EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 December 2013
EMA/CHMP/138212/2014
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Winfuran

International non-proprietary name: nalfurafine

Procedure No. EMEA/H/C/002683/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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An agency of the European Union

Product information

Name of the medicinal product:	Winfuran
Applicant:	Toray International U.K. Limited Verulam Gardens 70 Gray's Inn Road London WC1X 8NH UNITED KINGDOM
Active substance:	NALFURAFINE HYDROCHLORIDE
International Nonproprietary Name/Common Name:	NALFURAFINE
Pharmaco-therapeutic group (ATC Code):	nalfurafine (V03AX02)
Therapeutic indication(s):	Treatment of severe uraemic pruritus (UP) in patients of 18 years of age or older, with end stage renal disease (ESRD) undergoing regular dialysis.
Pharmaceutical form(s):	Concentrate for solution for infusion
Strength(s):	10 micrograms/ml
Route(s) of administration:	Intravenous use
Packaging:	ampoule (glass)
Package size(s):	5 ampoules, 6 ampoules, 10 ampoules and 12 ampoules

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List of abbreviations

ADR	Adverse drug reaction
AE	Adverse event
ANCOVA	Analysis of covariance
AUC	Area under the plasma concentration-time curve
AUC_{0-t}	Area under the curve from time 0 until time t
$AUC_{0-\infty}$	Area under the curve from time 0 to infinity
AUCD	AUC difference
BMI	Body mass index
CAPD	Continuous ambulatory peritoneal dialysis
CHF	Congestive heart failure
CI	Confidence interval
Cmax	Maximum drug concentration
CMH	Cochran-Mantel-Haenszel
C_{min}	Minimum drug concentration
CL	Body clearance
CNS	Central nervous system
CRI	Chronic renal insufficiency
CYP	Cytochrome P
de-CPM	17-decyclopropylmethylated nalfurafine
DEVD	Dialysis effect of VAS difference
ECG	Electrocardiography
EMA	European medicines Agency
ESRD	End stage renal disease
FAS	Full Analysis Set
GI	Gastrointestinal
HD	Haemodialysis
ICH	International Conference on Harmonisation
IgA	Immunoglobulin A
i.d.	Intradermal
i.m.	Intramuscular
i.v.	Intravenous
K_{elim}	Elimination rate constant
Ki	Inhibition constant
LC/MS/MS	Liquid chromatography/mass spectrometry/mass spectrometry
LOCF	Last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities

MPA	Medical Products Agency
MRT	Mean residence time
NDEVD	Non-dialysis effect of VAS difference
NF	Nalfurafine Toray
NFU	Nalfurafine Toray
NFA-G	3-glucuronide of nalfurafine
NNT	Number needed to treat
NYHA	New York Heart Association
PD	Pharmacodynamics
PI	Pain intensity
PIR	Pruritus intensity relief
PK	Pharmacokinetics
PL	Placebo
p.o.	Oral
PP	Per protocol
PPID	Peak pruritus intensity difference scores
PPIR	Percentage change in PIR
PPR	Peak pruritus relief
PT	Preferred term
PU	Pain bothering
QoL	Quality of life
QTc	Corrected QT
SAE	Serious adverse event
SD	Standard Deviation
s.c.	Subcutaneous
SOC	System Organ Class
SPID	Summed pruritus intensity difference score
t1/2	Elimination half-life
TEAE	Treatment emergent adverse event
T_{max}	Time to maximum drug concentration
TPR	Total pruritus relief
TRK-820	Nalfurafine Toray
UK	United Kingdom
UP	Uraemic pruritus
USA (US)	United States of America
VAS	Visual Analogue Scale
Vd	Volume of distribution
V_{ss}	Volume of distribution at steady state
Dose	All doses expressed in this document are per body e.g. 5 μg is a

fixed dose of 5 μg per body.

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Toray International U.K. Limited submitted on 28 June 2012 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Winfuran, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 April 2007.

Winfuran, was designated as an orphan medicinal product EU/3/02/115 on 11 September 2002. Winfuran was designated as an orphan medicinal product in the following indication: treatment of uraemic pruritus.

The applicant applied for the following indication:

Treatment of severe uraemic pruritus (UP) in patients of 18 years of age or older, with end stage renal disease (ESRD) undergoing regular dialysis.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application. The applicant indicated that nalfurafine was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0094/2012 on the agreement of a paediatric investigation plan (PIP).
At the time of submission of the application, the PIP was not yet completed as the measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's requests for consideration

Conditional Marketing Authorisation

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of the above mentioned Regulation and Art 4(1) of Commission Regulation

(EC) No 507/2006 based on the following claims:

(1) Nalfurafine falls within the scope of Article 3(1) and (2) of Regulation (EC) No 726/2004 and was designated as orphan medicinal product in accordance with Article 3 of regulation (EC) No 141/2000 on 11th September 2002 (Orphan Designation number: EU/3/02/115);

(2) The risk-benefit balance of Nalfurafine, as defined in Article 1(28a) of Directive 2001/83/EC, is positive;

(3) It is likely that the applicant will provide comprehensive clinical data;

(4) Treatment of UP is a currently unmet medical need, which will be fulfilled with Nalfurafine,

(5) The benefit to public health of the immediate availability of Nalfurafine outweighs the risks inherent in the fact that additional data are still required.

New active Substance status

The applicant requested the active substance nalfurafine hydrochloride contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Protocol Assistance

Scientific Advice was obtained for nalfurafine hydrochloride at MPA, Sweden in 1999 and 2002.

The applicant received Protocol Assistance from the CHMP in 2004. The Protocol Assistance pertained to clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur:	**Romaldas Mačiulaitis**	**Co-Rapporteur:**	**Kristina Dunder**

- The application was received by the EMA on 28 June 2012.
- The procedure started on 18 July 2012.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 8 October 2012. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 5 October 2012
- During the meeting on 15 November 2012, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 15 November 2012
- The applicant submitted the responses to the CHMP consolidated List of Questions on 23 May 2013.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 21 June 2013
- During the PRAC meeting on 11 July 2013, the PRAC agreed on a PRAC RMP advice and assessment overview
- During the CHMP meeting on 25 July 2013, the CHMP agreed on a List of Outstanding issues to be addressed in writing and in an oral explanation by the applicant
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 21 October 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 5 November 2013
- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 15 November 2013
- During the CHMP meeting on 20 November 2013, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 19 December 2013, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Winfuran.

2. Scientific discussion

2.1. Introduction

Problem statement

Uremic pruritus (UP) is a common and bothersome symptom among patients with end-stage-renal-disease (ESRD). The prevalence of patients with UP is approximately 3.5 in 10,000 persons in the EU. In one of the largest trials (the Dialysis Outcomes and Practice Patterns Study (DOPPS)), pruritus was experienced by 42% of dialysis patients.

