Pierre Fabre Médicament
Represented by: Institut de Recherche Pierre Fabre
45, Place Abel Gance
F-92100 Boulogne Cedex

1. TITLE PAGE

CLINICAL STUDY REPORT

EXPLORATORY STUDY OF L.S.E.S.r. (PERMIXON® 160 mg hard capsule) VERSUS TAMSULOSINE LP ACTIVITY ON INFLAMMATION BIOMARKERS IN THE TREATMENT OF URINARY SYMPTOMS RELATED TO BPH
A multinational, multicentric, randomised, double-blind, parallel-group prospective study

Investigational product: Lipidosterolic Extract of *Serenoa Repens* (L.S.E.S.r)

EudraCT number: **2011-005307-33**

Protocol number: **P00048 GP 4 03**

Phase of development: Phase IV

Date of first enrolment: 27 JUN 2012

Date of last completed: 08 OCT 2013

International Coordinator: Pr Alexandre DE LA TAILLE
CHU Henri Mondor
51, Avenue du Marechal de Lattre de Tassigny
94010 Creteil Cedex - France
Phone: +33 1 49 81 25 57
Fax: +33 1 49 81 25 52
E-mail: adelataille@hotmail.com

Sponsor Representative(s) for study report:

Research and Development Physician:
Marie-Thérèse PETRISSANS
Centre de Recherche & Développement Pierre Fabre
3, Avenue Hubert Curien - BP 13562
31035 Toulouse Cedex 1 – France
Phone : +33 5 34 50 63 42

Statistician:
Pierre BUNOUF
Pierre Fabre Biométrie
142, rue du Village d'Entreprises - BP 687
31319 Labège Innopole Cedex - France
Phone: +33 5 62 24 54 36

Date of report: Final Version – 22 OCT 2014

Study performed in compliance with Good Clinical Practice.

2. SYNOPSIS

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

Title of study:	**Exploratory study of L.S.E.S.r. (PERMIXON® 160 mg hard capsule) versus Tamsulosine LP activity on inflammation biomarkers in the treatment of urinary symptoms related to BPH; a multinational, multicentric, randomised, double blind parallel-group prospective study.**
Coordinating Investigator:	Pr Alexandre DE LA TAILLE, CHU Henri Mondor, Créteil, France
Investigators:	Urologists and general practitioners
Study centre(s):	20 centres in France (10 Urology departments/clinics and a network of 10 general practitioners), 8 centres in Italy (Urology departments), 3 centres in Portugal (Urology departments) and 11 in Spain (Urology departments)
Study period: **Date of first enrolment** **Date of last completed**	27 JUN 2012 08 OCT 2013
	Phase of development: **IV**
Objectives: **Primary:**	• **Main objective** : To evaluate the effect of L.S.E.S.r. 160 mg b.i.d. and Tamsulosine LP 0.4 mg o.a.d. at D30 and D90 on biomarkers of inflammation in patients suffering from BPH : ***- Urine inflammation markers on the first urine flow :*** - Gene (mRNA) expression profile of inflammation in BPH ***- Serum inflammation markers :*** - CRP and Sedimentation Rate
Secondary:	• **Secondary objectives** : ***Efficacy :*** - To assess the efficacy of L.S.E.S.r. 160 mg b.i.d. and Tamsulosine LP 0.4 mg o.a.d. through the assessment of I-PSS. - To assess the efficacy of L.S.E.S.r. 160 mg b.i.d. and Tamsulosine LP 0.4 mg o.a.d. through the assessment of QoL. - To assess the effect of L.S.E.S.r. 160 mg b.i.d. and Tamsulosine LP 0.4 mg o.a.d. on sexual function (MSF-4). - To assess the efficacy of L.S.E.S.r. 160 mg b.i.d. and Tamsulosine LP 0.4 mg o.a.d. through the assessment of Qmax. - To assess the efficacy of L.S.E.S.r. 160 mg b.i.d. and Tamsulosine LP 0.4 mg o.a.d. through the assessment of post-void residual urine volume. - To evaluate the effect of L.S.E.S.r. 160 mg b.i.d. and Tamsulosine LP 0.4 mg o.a.d. on prostate volume. - To perform an exploratory analysis of the link between inflammation biomarkers and BPH clinical symptoms on changes from baseline, if applicable. - To perform a supportive analysis on the protein expression profile of inflammation in BPH of the main expressed genes (mRNA), if applicable. ***Safety :*** - To assess the clinical safety of L.S.E.S.r. 160 mg b.i.d. and Tamsulosine LP 0.4 mg o.a.d. in patients suffering from BPH.

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

Methodology:	Double-blind, randomised, multicentric, parallel-group prospective study.
Number of patients (planned and analysed):	Planned: 2 x 100 Analysed: 203 (Full Analysis Set)
Diagnosis and main criteria for inclusion:	**Inclusion Criteria:** ***Demographic Characteristics and Other Baseline Characteristics:*** - Male patient. - Between 45 and 85 years old. ***Diagnostic Criteria:*** - Patient with bothersome lower urinary tract symptoms such as pollakiuria (daytime or night time), urgency, sensation of incomplete voiding, delayed urination or weak stream, existing for over 12 months. - I-PSS ≥ 10 at selection visit and ≥ 12 at randomisation visit (visit 2). - Stable patient's disease at randomisation defined as an absolute difference of 2 or less on I-PSS between selection and randomisation visits (visit 1 and visit 2). - I-PSS QoL score ≥ 3 evaluated at selection and randomisation visits. - 5 mL/s ≤ maximum urinary flow rate < 15 mL/s for a voided volume ≥ 150 mL and ≤ 500 mL evaluated at randomisation visit (2 measurements if necessary). - Prostatic volume ≥30 cm^3 determined by transrectal ultrasound at randomisation visit (visit 2). - Serum total PSA at randomisation visit (visit 2) : a) ≤ 4 ng/mL or b) ≤ 10 ng/mL and PSA (free) / PSA (total) ≥ 25% or negative prostate biopsy within the past 6 months prior to selection visit. ***Ethical /legal considerations:*** - Patient able to understand and sign the informed consent and understand and fill in self-questionnaires. - Having signed his written informed consent. - Affiliated to a social security system, or is a beneficiary (if applicable in the national regulation). **<u>Non-Inclusion Criteria</u>:** ***Target disease characteristics:*** - Post-void residual urine volume > 200 mL (by suprapubic ultrasound) at randomisation visit (visit 2). - Urological history : • Urethral stricture disease and/or bladder neck disease. • Active (at selection and randomisation visits) or recent (< 3 months) or recurrent urinary tract infection. • Urinary retention with indwelling catheter or intermittent catheterisation. • History of unprompted acute urinary retention in the past. • Indication of BPH surgery.

