasitibant 5 mg versus placebo	0.927	0.768

Conclusions:

The overall tolerability and safety profile of fasitibant were very good at all tested doses, both when administered as single agent (fasitibant 1 mg, 2.5 mg, 5 mg), and in combination with sodium hyaluronate (fasitibant 5 mg + sodium hyaluronate 20 mg). No SAEs or discontinuations due to AEs have been reported in this study. The type and frequency of TESS reported across treatment groups did not show any apparent dose-proportionality. Any dose of fasitibant did not elicit any clinically relevant changes in safety laboratory tests, ECG parameters, or vital signs.

Fasitibant appeared rapidly into the plasma following its intra-articular administration in the knee, reaching peak concentrations between 1.67 and 4 hours. Parameters directly related to systemic exposure (Cmax and AUC) increase in the range of the tested doses with a linear dose-relationship. The apparent terminal half-life (t1/2) was long (> 90 hours) and the volume of distribution limited to approximately 7-8 L, with a low apparent clearance of approximately 44-58 mL/h.

The combined administration of fasitibant (5 mg) with sodium hyaluronate (20 mg) did not alter the pharmacokinetics of fasitibant. The mean concentration-time courses, as well as main PK parameters of fasitibant 5 mg as single agent were virtually super imposable with those obtained following its extemporaneous combination with sodium hyaluronate.

Among different OA biomarkers exploratory investigated in the study, MMP-2 was statistically significant lower in the OA group treated with 1 mg (p=0.0533) and 2.5 mg (p=0.0079) of fasitibant versus placebo; interestingly, in different preclinical models BK has been shown to promote MMP-2 activation that can be prevented by B2 receptor antagonists. TIMP-2, instead, approached only the statistical significance due the α level adjustment (p=0.0209 for 1 mg and p=0.0651 for 2.5 mg).

The exploratory PK/PD analysis highlighted that a relationship between WOMAC A pain sub score and fasitibant AUC can be demonstrated for 2.5 mg dose ($p<0.005$), suggesting a statistically significant reduction of pain versus placebo at this dose.

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