The pathophysiology of uremic pruritus is poorly understood. Hypotheses implicating immunologic and opioidergic systems have been proposed and the therapies are usually seen as the ones targeting those paths. The immunohypothesis proposes that UP is the result of systemic inflammation rather than a local skin disorder. Immunomodulating therapies such as ultraviolet B phototherapy, thalidomide and calcineurin inhibitors have been shown to decrease UP in some studies. The opioid hypothesis proposes that imbalances in the expression of mu and kappa opioid receptors cause pruritus. Thus, pruritus is increased by mu-receptor activation and kappa-receptor blockade, and decreased by kappa-receptor activation and mu-receptor blockade (Ikoma A et al. 2006). This dysbalance hypothesis is supported by the observation that the ratio of the mu-receptor agonist (beta-endorphin) to the kappa-receptor agonist (dynorphin-A) is increased in hemodialysis patients compared with healthy controls, and this ratio increased with severity of pruritus. Number of contributing factors for UP pathogenesis are considered: mast cell release of histamine/other pruritogens and xerosis.

No single cause underlying UP has been identified. Multiple factors have been associated in observational studies and supportive therapies that are used to treat UP have targeted such factors. A number of risk factors have been identified in multiple studies: (i) inadequate dialysis, (ii) hyperparathyroidism, (iii) elevated calcium x phosphorus product, (iv) xerosis, (v) elevated serum magnesium and aluminium

concentrations. Less convincing associations have also been made to anemia, male gender, hypervitaminosis-A, increased beta 2 microglobulin levels, serotype HLA-B35, and comorbidities including congestive heart failure, neurologic disease, and ascites. Risk appears to be independent of age, ethnicity, type of dialysis, and underlying renal disease.

Recommendations for the treatment of UP are limited and based on anecdotal reports and small uncontrolled clinical trials. Treatment is not unified while one of the options is to use a stepwise approach that depends upon the severity of symptoms and the response to initial therapies.

(1) The initial therapy for all dialysis patients with UP includes: (i) optimal dialysis since underdialysis is commonly associated with pruritus; (ii) optimal treatment of hyperparathyroidism, hyperphosphatemia and hypermagnesemia and (iii) the regular use of emollients, and/or topical analgesics, such as pramoxine lotion. For the dialysis, a modification to the prescription that has been reported to lessen pruritus is switching to a biocompatible dialysis membrane (such as polymethylmethacrylate [PMMA]) in the rare patient who is being dialyzed with a bioincompatible membrane (Lin et al., 2008, Kato et al, 2001 and Aucella et al., 2007).

(2) Second step in UP therapy is considered to for so called "Resistant UP" cases. The definition of resistant UP is arbitrary and one of those employed is "continued symptoms despite adequate dialysis, optimization of metabolic parameters, and the use of topical emollients and analgesics for approximately four weeks" (Tzeremas T and Kobrin SM. Uremic pruritus. 2012 UpToDate [serial online]). For such patients various treatment options are tried starting with an oral antihistamine, and if that is ineffective after a one to two week trial, gabapentin is used. All these treatments are off-label.

(3) Third step is considered to so called "Refractory UP" that are refractory to emollients, topical analgesics and oral antihistamines or gabapentin. For patients who are refractory to these agents, ultraviolet B phototherapy is a therapeutic option. Kidney transplantation is definitive therapy for UP.

Whereas no satisfactory treatment exists, the Applicant recognised that there is a medical need for effective treatment for severe UP that could improve quality of life in those patients.

About the product

Nalfurafine hydrochloride (NFU) is a novel derivative of the opioid receptor antagonist, naltrexone. Mechanistically, NFU is a potent and selective agonist for the kappa-opioid (κ-opioid) receptor without any appreciable significant action on the μ- or δ- opioid receptors. Thus, NFU differs from other existing opioid drugs in terms of kappa-opioid receptor selectivity, absence of euphoric effects and a little or no tolerance over prolonged treatment.

During the R&D process NFU was tested and/or used in various dosages and for several routes of administration, while the clinical development programme has been focussed on the development of an intravenous (i.v.) dosing formulation of Nalfurafine. Some studies have also been performed on intramuscular (i.m.) and oral (p.o.) formulations. Haemodialysis (HD) patients have ready access for i.v. dosing via the dialysis vascular access and attend a clinic regularly for dialysis sessions. Thus, the proposed administration of 3 times a week follows the usual scheduled HD programme and so facilitates drug administration both for patients and for nurses. In addition, fixed dose of 5 μg facilitates administration in clinical practice compared to a body weight dosing schedule.

The i.v. formulation of Nalfurafine was designated as an Orphan Medicinal Product for the treatment of UP on 11 September 2002. According to the Applicant, the clinical development programme demonstrated that Nalfurafine is most effective in patients with severe UP (defined as intolerable itching or itching unrelieved by scratching) in patients undergoing regular HD or haemofiltration.

The Applicant provided 5 main efficacy studies (LCRC/G/028, STTOR002, STTOR003, EU820UPV01 and STTOR004) to support submission of the indication "Treatment of severe uraemic pruritus (UP) in patients

of 18 years of age or older, with end stage renal disease undergoing regular haemodialysis, haemodiafiltration or haemofiltration (dialysis). Severe UP is defined as intolerable itching or itching unrelieved by scratching". The current dose regimen is up to 5 µg/body (i.e. approximately 0.08 µg/kg), three times weekly immediately after dialysis.

Nalfurafine hydrochloride is a novel, potent and selective agonist for the kappa-(κ-) opioid receptor without appreciable action on other types of opioid receptors. Nalfurafine hydrochloride (INN) is the recommended name for (E)-N-[17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6β-yl]-3-(furan-3-yl)-N-methylprop-2-enamide monohydrochloride (chemical name), andit is not described in an pharmacopoeia. Nalfurafine hydrochloride is manufactured in Japan.

Nalfurafine is a 9.3 micrograms/mL concentrate for solution for infusion. The product is delivered in a 1-mL, Type I, amber glass ampoule.

Type of application and aspects on development

The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application. The applicant indicated that nalfurafine hydrochloride was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of the above mentioned Regulation based on the following claims:

(1) Nalfurafine falls within the scope of Article 3(1) and (2) of Regulation (EC) No 726/2004 and was designated as orphan medicinal product in accordance with Article 3 of regulation (EC) No 141/2000 on 11th September 2002 (Orphan Designation number: EU/3/02/115);

(2) The risk-benefit balance of Nalfurafine, as defined in Article 1(28a) of Directive 2001/83/EC, is positive;

(3) It is likely that the applicant will provide comprehensive clinical data;

(4) Treatment of UP is a currently unmet medical need, which will be fulfilled with Nalfurafine Toray,

(5) The benefit to public health of the immediate availability of Nalfurafine outweighs the risks inherent in the fact that additional data are still required.

Scientific Advice was obtained for nalfurafine hydrochloride in MPA, Sweden.

The applicant received Protocol Assistance from the CHMP. The Protocol Assistance pertained to clinical aspects of the dossier such as study design, trial duration, primary endpoint, secondary endpoints, dosing regimen and targeted patient population.

2.2. Quality aspects

2.2.1. Introduction

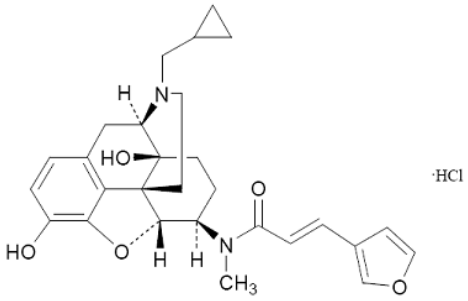
The finished product was proposed as a concentrate for solution containing 4.6 micrograms of nalfurafine as active substance.

Other ingredients are: mannitol, sodium thiosulfate and water for injections.

The product is available in a 1 ml Type I amber glass ampoule.