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

- Stone in bladder or urethra
- Acute or chronic (documented) prostatitis.
- Prostate cancer treated or untreated.
- Bladder cancer.
- Interstitial cystitis (documented by symptoms and/or biopsy).
- Active upper tract stone disease causing symptoms.

- Patient with history of surgery of the prostate, bladder neck or pelvic region.

Other diseases:

- Any local and/or systemic inflammation disorders at selection and randomisation visit.
- Any neurologic or psychiatric disease/disorder interfering with detrusor or sphincter muscle.
- Insulin-dependent diabetes mellitus and non-controlled non insulin-dependent diabetes mellitus.
- Chronic renal insufficiency, with serum creatinine ≥ 30 % above the upper normal range at randomisation visit (visit 2).
- History of severe hepatic failure.
- Orthostatic hypotension defined as a decrease of at least 20 mmHg in SBP or 10 mmHg in DBP between supine and 2-minute standing positions at selection (visit 1) and randomisation visit (visit 2).
- Patient with any severe underlying disease considered as life threatening in the short or medium term.

Relating to treatments:

- Known hypersensitivity to one of the constituents of the study drugs.
- Concomitant medication at selection visit (visit 1) :

- Anti-androgens (must be discontinued at least 6 months prior to selection).
- LH-RH analogues (must be discontinued at least 6 months prior to selection).
- 5 alpha-reductase inhibitors (must be discontinued at least 3 months prior to selection).
- Plants extracts used for treatment of LUTS (must be discontinued at least 3 months prior to selection).
- Alpha blockers and alpha/beta blockers (must be discontinued at least 1 month prior to selection).

The following treatments must be discontinued at V1 (wash-out period of 2 Weeks)

- NSAIDs by systemic route (except aspirin up to 325 mg/day for cardiovascular prophylaxis).
- Corticosteroids by systemic route.
- Antibiotics by systemic route.
- 5-PDE inhibitors for BPH treatment.
- Mepartricine.

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

	• ACE inhibitors, calcium antagonists, beta blockers, diuretics, sympathomimetics, antihistamines, antidepressants (anticholinergic), atropine, antispasmodic drugs, antiparkinsonism drugs, pseudoephedrine, chlorpheniramine or spironolactone (if non stable dose or initiated 6 weeks or less prior to selection). ***Habits:*** - Patient with a history of drug or alcohol abuse. ***Others:*** - Patient whose follow-up would be difficult because of psychological, family, social or geographical reasons. - Any disorders preventing the patient from decision making and outcome assessment. - Is a family member or work associate (secretary, nurse, technician,…) of the Investigator. - Has participated in another clinical trial within the last 3 months, has received treatment with known remnant effects or undergone investigation liable to interfere with the present clinical trial. - Is participating in another clinical trial. - Mentally unable to understand the nature, objectives and possible consequences of the trial; or refusing to subject himself to its constraints. - Has forfeited his freedom by administrative or legal award or is under guardianship.
Test product, **Dose,** **Mode of administration,** **Batch number:**	PERMIXON ® 160 mg hard capsule. 160 mg b.i.d Per os G06340, expiry date 12/2014
Other product, **Dose,** **Mode of administration,** **Batch number:**	Placebo of Tamsulosine Arrow LP o.a.d. Per os CFS256, expiry date 12/2014
Duration of treatment:	90 days
Reference therapy, **Dose,** **Mode of administration,** **Batch number:**	Tamsulosine Arrow LP 0.4 mg o.a.d. Per os F13037A, expiry date 03/2014
Other product, **Dose,** **Mode of administration,** **Batch number:**	Placebo of Permixon® 160 mg b.i.d. Per os SB0879, expiry date 12/2013
	P00048 GP 4 03 – synopsis page 4/17

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

Criteria for evaluation: **Efficacy:** **Safety:**	**Efficacy criteria** **Main efficacy criteria:** Inflammation biomarkers assay in patients suffering from BPH: o Urine Inflammation markers on the first urine flow after Digital Rectal Examination (DRE) at D1, D30 and D90. o Serum Inflammation markers at D1, D30 and D90: - CRP and Sedimentation Rate. **Secondary efficacy criteria :** - Assessment of I-PSS at selection, D1, D30 and D90. - Assessment of QoL at selection, D1, D30 and D90. - Assessment of sexual function (MSF-4) at D1, D30 and D90. - Assessment of Qmax at D1, D30 and D90, by uroflowmetry. - Assessment of post-void residual urine volume at D1, D30 and D90, by supra- pubic ultrasound. - Assessment of prostate volume at D1, D30 and D90, by transrectal ultrasound. - Link between inflammation biomarkers and BPH clinical symptoms on changes from baseline. - Protein expression profile of urine inflammation biomarkers. **Safety criteria** - Adverse events. - Physical examination at selection visit and at each visit including body temperature, weight and height (at selection visit only). - Vital signs at selection visit and at each visit.
Central Lab methods of urine analysis	The Central assay centre followed the standard instructions for mRNA quantification method using standard reverse transcription-quantitative polymerase chain reaction (RT-qPCR) protocol. RT-qPCR is a sensitive method for the detection of mRNA expression levels. Traditionally RT-qPCR involves two steps: the RT reaction and PCR amplification. RNA is first reverse transcribed into cDNA using a reverse transcriptase, the resulting cDNA is used as templates for subsequent PCR amplification using. Quantification of KLK3 (PSA) specific of prostatic cells was also performed to confirm that results of biomarkers reflected only the expression of these markers in prostatic cells.
	P00048 GP 4 03 – synopsis page 5/17