2.2.2. Active substance

The chemical name of nalfurafine hydrochloride is (E)-N-[17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6β-yl]-3-(furan-3-yl)-N-methylprop-2-enamide monohydrochloride and has the following structure:



The chemical structure of nalfurafine hydrochloride has been confirmed through spectroscopic measurements (UV, IR, NMR (1H and 13C) and MS), X-ray crystal structure analysis and from elemental analysis. Satisfactory spectra and data together with interpretations were provided.

The active substance is a highly hygroscopic amorphous white powder freely soluble in water and methanol, slightly soluble in ethanol and practically insoluble in ethyl acetate and diethyl ether.

Nalfurafine hydrochloride exhibits stereoisomerism due to the presence of 5 chiral centres. Enantiomeric purity is controlled routinely by specific optical rotation. Polymorphism has not been observed, the active substance is amorphous.

The information on the active substance is provided according to the Active Substance Master File (ASMF) procedure.

Manufacture

The active substance is supplied by one source only.

Nalfurafine hydrochloride is synthesized in 4 main steps using two well defined starting materials (introduced at step I and III of the synthesis) with acceptable specifications.

The penultimate step of the synthesis was defined as critical as its isolated intermediate has a significant impact on the final purity of the active substance with regards related substances and residual solvents.

It was concluded that the formation of the stereochemical configuration of the active substance is strictly controlled during the manufacturing process and purification steps.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

Specification

The active substance specification includes tests for: appearance, identity (IR, UV), assay (HPLC), impurities (HPLC), residual solvents (GC), water content (KF), heavy metals (Ph. Eur.), residue on ignition (Ph. Eur.), specific optical rotation (Ph. Eur.), microbiological quality (Ph. Eur.), endotoxins (Ph. Eur.), hydrochloride analysis (Ph. Eur.).

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines.

Batch analysis data (up to a number of pilot and commercial scale batches) of the active substance from the active substance manufacturer are provided. The results are within the specifications and consistent from batch to batch.

The absence of batch data from the finished product manufacturer was raised during the procedure as a minor concern however never satisfactorily addressed by the applicant.

Stability

Stability data on a number of pilot and commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 72 months under long term conditions at 5 ºC and for up to 6 months under accelerated conditions at 25 ºC / 60 % RH according to ICH guidelines were provided.

Photostability testing following ICH guideline Q1B and stability studies under stress conditions (high temperature, humidity, oxygen) were also provided for up to a number of batches. Results show that the substance is sensitive to light, moisture and oxygen.

The following parameters were tested: colour and appearance, pH, related substances and assay. Identification, hydrochloride analysis and microbiological quality were tested at the start of the studies and for some additional time points during the studies. Water content, bacterial endotoxins and clarity of solution was monitored at some time points for some batches. The analytical methods used were the same as for release and were stability indicating.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period in the proposed container.

2.2.3. Finished medicinal product

Pharmaceutical development

As it is currently proposed the product is intended to be diluted in an isotonic saline solution before intravenous infusion. The pharmaceutical development satisfactorily addresses the concerns over exposure of the active substance to direct light and oxygen, justifying the use of the antioxidant (sodium thiosulfate) and amber ampoules.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

The primary packaging is amber glass ampoule. The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Adventitious agents

No excipients derived from animal or human origin are used.

Manufacture of the product

The manufacturing process consists of five main steps: preparation of batch solution, filtration, filling of the ampoules, terminal sterilisation and final packaging. The process is considered to be a standard manufacturing process.

Major steps of the manufacturing process have been validated by a number of studies. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this of type of manufacturing process and pharmaceutical form.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form, e.g. description, identification (HPLC, UV), pH (Ph. Eur.), assay and related substances (HPLC), particulate matter (Ph. Eur.), sterility (Ph. Eur.), endotoxins (Ph. Eur.) and extractable volume (Ph. Eur.).

Batch analysis results are provided for up to a number of pilot and commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

Stability of the product

Stability data of up to a number of pilot and commercial scale batches of finished product stored under long term conditions for up to 53 months at 25 ºC / 60% RH and for up to 6 months under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines were provided. The batches of the nalfurafine concentrate are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for identification, pH, assay and related substances, particulate matter, sterility and endotoxins. The analytical procedures used are stability indicating.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products.
With the exception of the increase of impurity no other time depended change was observed.

Based on available stability data, the shelf-life and storage conditions as stated in the SmPC are acceptable.

2.2.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

At the time of the CHMP opinion, there were one minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.2.6. Recommendation(s) for future quality development

Not applicable

2.3. Non-clinical aspects

2.3.1. Introduction

Nalfurafine hydrochloride is a novel, potent and selective agonist for the kappa-(κ-) opioid receptor without appreciable action on other types of opioids receptors. It is a white to slightly yellowish powder that is freely soluble in water and methanol, slightly soluble in ethanol, and practically insoluble in ethyl acetate and in diethyl ether.

The proposed indication in SmPC section 4.1 is for the treatment of severe uraemic pruritus (UP) in patients of 18 years of age or older, with end stage renal disease undergoing regular haemodialysis, haemodiafiltration or haemofiltration (dialysis). Severe UP is defined as intolerable itching or itching unrelieved by scratching. The pharmaceutical form is Concentrate for solution for infusion 10 micrograms/ml.

Extensive research and development with this indication has resulted in nalfurafine hydrochloride being established as an effective treatment for uraemic pruritus in patients with end stage renal disease (ESRD) by the i.v. route. Since the original route of administration in humans was to be i.m., many of the early toxicological studies were conducted using this route and the s.c. route also. However, according to the Applicant, since pharmacokinetic studies have clearly demonstrated that nalfurafine hydrochloride is rapidly absorbed and widely distributed in the body from all major routes of administration tested, these i.m. and s.c. toxicological studies are relevant to the safety assessment of nalfurafine hydrochloride by the i.v route, and together with subsequent p.o. and i.v. studies, more than adequately support the intended clinical dosing regimen.

Scientific Advice was obtained for nalfurafine hydrochloride in MPA, Sweden in 1999 and 2002 as well as from the CHMP in 2001.

Pharmacodynamics, safety pharmacology, pharmacokinetic and toxicological studies have been conducted in accordance with either Standard Operating Procedures established at the Applicants laboratories by adopting the GLP regulations enforced in Japan or some studies in strict compliance with the GLP regulations enforced in Japan. The studies data submitted and presented in this assessment report are well described in study reports and are of acceptable quality. All toxicology studies were conducted according to GLP.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Pharmacodynamics of nalfuarfine hydrochloride *in vitro*

Binding affinities of nalfuarfine hydrochloride for the recombinant human opioid receptors were evaluated in two independent binding assays. [3H]diprenorphine (κ- and μ-opioid receptors) and [3H]naltrindole (δ-opioid receptor) were used as radioligands.

Agonistic activities for human opioid receptors were investigated in cells expressing the human κ-opioid receptors, human μ-opioid receptors and human δ-opioid receptors (pP5).

Binding affinity for other receptors (M_1, M_2, M_3, A_1, A_2, α_1, α_2, glutamate AMPA and NMDA, adenosine, angiotensin II, L type calcium channels, cholescystokinin A and B, histamine, serotonin $5HT_1$, $5HT_{1A,}$ $5HT_2$, $5HT_{3,}$ vasopressin V1, atrial natriuretic peptide, interleukines 1β and 8, TNFα, CC chemokine receptor (CCR1, CCR2 types) was investigated (pP13, pP14, pP15). Nalfurafine hydrochloride did not show any detectable affinity to these receptors except for a low affinity (Ki value of 1700 nmol/L) for the muscarinic M1 receptor.

Table 1. Summary of selectivity for opioid receptors studies of nalfurafine hydrochloride.

Type of Study, report	Test System	Results / Conclusion
Electrically-induced contraction of mouse vas deferens *in vitro* (pP1 /TM-97011)	Mouse vas deferens (n=4). The selectivity for the opioid receptors was evaluated by comparison of the inhibitory effect of nalfurafine hydrochloride in the absence and presence of Nor-BNI, naloxone and NTI used as κ-, mu- and δ-opioid receptor antagonist, respectively.	Nalfurafine hydrochloride inhibited the electrically-induced contraction of mouse vas deferens with the IC50 of 0.080 nmol/L and 0.12 nmol/L in two independent experiments *in vitro*. The Ke values for nor-BNI were less than those for the other two antagonists. **Conclusion:** Results suggested that nalfurafine hydrochloride had a potent and selective agonistic activity for the κ-opioid receptor in mouse. Statistical differences of mean values are not presented.

<table>
<tr>
<td>Electrically-induced contraction of guinea pig ileum in vitro (pP2 /TM-97014)</td>
<td>Guinea pig ileum (n=4). The selectivity for the opioid receptors was evaluated by comparison of the inhibitory effect of nalfurafine hydrochloride in the absence and presence of Nor-BNI and naloxone used as κ-, mu- opioid receptor antagonist, respectively.</td>
<td>Nalfurafine hydrochloride inhibited the electrically-induced contraction of GPI preparations in a concentration-dependent manner, and the IC50 value was estimated to be 0.0081 nM.

Using nor-BNI as the antagonist, the concentration-response curve determined with TRK-820 alone was shifted to a high dose region in the presence of nor-BNI at all of the concentrations examined.

Therefore, it was suggested that TRK -820 possessed K agonist activity.

In the experiments using naloxone as the antagonist, the concentration-response curve determined with TRK-820 alone was shifted in parallel to a high dose region dependent on the concentration of naloxone. However, the shift was observed to a lesser extent than that seen with nor-BNI. From these results, it appeared that TRK-820 possessed weak mu agonist activity.

The Ke values for nor-BNI were less than those for naloxone (see table below).
<table>
<tr><th>Antagonist</th><th>Concentration of antagonist (nmol/L)</th><th>Dose ratio[a]</th><th>Ke (nmol/L)[b]</th></tr>
<tr><td>nor-BNI</td><td>3</td><td>21 (13 - 34)</td><td>0.15 (0.090 - 0.24)</td></tr>
<tr><td></td><td>10</td><td>143 (94 - 225)</td><td>0.070 (0.045 - 0.11)</td></tr>
<tr><td></td><td>30</td><td>347 (217 - 593)</td><td>0.087 (0.051 - 0.14)</td></tr>
<tr><td>Naloxone</td><td>10</td><td>3.9 (2.0 - 8.0)</td><td>3.4 (1.4 - 9.6)</td></tr>
<tr><td></td><td>30</td><td>6.1 (3.9 - 10)</td><td>5.8 (3.2 - 10)</td></tr>
<tr><td></td><td>100</td><td>19 (11 - 38)</td><td>5.5 (2.7 - 9.6)</td></tr>
</table>
a: Dose ratio was defined as the degree of parallel shift in the concentration-response curve induced by an antagonist.

b: Ke (nmol/L) = [Concentration of antagonist (nmol/L)]/(Dose ratio - 1). Values in parentheses represent 95% confidence interval.

Conclusion: Results suggested that nalfurafine hydrochloride had a potent and selective agonistic activity for the κ-opioid receptor in guinea pig.

Statistical differences of mean values are not presented.</td>
</tr>
<tr>
<td>Binding ability for human opioid receptors in vitro (pP3 /TG-05002)</td>
<td>Human CHO-KI cells expressing human mu opioid receptors

Human CHO cells expressing human sigma opioid receptors

Human HEK-293 cells expressing</td>
<td>Nalfurafine hydrochloride displaced radioligands from the κ-, mu- or δ-opioid binding sites, having Ki values of 0.244, 2.21 and 484 nmol/L, respectively. The results indicate that nalfurafine hydrochloride preferentially bound to the human κ-opioid receptor.

The Applicant is asked to discuss what influence for final results consistency may have using different Human cell lines (CHO and HEK) expressing different opioid receptors (other concern).</td>
</tr>
</table>

<table>
<tr><td></td><td>human K opioid receptors

[3H]diprenorphine (κ- and mu-opioid receptors) and [3H]naltrindole (δ-opioid receptor) used as the radioligands.</td><td></td></tr>
<tr><td>Binding ability for human opioid receptors in vitro (pP4/146924)</td><td>Human cells expressing K, mu and δ opioid receptors

[3H]diprenorphine (κ- and mu-opioid receptors) and [3H]naltrindole (δ-opioid receptor) used as the radioligands.</td><td>Nalfurafine hydrochloride displaced radioligands from the κ-, mu- or δ-opioid binding sites, having Ki values of 0.114, 1.9 and 423 nmol/L, respectively. The results indicate that nalfurafine hydrochloride preferentially bound to the human κ-opioid receptor.

From presented data is not clear which type of cell lines were used.</td></tr>
<tr><td>Agonistic activities for human opioid receptors in vitro (pP5/TG-05006)</td><td>Agonistic activities of TRK-820, morphine, buprenorphine, butorphanol, and standard full agonists of each receptor (DAMGO, DPDPE, or U-69593) were evaluated using CHO cells that stably express human opioid receptor subtypes (fl, 8, or K receptors) according to their inhibitory effects on forskolin-stimulated cAMP accumulation.</td><td>Agonistic activity for human opioid receptors
<table>
<tr><th rowspan="2">Test compound</th><th colspan="2">κ-opioid receptor</th><th colspan="2">μ-opioid receptor</th><th colspan="2">δ-opioid receptor</th></tr>
<tr><th>EC_{50} (nmol/L)</th><th>I_{max} (%)</th><th>EC_{50} (nmol/L)</th><th>I_{max} (%)</th><th>EC_{50} (nmol/L)</th><th>I_{max} (%)</th></tr>
<tr><td>Nalfurafine hydrochloride</td><td>0.00816 ± 0.00138</td><td>91.3 ± 0.5</td><td>1.66 ± 0.09</td><td>53.2 ± 1.3 [a,b,d]</td><td>21.3 ± 1.0</td><td>77.9 ± 1.6 [d,e]</td></tr>
<tr><td>Morphine</td><td>391 ± 33</td><td>80.4 ± 0.7</td><td>35.7 ± 2.6</td><td>75.6 ± 0.5</td><td>N.C.</td><td>(80.5 ± 0.7) [f]</td></tr>
<tr><td>Buprenorphine</td><td>4.13 ± 0.24</td><td>47.7 ± 1.7</td><td>1.59 ± 0.26</td><td>56.1 ± 1.1 [a,b]</td><td>2.40 ± 0.19</td><td>79.9 ± 1.5 [e]</td></tr>
<tr><td>Butorphanol</td><td>0.752 ± 0.050</td><td>84.5 ± 0.8</td><td>3.34 ± 0.23</td><td>46.5 ± 1.0 [a,b,c]</td><td>4.88 ± 0.41</td><td>83.3 ± 1.0</td></tr>
<tr><td>Full agonist of each receptor [g]</td><td>0.642 ± 0.022</td><td>91.2 ± 0.3</td><td>5.63 ± 0.31</td><td>77.2 ± 0.8</td><td>0.186 ± 0.045</td><td>87.3 ± 0.6</td></tr>
</table>
Each value represents mean ± S. E., n=5