Name of Company: Pierre Fabre Médicament	Individual Study Table	(For National Authority Use Only)
Name of finished product: Permixon® 160 mg hard capsule	Referring to Module 5 of the Dossier	
Name of active substance: Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)	Vol.: .....Page:	

Statistical methods:

All results provided are exploratory.

The sample size was not based on statistical calculations as the study was exploratory.

Evaluation of efficacy

Primary criteria:

- Urine inflammation markers:

Expression level (up-regulated, no change, down-regulated) on each mRNA marker at D90 defined as:

- Down-regulated = fold change between D90 and baseline ≤ 0.5.
- No change = fold change between D90 and baseline > 0.5 and < 2.
- Up-regulated = fold change between D90 and baseline ≥ 2.

For each mRNA marker, the number and percentage of patients experiencing up-regulated, no change or down-regulated at D30 and D90 were tabulated by treatment group, on the FAS (main descriptive analysis) and on the 3 PP sets (supportive descriptive analysis).

The expression level at D30 was defined in the same way.

If less than 4 markers were available (non missing data) on a urine sample (raw data), the sample was considered as non exploitable (poor quality) at the concerned visit.

Data analysed at D30 and D90 were exploitable data normalised on KLK3 gene (PSA) and considering that baseline is equal to 1 (data available at follow-up visits only if baseline measurable).

It is to be noted that a marker with a value at D90 equal to NI (weakly expressed gene) was considered as down-regulated.

A value equal to NI was considered as non missing data.

- Serum inflammation markers:

For CRP and Sedimentation rate at 1 hour, shift tables (analysis in classes) by treatment group on the Full Analysis Set (FAS) show the number of patients who are normal or abnormal at baseline and then at D30 (respectively D90) (main descriptive analysis).

Sedimentation rate at 2 hours: listing of individual data.

Secondary criteria:

All the secondary efficacy criteria were analysed on the FAS.

- Descriptive statistics were provided for all criteria, by treatment group and assessment time.

Moreover changes from baseline to D30 and D90 of those criteria were analysed using an analysis of covariance with treatment group as fixed effect and the baseline as covariate to compare the effect of L.S.E.S.r. 160 mg versus tamsulosine LP 0.4 mg. The analysis was performed using the following model:

$$\Delta \text{ "criterion"} = \mu + \text{treatment group} + \text{baseline "criterion"} + \text{error}$$

using the MIXED procedure of SAS® software and the type III sums of squares.

- Exploratory analysis of the link between inflammation biomarkers and BPH clinical symptom

Changes from baseline of clinical symptoms (I-PSS score, QoL, MSF-4 and Qmax) were described according to changes from baseline of urinary inflammatory markers of interest (expressed in 3 classes: down-regulated / no change / up-regulated) by treatment group on the PP30 at D30 and on the PP90 at D90.

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

- Exploratory analysis of protein expression profile

It was mentioned in the protocol (see § 4.2.2 and 8.1.1.2.5 of the protocol) that the analysis of protein expression profile was to be supportive and was to be done on the main expressed mRNA genes.

Therefore, out of the main expressed mRNA genes in this study, 10 proteins were selected including 5 cellular proteins (HIF1A, NFKB, PTPRC, ALOX15B and ALOX5) and 5 proteins potentially excreted in urine (IL1B, IL8, CCL2, CXCL10 and MIF). Changes between V2 (baseline) and V4 (D90) were categorised into the 6 following classes and the number and percentage of patients in each category was tabulated by treatment group, on the FAS :

- Not detected to Not detected, i.e. 0 pg/ml at both visits
- Expressed to Not detected, i.e. 0 pg/ml at V4
- Expressed to Expressed (down-regulated), i.e. percent change from baseline < -25%
- Expressed to Expressed (no change), i.e. percent change from baseline between [-25%; +25%]
- Expressed to Expressed (up-regulated), i.e. percen change from baseline > +25%
- Not detected to Expressed, i.e. 0 pg/ml at V2.

- Complementary exploratory analyses:

o *GEE Model (Generalized Estimated Equations)*

At each assessment visit (V2, V3, V4), for each urine inflammation marker of an exploitable* sample, the original value** was categorized as "expressed" (value>0) or "not detected" (missing value or value=NI). Then, changes from baseline (V2) were described at V3 and V4 in 4 classes (Not detected to Not detected / Not detected to Expressed / Expressed to Not detected / Expressed to Expressed).

The GEE model was used to analyse changes from baseline clustered in a binary variable as following :

- first model : "not detected to expressed" *vs.* "the others"
- second model : "expressed to not detected" *vs.* "the others"

In each model, changes were analyzed as repeated measures using an unstructured correlation structure.