N.C.: not calculated

a: p<0.05 vs. DAMGO, b: p<0.05 vs. morphine, c: p<0.05 vs. buprenorphine, d: p<0.05 vs. butorphanol, e: p<0.05 vs. DPDPE (Tukey's multiple comparison)

f: Although the reaction at the highest concentration did not reach a maximal effect, the inhibitory rate at the highest concentration (10000 nmol/L) was shown as a reference value.

g: U-69593 (k-opioid receptor), DAMGO (μ-opioid receptor), DPDPE (δ-opioid receptor)

In cells expressing the human κ-opioid receptors, the Imax of nalfurafine hydrochloride against forskolin-stimulated cAMP accumulation was 91%. This rate was similar to that of U-69593</td></tr>
</table>

		(Imax: 91%), a κ-opioid receptor full agonist. The Imax of morphine and butorphanol was 80 and 85%, respectively, which were slightly lower than those of nalfurafine hydrochloride and U-69593. The Imax of buprenorphine was 48%, which was half of those of nalfurafine hydrochloride and U-69593, but there were no significant differences. The order of EC50 was nalfurafine hydrochloride < U69593 < butorphanol < buprenorphine < DAMGO < morphine. In cells expressing the human μ-opioid receptors, the Imax of nalfurafine hydrochloride was 53%, which was significantly lower than those of DAMGO (Imax: 77%), a μ-opioid receptor full agonist, and morphine (Imax: 76%), but which was similar to those of buprenorphine (Imax: 56%) and butorphanol (Imax: 47%). The order of EC50s was buprenorphine ≈ nalfurafine hydrochloride < butorphanol < morphine. In cells expressing the human δ-opioid receptors, the Imax of nalfurafine hydrochloride was 78%, which was significantly lower than those of DPDPE (Imax: 87%), a δ-opioid receptor full agonist, and butorphanol (Imax: 83%). The Imax of buprenorphine (Imax: 80%) was similar to that of nalfurafine hydrochloride. The order of EC50s was DPDPE < buprenorphine < butorphanol < nalfurafine hydrochloride. Since morphine did not show a maximal response at the highest dose (10 000 nmol/L), the Imax and EC50 were not calculated in this study. The inhibitory rate at 10 000 nmol/L was about 80%. The ratio of EC50 (κ : μ : δ) for each test compound was as follows: 1 : 203 : 2610 for nalfurafine hydrochloride, 1 : 0.1 : 1.0 for morphine, 1 : 0.4 : 0.6 for buprenorphine and 1 : 4.4 : 6.5 for butorphanol. Therefore, it was suggested that the *in vitro* pharmacological profile of nalfurafine hydrochloride was markedly distinct from those of morphine, buprenorphine and butorphanol and that nalfurafine hydrochloride possessed a selective and high potential κ-opioid receptor agonistic activity.

Pharmacodynamics of nalfuarfine hydrochloride *in vivo*

Injection of substance P (one of the most common peripheral pruritogens) to mice induces scratching behaviour and is therefore used as an experimental animal model for itching.

Intravenous administration of nalfurafine hydrochloride (2.5, 5, 7.5 and 10 μg/kg) to mice dose-dependently inhibited substance P-induced scratching behaviour. Significant inhibitions were observed at 7.5 and 10 μg/kg and the ED50 was 3.77 μg/kg (pP6). In addition, s.c. dosing of nalfurafine hydrochloride (0.3, 1, 3 and 10 μg/kg) also dose-dependently inhibited substance P-induced scratching behaviour, and significant inhibition was observed at 10 μg/kg. The ED50 following s.c. dosing was 1.65 μg/kg (pP7).

Furthermore, the antipruritic effects of histamine H1 receptor antagonists (antihistamines) were evaluated. The antagonists ketotifen and chlorpheniramine were administered p.o. to mice, and 60 min

later, substance P (250 nmol/site) was injected i.d. Chlorpheniramine (1, 3, 10 and 30 mg/kg) did not inhibit substance P-induced scratching behaviour (pP8). On the other hand, ketotifen (0.1, 1, 10 and 100 mg/kg) dose-dependently inhibited substance P-induced scratching behaviour. Inhibitions at 10 and 100 mg/kg were 50.9 and 66.0%, respectively, and the ED50 was 9.61 mg/kg.

The antipruritic effect of nalfurafine hydrochloride was evaluated in the morphine-induced mouse scratching model and compared with that of the antihistamine ketotifen. Nalfurafine hydrochloride (1.25, 2.5, 5 and 10 µg/kg, s.c.) dose-dependently inhibited morphine-induced scratching behaviour, and the ED50 was 2.34 µg/kg while ketotifen (0.01, 0.1, 1 and 10 mg/kg, i.p.) did not significantly inhibit scratching behaviour (pP10, pP11).

A possible development of tolerance was evaluated in the substance P-induced mouse scratching model. Vehicle or nalfuarfine hydrochloride (100 µg/kg, p.o.) was repeatedly administered to mice (twice daily for 7 days). The effect of nalfuarfine F hydrochloride (25, 50, 100 and 200 µg/kg, p.o.) on substance P-induced scratching was evaluated on day 8. Nalfuarfine hydrochloride produced an inhibitory effect on substance P-induced scratching behaviour. ED50 values of 30.4 µg/kg and 56.0 µg/kg were achieved for vehicle and nalfurafine hydrochloride respectively, showing that upon repeated p.o. administration of nalfuarfine hydrochloride, a tolerance towards its antipruritic effect is probable (pP12).