The table of parameter estimates also contains the associated p-value. For a given urine marker, a p-value ≤ 0.05 for the variable “treatment” indicates a difference between treatment groups. "Tamsulosine" being considered as the reference group (estimate=0), an estimate < 0 for "Permixon®" indicates that there is less of "not detected to expressed" (first model) in Permixon® *vs.* Tamsulosine. Nevertheless, results have to be interpreted with regard to the baseline status.

** at least 4 markers available on a urine sample*

*** raw data i.e. before "normalization" on LnCap and V2*

o *Analysis of genes expression profile by classes*

In order to get an overview of the expression/non-detection of genes from D1 to D90, a shift-table was provided for each of the 29 urinary biomarkers on the FAS. This table is complementary to the main analysis and to the exploratory analysis above on the binary variable ("expressed" / "not detected").

Name of Company: Pierre Fabre Médicament	Individual Study Table	(For National Authority Use Only)
Name of finished product: Permixon® 160 mg hard capsule	Referring to Module 5 of the Dossier	
Name of active substance: Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)	Vol.: .....Page:	

- *Wilcoxon Rank-Sum test*

For each urine inflammation marker, changes from baseline (V2) were also calculated at V3 and V4 on values normalised with LnCap. Beforehand, for exploitable* samples, missing values were considered equal to 0 (not detected) while NI values were considered equal to 0.001.

Then for each marker, a Wilcoxon rank-sum test was performed on changes from baseline at each visit (resp. V3 and V4) in order to compare the two treatment groups. This non-parametric test was used because of the non-normal data distributions. This test is based on analysis of the Wilcoxon scores (ranks of the observations).

In case of a significant p-value (≤ 0.05), the median which is a location indicator permits the interpretation of the results.

On the other side, if the number of non-null values is to low and the location indicators such as Q1, the median and Q3 take the value 0, neither the median nor the mean are relevant to express the difference between treatment groups.

- *Exploratory analysis of urinary markers on patients subgroups according to I-PSS scores*

In addition to the exploratory analyses planned in the protocol, a descriptive analysis of the urine inflammation markers (fold change) was performed at D30 and D90 by treatment group on two subgroups of FAS defined according to change in I-PSS total score at D90 :

- change $\leq$ -5 (corresponding to the median under Permixon),
- change > - 5.

- *Number of genes expressed at D1, D30 and D90*

The number of genes expressed at D1, D30 and D90 was determined for each patient and described by treatment group on the PP set.

- *Exploratory analysis of the subgroups according to the 3rd quartile of biomarkers at V2*

Exploratory analyses were performed by treatment group on IPSS changes and, on urine biomarkers fold-changes, at D30 and D90 on the Full Analysis Set. Thus a description was done on subgroups defined according to the overall value of the 3rd quartile of markers of interest (normalised on LnCap) at V2. The objective was to described the changes between D1 (baseline) and follow-up visits by treatment group on a subgroup of patients who overexpressed one inflammation biomarker at V2 (with a value > Q3) versus the other patients.

Evaluation of safety

Descriptive statistics on all randomised patients having received at least once the trial drug (FAS).

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

Summary - Conclusions:

The conduct of this international, multicentric, randomised, double-blind, parallel group prospective study was satisfactory and in accordance with the Good Clinical Practice Guidelines.

Disposition of patients:

A total of 323 patients were **screened** (i.e. signature of a consent form). Out of these patients, 303 were **retained at Selection Visit** (V1) .

Among them, 206 were **randomised** (included in the study). Three patients did not take the study treatment and were excluded from the Full Analysis Set (FAS). Therefore, there were 203 patients in the FAS (102 with Permixon® and 101 with Tamsulosine) including 83 completers with Permixon® and 86 with Tamsulosine.

Both treatment groups had similar demographics and other BPH baseline characteristics; mean age was 65.8 years, mean duration of BPH 3.96 years, mean I-PSS 17.2, mean QoL 3.8, mean MSF-4 7.2, mean Qmax 10.74 mL/s, mean PVR 47.99 cm^3 and mean prostate volume 47.55 cm^3. In spite of an important variability observed in expression of each available urinary inflammation marker, this expression was globally similar in both groups. Compliance to the study treatment during the double-blind period was very good and similar in both groups (98.3 and 95.3%).

One patient (1104002) presented an error of treatment allocation at visit 3 and, thus, took both treatments along the study (Tamsulosine and then Permixon®). He was assigned, for both efficacy and safety analysis, in the treatment group corresponding to the first treatment taken (i.e. group in which he was randomised).

Efficacy results

As this study was exploratory, it was not powered to highlight differences between the two groups.

Primary criteria:

Analysis of urinary inflammation markers on the FAS compared Permixon® versus Tamsulosine activity on each inflammation biomarker between V2 (baseline) and follow up visits (V3 and V4) in regards to regression (down-regulation) and progression (up-regulation) of gene expression.

Out of the 29 inflammatory markers tested, 15 were expressed at V2 and V4 in a number of patients considered sufficient per group (at least 30 patients per group) : IL-1b, IL-8, PLA2G2A, CCL2, ALOX15B, ALOX5, CAT, HIF1A, MIF, NFKB1, PTGES2, PTGES3, PTGS2, PTPRC and STAT3.

According to the FAS and after a 3-month treatment period, Permixon® had a positive effect (defined as a difference of at least 5% in favour of Permixon® compared to Tamsulosine treatment) on 9 of these 15 biomarkers. For 3 of them (HIF1A, PTGES3, PTPRC), the positive effect of Permixon® was observed on both regression and less progression of gene expression compared to Tamsulosine group. For the other 6 ones (ALOX15B, CAT, CCL2, IL8, NFKB1, STAT3), the positive effect of Permixon® was observed on either regression or less progression of gene expression.