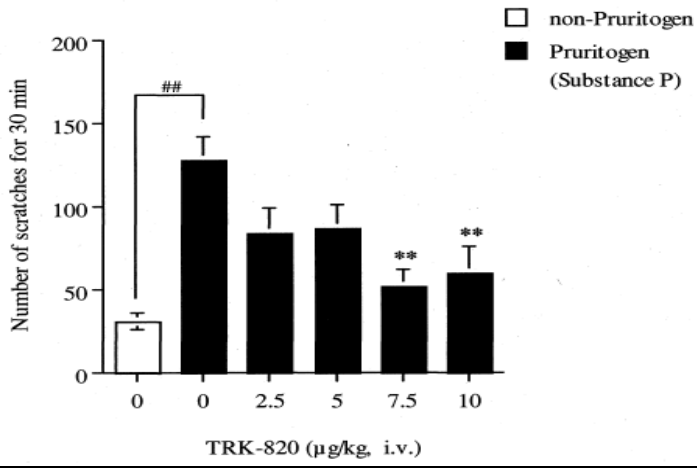
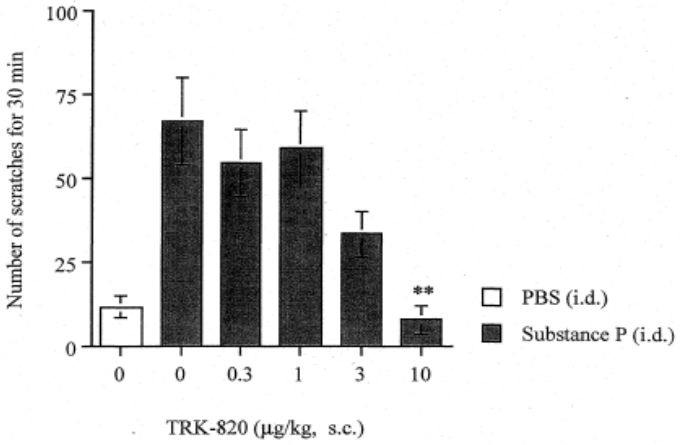
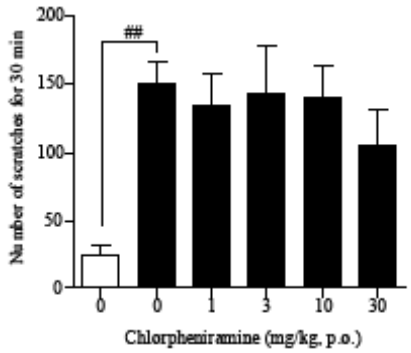
The effect of the κ-opioid receptor antagonist, nor-BNI, on the scratching inhibitory activity of nalfurafine hydrochloride was evaluated in the substance P-induced scratching model. Nor-BNI (1, 3 and 10 mg/kg) was administered s.c. 1 day before nalfurafine hydrochloride administration. Nor-BNI dose-dependently antagonised the scratching inhibitory activity of nalfurafine hydrochloride (10 µg/kg, s.c.), and significant antagonisms were seen at 3 and 10 mg/kg of nor-BNI (pP16). In addition, i.c.v. injection of nor-BNI (10 µg/site) 1 day before nalfurafine hydrochloride administration (10 µg/kg, s.c.) completely antagonised the inhibitory activity of nalfurafine hydrochloride on substance P-induced scratching behaviour (pP17).

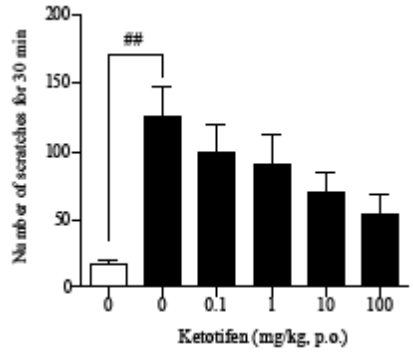
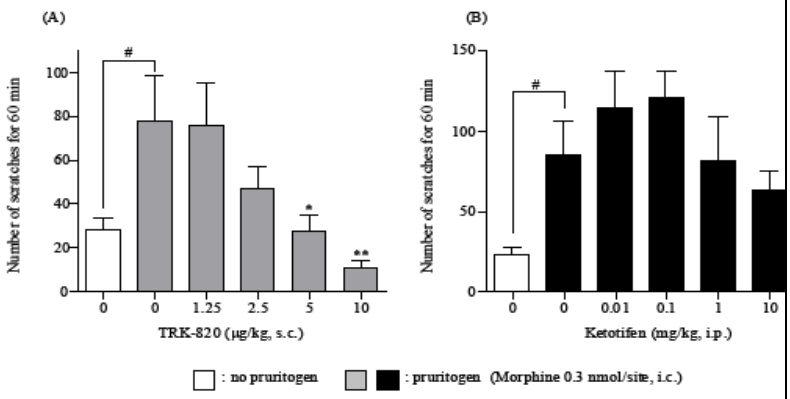
With regards to the effect on inflammatory mediators, nalfurafine hydrochloride had little or no inhibitory effect on prostaglandin E2 secretion, prostaglandin D2 secretion, tumour necrosis factor α secretion, IL-1β secretion, IL-6 secretion, compound 48/80-stimulated histamine release or substance P-stimulated histamine release. In addition, nalfurafine hydrochloride did not influence the inducible NOS or constitutive NOS activities (pP13).

The pharmacological profiles of the impurity in the injectable formulation, impurity x, and the main metabolites, de-CPM, NFA-G and de-CPM-G, were determined Impurity x had an affinity similar to that of nalfurafine hydrochloride for three opioid receptors (pP18). and had agonistic activities for the κ- and µ-opioid receptors, and partial agonistic activity for the δ-opioid receptor (pP19). de-CPM (pP20) or de-CPM·T (pP21) and NFA-G (pP20, pP22) bound to some opioid receptors with lower affinities than that of nalfurafine hydrochloride. de-CPM-G did not have any significant affinity for any of the opioid receptors (pP20, pP23). Agonistic activities of the main metabolites of nalfurafine hydrochloride (pP24) were also lower than that of nalfurafine hydrochloride. Neither Impurity x (0.3, 1, 3 and 10 µg/kg, s.c.) nor the main metabolites (1, 10, 100 and 1000 µg/kg, s.c.) inhibited substance P-induced scratching behaviour (pP25, pP26, pP27, pP28).

Table 2. Summary of nalfurafine hydrochloride Antipruritic Effect studies.

Type of Study, report	Test System	Results / Conclusion
Substance P-induced scratching	Mouse ICR, male, 6 groups, 10 mice/group.	Nalfurafine hydrochloride dose-dependently inhibited substance P-induced scratching behaviour, and significant inhibitions were observed at 7.5 and 10 mcg/kg. See figure. The ED50 was 3.77

behaviour (pP6 /TG-01007)	Nalfurafine hydrochloride i.v. (2.5, 5, 7.5 and 10 mcg/kg) and 30 min later, substance P (250 nmol/site) was injected i.d. to induce scratching behaviour	mcg/kg.  ## p<0.01 (non-paired t-test or Welch's test), ** p<0.01 (Tukey-Kramer's multiple comparison test or Dunnett's multiple comparison test)
Substance P-induced scratching behaviour (pP7 /TG-97010)	Mouse ddY., male, 6 groups, 9 mice/group. Nalfurafine hydrochloride s.c. (0.3, 1, 3 and 10 mcg/kg) and 30 min later, substance P (250 nmol/site) was injected i.d. to induce scratching behaviour	Nalfurafine hydrochloride s.c. dose-dependently inhibited substance P-induced scratching behaviour, and significant inhibition was observed at 10 mcg/kg. The ED50 was 1.65 mcg/kg.  ** p<0.01
Substance P-induced scratching behaviour (pP8 /TG-98011)	Mouse ICR, male, 6 groups, 8 mice/group. Chlorpheniramine (1, 3, 10 and 30 mg/kg p.o.) and 60 min later, substance P (250 nmol/site) was injected i.d. to induce scratching behaviour	Chlorpheniramine did not inhibit substance P-induced scratching behaviour. 