Conversely, the effect of Tamsulosine was positive for 3 of the 15 markers (PTGES2, PTGS2, IL1B), the 2 first ones on less progression of gene expression and the last one on both.

For the 3 last markers expressed in at least 30 patients/group (ALOX5, MIF and PLA2G2A), there was no difference between groups.

In the table below, is presented for each of these biomarkers the most frequently expressed the number of patients by expression profile at D90.

Name of Company: Pierre Fabre Médicament	Individual Study Table	(For National Authority Use Only)
Name of finished product: Permixon® 160 mg hard capsule	Referring to Module 5 of the Dossier	
Name of active substance: Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)	Vol.: .....Page:	

Gene expression profiles expressed at least in 30 patients per group: number of patients by expression level at D90 (FAS)

	Permixon n=102	Tamsulosine n=101
HIF1A Fold change		
V4 (D90)		
Number of available data	82	79
Down-regulated	21 (25.6 %)	12 (15.2 %)
No change	49 (59.8 %)	42 (53.2 %)
Up-regulated	12 (14.6 %)	25 (31.6 %)
PTGES3 Fold change		
V4 (D90)		
Number of available data	80	79
Down-regulated	21 (26.3 %)	12 (15.2 %)
No change	46 (57.5 %)	46 (58.2 %)
Up-regulated	13 (16.3 %)	21 (26.6 %)
PTPRC Fold change		
V4 (D90)		
Number of available data	40	36
Down-regulated	20 (50.0 %)	13 (36.1 %)
No change	6 (15.0 %)	8 (22.2 %)
Up-regulated	14 (35.0 %)	15 (41.7 %)
ALOX15B Fold change		
V4 (D90)		
Number of available data	75	62
Down-regulated	10 (13.3 %)	11 (17.7 %)
No change	53 (70.7 %)	36 (58.1 %)
Up-regulated	12 (16.0 %)	15 (24.2 %)
CAT Fold change		
V4 (D90)		
Number of available data	80	80
Down-regulated	12 (15 %)	11 (13.8 %)
No change	53 (66.3 %)	46 (57.5 %)
Up-regulated	15 (18.8 %)	23 (28.8 %)
CCL 2 Fold change		
V4 (D90)		
Number of available data	37	36
Down-regulated	17 (45.9 %)	16 (44.4 %)
No change	9 (24.3 %)	6 (16.7 %)
Up-regulated	11 (29.7 %)	14 (38.9 %)
IL8 Fold change		
V4 (D90)		
Number of available data	73	68
Down-regulated	35 (47.9 %)	33 (48.5 %)
No change	18 (24.7 %)	13 (19.1 %)
Up-regulated	20 (27.4 %)	22 (32.4 %)
NFKB1 Fold change		
V4 (D90)		
Number of available data	75	68
Down-regulated	22 (29.3 %)	20 (29.4 %)
No change	41 (54.7 %)	31 (45.6 %)
Up-regulated	12 (16.0 %)	17 (25.0 %)

1/2 (CONTINUED)

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

		Permixon n=102	**Tamsulosine n=101**
STAT3 Fold change			
V4 (D90)			
	Number of available data	82	72
	Down-regulated	16 (19.5 %)	9 (12.5 %)
	No change	51 (62.2 %)	48 (66.7 %)
	Up-regulated	15 (18.3 %)	15 (20.8 %)
ALOX5 Fold change			
V4 (D90)			
	Number of available data	75	68
	Down-regulated	23 (30.7 %)	18 (26.5 %)
	No change	32 (42.7 %)	34 (50.0 %)
	Up-regulated	20 (26.7 %)	16 (23.5 %)
MIF Fold change			
V4 (D90)			
	Number of available data	82	83
	Down-regulated	9 (11 %)	8 (9.6 %)
	No change	56 (68.3 %)	56 (67.5 %)
	Up-regulated	17 (20.7 %)	19 (22.9 %)
PLA2G2A Fold change			
V4 (D90)			
	Number of available data	75	74
	Down-regulated	13 (17.3 %)	16 (21.6 %)
	No change	48 (64.0 %)	42 (56.8 %)
	Up-regulated	14 (18.7 %)	16 (21.6 %)
PTGES2 Fold change			
V4 (D90)			
	Number of available data	77	69
	Down-regulated	18 (23.4 %)	14 (20.3 %)
	No change	39 (50.6 %)	42 (60.9 %)
	Up-regulated	20 (26.0 %)	13 (18.8 %)
PTGS2 Fold change			
V4 (D90)			
	Number of available data	54	51
	Down-regulated	19 (35.2 %)	19 (37.3 %)
	No change	21 (38.9 %)	23 (45.1 %)
	Up-regulated	14 (25.9 %)	9 (17.6 %)
IL1B Fold change			
V4 (D90)			
	Number of available data	60	57
	Down-regulated	24 (40.0 %)	32 (56.1 %)
	No change	16 (26.7 %)	10 (17.5 %)
	Up-regulated	20 (33.3 %)	15 (26.3 %)

2/2

Name of Company: Pierre Fabre Médicament	Individual Study Table	(For National Authority Use Only)
Name of finished product: Permixon® 160 mg hard capsule	Referring to Module 5 of the Dossier	
Name of active substance: Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)	Vol.: .....Page:	

These data were confirmed on the Per Protocol Set at D90 (PP90).

Regarding serum inflammatory markers (CRP and sedimentation rate), in both treatment groups, these parameters remained normal throughout the study in most cases when comparing D90 to baseline (81.7% with Permixon®, 82.7% with Tamsulosine for CRP and 77.6% with Permixon® and 84.9% with Tamsulosine for sedimentation rate).

Secondary criteria:

- Clinical symptoms

An improvement of the clinical symptoms I-PSS, QoL, Qmax and prostate volume was observed after a 3-month treatment period in both groups.

Regarding the I-PSS score, the mean corresponding change adjusted with respect to baseline was – 4.28 and – 6.56 in the Permixon® and the Tamsulosine groups, respectively.

The adjusted mean of Quality of Life score change was - 0.87 with Permixon® and - 1.29 with Tamsulosine. The adjusted mean of Qmax change was + 1.77 mL/s with Permixon® and + 2.09 with Tamsulosine and the adjusted mean of prostate volume change was - 0.99 cm^3 with Permixon® and - 0.53 with Tamsulosine.

These results at D90 prolonged those at D30 in most instances.

In contrast, no clear effect was observed at D90 for the MSF4 score and the post void urine volume. The adjusted mean of MSF4 change was +0.36 with Permixon® and + 0.64 with Tamsulosine. For the post void urine volume (PVR), we consider the results not reliable enough to draw conclusions in view of the wide variations observed within each group.

The activity of both treatments on clinical symptoms is summarized in the table below. Statistics at baseline are those obtained on the entire Full Analysis Set .

Name of Company: Pierre Fabre Médicament	Individual Study Table	(For National Authority Use Only)
Name of finished product: Permixon® 160 mg hard capsule	Referring to Module 5 of the Dossier	
Name of active substance: Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)	Vol.: .....Page:	

Clinical symptoms at each assessment visit and adjusted means on changes from baseline at D90

		Permixon n=102	Tamsulosine n=101
I-PSS: Total score	Baseline Mean (SD)	17.7 (4.4)	16.8 (4.5)
	Value D30 Mean (SD) OC*	13.9 (5.5)	11.8 (5.4)
	Value D90 Mean (SD) LOCF**	13.2 (6.0)	10.3 (5.5)
	Change D90-Baseline LSMean*** (SE)	-4.28 (0.55)	-6.56 (0.55)
QoL score	Baseline Mean (SD)	3.9 (0.9)	3.8 (0.9)
	Value D30 Mean (SD) OC	3.3 (1.3)	2.8 (1.3)
	Value D90 Mean (SD) LOCF	3.0 (1.4)	2.5 (1.2)
	Change D90-Baseline LSMean*** (SE)	-0.87 (0.12)	-1.29 (0.12)
MSF4 score	Baseline Mean (SD)	7.4 (4.5)	6.9 (4.5)
	Value D30 Mean (SD) OC	7.2 (4.5)	7.8 (4.4)
	Value D90 Mean (SD) LOCF	7.7 (4.8)	7.7 (4.7)
	Change D90-Baseline LSMean*** (SE)	0.36 (0.35)	0.64 (0.35)
Qmax	Baseline Mean (SD)	10.88 (2.69)	10.60 (3.03)
	Value D30 Mean (SD) OC	11.86 (4.76)	13.08 (4.57)
	Value D90 Mean (SD) LOCF	12.53 (5.21)	12.73 (4.42)
	Change D90-Baseline LSMean*** (SE)	1.77 (0.46)	2.09 (0.45)
Transrectal prostate volume	Baseline Mean (SD)	48.82 (20.80)	46.29 (13.88)
	Value D30 Mean (SD) OC	46.61 (17.85)	47.72 (16.08)
	Value D90 Mean (SD) OC	47.95 (20.05)	46.73 (16.83)
	Change D90-Baseline LSMean*** (SE)	-0.99 (1.08)	-0.53 (1.05)
Supra-pubic PVR volume (cm3)	Baseline Mean (SD)	53.82 (57.07)	42.04 (47.61)
	Value D30 Mean (SD) OC	62.88 (67.14)	55.73 (55.18)
	Value D90 Mean (SD) OC	64.11 (63.31)	47.41 (51.29)
	Change D90-Baseline LSMean*** (SE)	15.22 (5.80)	4.04 (5.84)

*: Observed case method (OC)
**: Last Observation Carried Forward method (LOCF)
***: Adjusted means from the ANCOVA model : Change = Baseline + Treatment

- Exploratory analysis of the link between inflammation biomarkers and BPH clinical symptoms

This analysis was performed on the PP30 at D30 and on the PP90 at D90 according to the 11 following markers: CCR7, ALOX15B, ALOX5, HIF1A, NFKB1, PTPRC, CCL2, IL8, MIF, IL1B and CXCL10. The choice of these markers was based on the results of the different analyses performed. No apparent relationship was identified between changes in clinical symptoms and changes of these 11 markers. It is to note the variability observed within the groups and the small number of patients in each sub group.

Name of Company: Pierre Fabre Médicament	Individual Study Table	(For National Authority Use Only)
Name of finished product: Permixon® 160 mg hard capsule	Referring to Module 5 of the Dossier	
Name of active substance: Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)	Vol.: .....Page:	

- Exploratory analysis of protein expression profile

Out of the 10 proteins selected (CXCL10 was also included in this selection because of a mRNA expression frequency close to the choosen limit of 30 patients/group i.e 29 patients/group), 7 were not detected neither in urine nor in cell pellets.
Finally, 3 proteins were successfully analysed CCL2, CXCL10 and MIF.

Regarding CCL2 protein, after a 3-month treatment period, we observed a decrease in the number of patients who expressed this protein (from 54.8 % at baseline to 35.6 % at D90) in the Permixon® group contrary to Tamsulosine group (from 46.5 % at baseline to 47.9 % at D90). Furthermore, for 27.4% of patients treated by Permixon®, this protein was no longer expressed at D90 (*vs.* 15.5% in the Tamsulosine group).