		## p<0.01 (non-paired t-test or Welch's test)
Substance P-induced scratching behaviour (pP9 /TG-98013)	Mouse ICR, male, 6 groups, 8 mice/group. ketotifen (0.1, 1, 10 and 100 mg/kg p.o.) and 60 min later, substance P (250 nmol/site) was injected i.d. to induce scratching behaviour	Ketotifen (0.1, 1, 10 and 100 mg/kg) dose-dependently inhibited substance P-induced scratching behaviour. Inhibitions at 10 and 100 mg/kg were 50.9 and 66.0%, respectively, and the ED50 was 9.61 mg/kg. Ketotifen, however, did not show any significant inhibition.  ## p<0.01 (non-paired t-test or Welch's test)
Morphine-induced scratching behaviour (pP10 /TG-01008)	Mouse ddY, male, 6 groups, 16 mice/group. Nalfurafine hydrochloride (1.25, 2.5, 5 and 10 mcg/kg, s.c.) and 30 min later, morphine (0.3 nmol/site) was injected into the cerebellomedullary cistern (cisterna magna) to induce scratching behaviour	Nalfurafine hydrochloride dose-dependently inhibited morphine-induced scratching behaviour, and the ED50 was 2.34 mcg/kg. See figure below (A).  Each column represents the mean ± S.E. n=11-15 (A), n=10-14 (B). # p<0.05 (Welch's test), * p<0.05, ** p<0.01 (Dunnett's multiple comparison test)
Morphine-induced scratching behaviour (pP10 /TG-02001)	Mouse ddY, male, 6 groups, 16 mice/group. Ketotifen (0.01, 0.1, 1 and 10 mg/kg, i.p.) and 30 min later, morphine (0.3 nmol/site) was injected into the cerebellomedullary cistern (cisterna magna)	Ketotifen did not significantly inhibit scratching behaviour. See figure above (B).

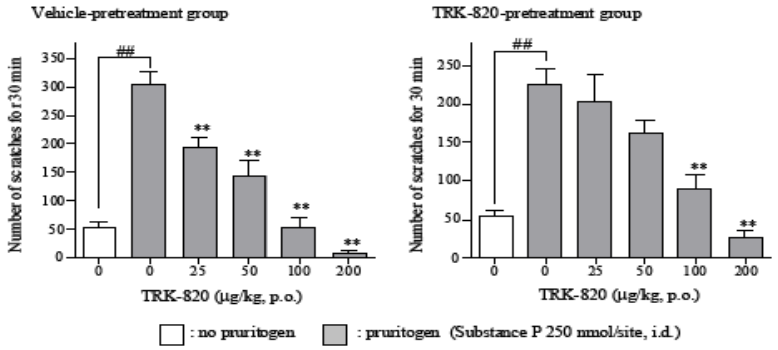
	to induce scratching behaviour	
Tolerance formation toward the antipruritic effect (pP12 /TG-00002)	Mouse ICR, male, 2 groups, 50 mice/group. Vehicle or nalfurafine hydrochloride (100 mcg/kg, p.o.) was repeatedly administered twice a day for 7 days. Nalfurafine hydrochloride (25, 50, 100 and 200 mcg/kg, p.o.) and 30 min later, substance P (250 nmol/site) was injected i.d. to induce scratching behaviour on Day 8.	In the vehicle- and nalfurafine hydrochloride-pretreatment groups, nalfurafine hydrochloride produced an inhibitory effect on substance P-induced scratching behaviour. However, pretreatment of nalfurafine hydrochloride induced a 1.84-fold increase in the ED50 (30.4 mcg/kg in vehicle-pretreatment group and 56.0 mcg/kg in nalfurafine hydrochloride-pretreatment group). See figure below. These results suggested that repeated administration of nalfurafine hydrochloride could result in the formation of tolerance towards its own antipruritic effect. However, the degree of tolerance induced was much lower than that reported with the analgesic effect of morphine. Therefore, it was considered unlikely that nalfurafine hydrochloride would induce significant tolerance in humans.  Each column represents the mean ± S.E. (n=7-8). ## p<0.01 (non-paired t-test or Welch's test), ** p<0.01 (Dunnett's multiple comparison test).

Table 3. Summary of nalfurafine hydrochloride mechanism of antipruritic effects studies.

Type of Study, report	Test System	Results / Conclusion
Effect on inflammatory mediators *in vitro* (pP13 / 2897)	The membranes or cells (13). TRK-820 was tested at 10^{-6} M or at 10^{-9} M and 10^{-8} M. Bound radioactivity was measured with a scintillation counter	Nalfurafine hydrochloride had little or no inhibitory effect on prostaglandin E2 secretion, prostaglandin D2 secretion, tumour necrosis factor α secretion, IL-1β secretion, IL-6 secretion, compound 48/80-stimulated histamine release or substance P-stimulated histamine release. In addition, nalfurafine hydrochloride did not influence the inducible NOS or constitutive NOS activities.
Affinity for receptors other than	The membranes or cells. TRK-820 was tested at	Nalfurafine hidrochloride displaced the radioligand from muscarinic M1 binding sites, having a Ki value of 1700 nmol/L. On the other hand, nalfurafine hydrochloride did not displace

opioid receptors *in vitro* (pP13 / 2897), (pP14 / 2786), (pP15 /107142-ADD, 107145-ADD, 106115-ADD, 107774-ADD)	different concentrations. Displacement of radioligand measured with a scintillation counter	radioligands from adenosine A1, A2A, adrenergic α1, α2, angiotensin II, atrial natriuretic peptide, bombesin, calcitonin gene related peptide, L type calcium channel, CC chemokine receptor CCR1, CCR2, cholecystokinin A, B, dopamine D1, D2, GABAA-agonist site, glutamate (non-selective), glutamate AMPA, glutamate kainate, glutamate NMDA-agonist site, glutamate NMDA-phencyclidine site, histamine H1, H2, H3, IL-1β, IL-8, leukotriene D4, muscarinic M2, M3, neurokinin NK1, NK2, NK3, neuropeptide Y2, platelet activating factor, serotonin 5HT1, 5HT1A, 5HT2, 5HT3, sigma (non-selective), somatostatin, tumour necrosis factor α, vasoactive intestinal peptide, or vasopressin V1, V2 binding sites. These results indicated that nalfurafine hydrochloride showed no detectable affinity for a series of general receptors, except for a low affinity for the muscarinic M1 receptor. See table below.

Receptor / Ion channel	Inhibitory rate (%)	
	TRK-820 1000 nmol/L	TRK-820 10000 nmol/L
Adenosine A_1	N.E.	< 10
Adenosine A_{2A}	N.E.	13
Adrenergic α_1 (non-selective)	N.E.	27
Adrenergic α_2 (non-selective)	N.E.	11
Angiotensin II	N.E.	< 10
Atrial natriuretic peptide (ANP)	< 10	N.E.
Bombesin	N.E.	12
Calcitonin gene related peptide (CGRP)	10	N.E.
Calcium channel Type L (Dihydropyridine site)	N.E.	< 10
CC Chemokine receptor CCR_1	< 10	N.E.
CC Chemokine receptor CCR_2	< 10	N.E.
Cholecystokinin A	N.E.	< 10
Cholecystokinin B	N.E.	< 10
Dopamine D_1	N.E.	< 10
Dopamine D_2	N.E.	20
$GABA_A$ (agonist site)	N.E.	< 10
Glutamate (non-selective)	N.E.	< 10
Glutamate AMPA	N.E.	< 10
Glutamate kainate	N.E.	< 10
Glutamate NMDA (agonist site)	N.E.	10
Glutamate NMDA (phencyclidine site)	N.E.	< 10
Histamine H_1	N.E.	< 10
Histamine H_2	< 10	N.E.
Histamine H_3	< 10	N.E.