This was also observed with CXCL10 and MIF. Indeed, regarding CXCL10 protein, after a 3-month treatment period, we observed a decrease in the number of patients who expressed this protein (from 74.0 % at baseline to 63.0 % at D90) in the Permixon® group contrary to Tamsulosine group (from 64.8 % at baseline to 67.6 % at D90). Furthermore, for 20.5% of patients with Permixon®, this protein was no longer expressed at D90 (*vs.* 12.7% in the Tamsulosine group).

Regarding MIF protein, if all patients expressed this protein at D90, a higher decrease of the expression of this protein was observed in the Permixon® group compared to the Tamsulosine group (42.5% *vs.* 23.9% of the patients respectively).

- Complementary exploratory analyses

o *GEE Model (Generalized Estimating Equations)*

On the FAS, a significant statistical difference between treatment groups was highlighted for 2 markers (ALOX5 and ALOX15B) in favour of Permixon®. The proportion of patients for whom those markers were not detected at V2 (baseline) but expressed at the time of both follow-up visits is greater ($p < 0.05$) in the Tamsulosine group than in the Permixon® group suggesting a reduction in the increased expression of these 2 markers with Permixon®. However, the proportion of « not detected » was greater in the Tamsulosine group at V2.

Moreover, for 2 other markers (CCR7 and NFKB1), a trend is observed in favour of Permixon®. The proportion of patients for whom CCR7 and NFKB1 were expressed at baseline but not detected at both follow-up visits tends to be greater in the Permixon® group than in the Tamsulosine group over the limit of statistical significance ($p = 0.082$ and $p = 0.075$ for CCR7 and NFKB1, respectively).

These results were confirmed in the Per Protocol set. In addition, in this set, a statistical difference in favour of Permixon® was observed in IL8 expression for the patient class “expressed to not detected”.

o *Wilcoxon rank-sum test*

On the FAS at D90, for 4 markers (ALOX15B, HIF1A, CCR7 and CXCL6) p-values indicate a statistically significant difference or a difference at the limit of the significance between treatment groups. But for CCR7 and CXCL6 (expressed in very few patients), as the location indicators (Q1, Q2 and Q3) take the value 0, neither the mediane nor the mean are relevant to express the difference between treatment groups. Therefore, only for ALOX15B and HIF1A, it may be concluded that treatment with Permixon® (*vs.* Tamsulosine) tends to reduce the expression of both markers.

Name of Company: Pierre Fabre Médicament	Individual Study Table	(For National Authority Use Only)
Name of finished product: Permixon® 160 mg hard capsule	Referring to Module 5 of the Dossier	
Name of active substance: Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)	Vol.: .....Page:	

- *Exploratory analysis on urinary markers on patient subgroups according to I-PSS scores*

Patient subgroups were defined according to the I-PSS change at D90 considering that patients with a change in IPSS total score lower than or egal to - 5 points correspond to the subgroup of patients with the best clinical response to treatment.
After a 3-month treatment period, in this subgroup of best I-PSS response with Permixon®, we observed either a more important down-regulation or a less up-regulation (defined as a difference of at least 5%) of 7 of the markers of interest (CCL2, CXCL10, PTPRC, PTGS2, IL1B, IL8 and PLA2G2A) compared to the subgroup of the other patients treated by Permixon®. These results suggest a relationship between the expression level of these markers and I-PSS evolution in patients treated with Permixon®.
To go further into these results, we explored the subgroup of patients who overexpressed (3rd quartile) the markers of interest at baseline (HIF1A, PTGES3, PTPRC, ALOX15B, CAT, CCL2, IL8, NFKB1, STAT3, ALOX5, MIF, PTGES2, PTGS2, PLA2G2A, IL1B and CXCL10).
At D90, we observed a better response to I-PSS (median and/or mean) in the Permixon® sub group who overexpressed CXCL10, CCL2, PTPRC and PTGS2 at baseline compared to the other Permixon® sub group, contrary to the Tamsulosine subgroups. The more favorable results were noted for CXCL10 (median of the change from baseline: – 7.0 in the Permixon® sub group who overexpressed this marker at baseline *vs.* – 4.0 in the other Permixon® sub group) and CCL2 (median of the change from baseline: – 6.0 *vs.* – 4.0 in the Permixon® sub group who overexpressed this marker at baseline and in the other Permixon® sub group respectively).

- *Number of genes expressed at D1, D30 and D90*

No apparent difference was observed between groups.

- *Exploratory analysis of the subgroups according to the 3rd quartile of biomarkers at V2*

In this analysis, in each treatment group, for each biomarker of interest at baseline (HIF1A, PTGES3, PTPRC, ALOX15B, CAT, CCL2, IL8, NFKB1, STAT3, ALOX5, MIF, PTGES2, PTGS2, PLA2G2A, IL1B and CXCL10), we compared the subgroup of the patients who overexpressed one of the biomarkers (> Q3) *vs.* the other patients and we observed the evolution of the other markers expression under treatment.

After a 3-month treatment period, the results generally showed that in the subgroup of patients treated by Permixon® and who overexpressed the markers, whatever the biomarker, there was a more important down-regulation and/or a less up-regulation of the other biomarkers (defined as a difference of at least 5%) than in the subgroup who does not overexpress the biomarkers. Likewise, this effect appeared to be similar in the Tamsulosine group except for the subgroup who over expressed MIF.
However, we also compared the subgroup of patients who over expressed one of the biomarkers in the Permixon® group to the corresponding subgroup in the Tamsulosine group.

After the 3-month treatment period, we observed, in the subgroups who overexpressed PTGS2, MIF, CAT, HIF1A and ALOX5, that the effect of Permixon® treatment on the other markers expression was increased compared to Tamsulosine.

Name of Company: Pierre Fabre Médicament	Individual Study Table	(For National Authority Use Only)
Name of finished product: Permixon® 160 mg hard capsule	Referring to Module 5 of the Dossier	
Name of active substance: Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)	Vol.: .....Page:	

Safety results

Analysis of adverse events was performed on the Full Analysis Set including all patients who took at least once the medicine tested or the control treatment.

The percentage of patients with at least one AE was 29.4% in Permixon® group *vs.* 30.7% in Tamsulosine group with 41 AEs reported with Permixon and 50 AEs with Tamsulosine. A total of 10.8% (Permixon®) *vs.* 8.9% (Tamsulosine) had at least one related treatment emergent AE. There were 8 SAEs reported during the study including 4 during treatment administration. Only one SAE with Tamsulosine (bilateral gynecomastia) was considered reasonably related and is unlisted as per the corresponding SmPC.

The most frequent (>2% of patients) treatment emergent AEs (preferred term) were retrograde ejaculation (4% of patients), constipation (3%) and back pain (3%) with Tamsulosine while no adverse event occurred at a frequency of more than 2% with Permixon®.

No related treatment emergent AE had a frequency over 1% in the Permixon® group contrary to the Tamsulosine group in which ejaculation failure (2%), retrograde ejaculation (2%) and asthenia (2%) were noted.

With respect to treatment discontinuation, respectively 7.8% of patients with Permixon® *vs.* 3% of patients with Tamsulosine had at least one AE leading to study drug discontinuation (table below).

Group	Subject-Sex -Age	Reported term
Permixon	0501008-M-57	Feeling stuffy nose
		Palpitation
	0502033-M-69	Rash
	0511013-M-74	Dizziness sensation
		Persistant tiredness
	0513007-M-73	Abdominal pain
		Dry mouth
		Insomnia
		Nightmare
	0802013-M-60	Erectyle disfunction
	0805014-M-68	Groin testicular. the patient suffered from pubic pain/ache
	1002002-M-67	Diarrhea
		Joint swelling of both hands
		Hypertension
	1111007-M-46	Epigastric pain
Tamsulosine	0502026-M-53	Bilateral gynecomastia
	0802012-M-61	Anejaculation
	1003002-M-62	Weight loss (between v2-v3)

Conclusion

The conduct of this international, multicentric, randomised, double-blind, parallel group prospective study was satisfactory and in accordance with the Good Clinical Practice Guidelines. The inclusion objectives were achieved.

The primary objective was to evaluate the effect of Permixon® and Tamsulosine on inflammation biomarkers in urines and serum of patients suffering from BPH.

Name of Company: Pierre Fabre Médicament	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **Permixon® 160 mg hard capsule**	**Referring to Module 5 of the Dossier**	
Name of active substance: **Lipidosterolic Extract of Serenoa repens (L.S.E.S.r)**	**Vol.: .....Page: .....**	

The results observed in this study with Permixon® on some inflammation mRNA markers and proteins confirm the previous in-vitro and in vivo studies on the inflammation pathway, one of the mechanisms of action of Permixon® on BPH symptoms treatment.

Indeed, the results of the main efficacy analysis on urinary biomarkers showed that the expression mRNA of several inflammation markers expressed in prostatic cells (HIF1A, PTGES3, PTPRC, ALOX15B, CAT, CCL2, IL8, NFKB1, STAT3) could be prevented or reduced by Permixon® treatment. The effect of Permixon® on either regression (down regulation) and/or progression (up regulation) of these inflammatory markers was more important than the α-blocker Tamsulosine (results on the FAS analysis set, confirmed on the Per Protocol Set at D90, PP90).

Three proteins were successfully analysed (CCL2, CXCL10 and MIF) with a positive effect on their expression after a 3-month treatment period observed in the Permixon® group only.

Analysis of clinical signs showed an improvement in both treatment groups. Specifically there was more than 2 points improvement in the global score I-PSS after 3 months of treatment. This improvement was noted in the two groups as soon as 1 month of treatment and was associated with a slight improvement in Quality of Life, an increase in Qmax and a slight decrease in prostatic volume.

Moreover, no clear effect was observed in the MSF4 scale. As for post-void residual volume, because of variabilities due to both the evaluators and the use of the devices we consider the results not sufficiently reliable to draw conclusions.

Furthermore, a link could be identified between I-PSS and 4 inflammation biomarkers (mRNA). Indeed, in the subgroups of patients treated with Permixon® and who overexpressed (3rd quartile) CXCL10, CCL2, PTPRC and or PTGS2 at baseline, we observed after a 3-month treatment period a better response to I-PSS compared to the other Permixon® sub group contrary to the Tamsulosine sub groups.

These results suggest a relationship between the expression level of these markers and I-PSS evolution in patients treated with Permixon®.

Altogether, these results suggest a positive effect of Permixon® on different inflammation pathways: central inflammation (i.e PTPRC, NFKB1, IL8, IL1B and STAT3), lipooxygenase / prostaglandin effect (i.e ALOX5, ALOX15B, PTGES3, PTGS2 and PLA2G2A), macrophages attraction and T-cell activities (i.e CCL2 and MIF) as well as hypoxic inflammation (i.e HIF1A and CAT).

There were 8 SAE reported during the study including 4 during the treatment period and no death. Only one SAE with Tamsulosine (bilateral gynecomastia) was considered reasonably related and is unlisted as per the SmPC.

The frequency of patients with AE was comparable between treatment groups (29.4% with Permixon® and 30.7% with Tamsulosine).

No related treatment emergent AE had a frequency over 1% in the Permixon® group contrary to the Tamsulosine group with ejaculation failure (2%), retrograde ejaculation (2%) and asthenia (2%).

In this mid-term (90 days) study of treatment of BPH related symptoms with Permixon® *vs.* Tamsulosine, the safety profile of the two products can be considered satisfactory.

Date of report	Final Version – 22 OCT 2014