Interleukin-1β(IL-1β)	< 10	N.E.
Interleukin-8 (IL-8)	< 10	N.E.
Leukotriene D_4 (LTD_4)	< 10	N.E.
Muscarinic M_1	41	72 *
Muscarinic M_2	N.E.	< 10
Muscarinic M_3	N.E.	< 10
Neurokinin NK_1	N.E.	< 10
Neurokinin NK_2	< 10	N.E.
Neurokinin NK_3	< 10	N.E.
Neuropeptide Y_2	N.E.	< 10
Platelet activating factor (PAF)	< 10	N.E.
Serotonin 5-HT_1	N.E.	< 10
Serotonin 5-HT_{1A}	N.E.	< 10
Serotonin 5-HT_2	N.E.	12
Serotonin 5-HT_3	N.E.	< 10
Sigma (non-selective)	N.E.	14
Somatostatin	N.E.	< 10
Tumor necrosis factor α (TNF-α)	< 10	N.E.
Vasoactive intestinal peptide (VIP)	< 10	N.E.
Vasopressin V_1	< 10	< 10
Vasopressin V_2	< 10	N.E.

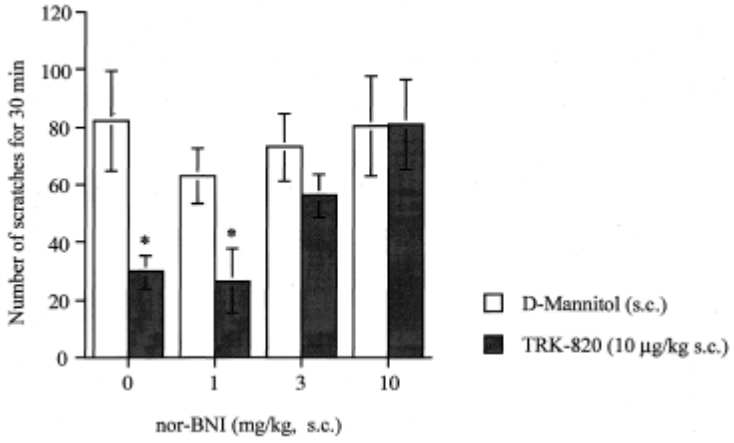
N.E.: not examined
a: Ki value = 1700 nmol/L. GABA : γ-aminobutyric acid, AMPA : α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid, NMDA : N-Methyl-D-aspartic acid

Effects of the test compounds on the specific radioligand binding to the receptors studied and IC_{50} values for the reference compounds

	ANP receptor			V_1 receptor			V_2 receptor		
Compounds	10^{-13} M	10^{-8} M	10^{-6} M	10^{-13} M	10^{-8} M	10^{-6} M	10^{-13} M	10^{-8} M	10^{-6} M
TRY-1001	–	–	–	–	–	–	–	–	–
TRY-1002	–	–	–	–	–	–	–	–	11
TRY-1003	–	–	–	–	–	68	24	49	98
TRY-1004	–	–	–	–	94	97	18	82	102
	IC_{50} ($\times 10^{-9}$ M)		*(nH)*	IC_{50} ($\times 10^{-9}$ M)		*(nH)*	IC_{50} ($\times 10^{-9}$ M)		*(nH)*
ANP	0.12		*(0.9)*						
V_1-antagonist				1.0		*(0.9)*			
AVP							4.0		*(1.0)*

For the test compounds, the results are expressed as a percent inhibition of control specific binding. (mean values ; n = 2).
The symbol – indicates an inhibition of less than 10 %.

Involvement of the κ-opioid receptor in the antipruritic effect (**pP16**/TG-97009)	Mouse, ddY, 8 animals/group. Nor-BNI (1, 3 and 10 mg/kg) administered s.c. 1 day before nalfurafine	Nor-BNI dose-dependently antagonised the scratching inhibitory activity of nalfurafine hydrochloride (10 mcg/kg, s.c.), and significant antagonisms were seen at 3 and 10 mg/kg of nor-BNI. See figure.

	hydrochloride (10 mcg/kg, s.c.) or D-mannitol and 30 min later, a percutaneous injection of substance P (300 nmol/body).	 *p<0.05.
Involvement of the κ-opioid receptor in the antripruritic effect (**pP17** / TG-02004)	Mouse, ddY, 10 animals/group, 8 groups. Nor-BNI (10 mg/kg) administered intracerebroventricularly or s.c. 1 day before nalfurafine hydrochloride (10 mcg/kg, s.c.) or Physiological saline, s.c. and 30 min later, a percutaneous injection of substance P (250 nmol/body).	Pretreatment with the K-opioid receptor antagonist, nor-BNI, by intracerebroventricular administration, antagonized the scratching inhibitory activity of TRK-820 in mice. These results suggested that the antipruritic effect of TRK-820 was mainly mediated via K-opioid receptors in the central nervous system.
Pharmacological profiles of the impurity and the main metabolites *in vitro* (pP18 /1018375)	Human tissue cells. Impurity x ·HCl	impurity x (1 nmol/L) displaced radioligands from κ-opioid receptors, having inhibition of 102%, displacedradioligands from µ-opioid receptors, having the Ki value of 0.785 nmol/L. ,displaced radioligands from sigma-opioid receptors, having inhibition of 55% and had an affinity similar to that of nalfurafine hydrochloride for three opioid receptors.
Pharmacological profiles of the impurity and the main metabolites *in vitro* (**pP19** / 1024475, 1024475-ADD	Electrically induced contraction of guinea pig ileum or mouse vas deferens. Impurity x ·HCl.	Agonist or antagonist activity: Impurity x demonstrated κ-, µ- and sigma-opioid receptor agonistų activities, having the EC50 of 4.5 ± 0.6 nmol/L, 2.8 ± 0.1 nmol/L, 170 ± 10 nmol/L, respectively. and demonstrated sigma-opioid receptor antagonist activity, having the IC50 of 68 ±15 nmol/L.

)		Selectivity for opioid receptors: Impurity x inhibited the electrically-induced contraction of guinea pig ileum concentration-dependently. The concentration response curve was shifted to a high concentration region in the presence of nor-BNI (1, 3, 10 nmol/L), having the Ke values of 0.10 nmol/L, 0.018 nmol/L, 0.0072 nmol/L, respectively. The concentration-response curve was shifted to a high concentration region in the presence of naloxone (10, 30, 100 nmol/L), having the Ke values of 5.92 nmol/L, 4.02 nmol/L, 8.70 nmol/L, respectively. Impurity x inhibited the electrically-induced contraction of mouse vas deferens concentration-dependently. The concentration response curve was not shifted in the presence of NTI (3, 10, 30 nmol/L). Impurity x had agonistic activities for the κ- and mu-opioid receptors, and partial agonistic activity for the δ-opioid receptor.
Pharmacological profiles of the impurity and the main metabolites *in vitro* (pP20 / TG-05005)	Human tissue cells. Binding assay. de-CPM 0.3 – 10000 (nmol/L) NFA-G 100 – 30000 (nmol/L) de-CPM-G 10000 (nmol/L)	de-CPM displaced radioligands from κ- and μ-opioid receptors, having the Ki values of 5.95 nmol/L and 133 nmol/L, respectively. de-CPM (10000 nmol/L) displaced radioligands from sigma-opioid receptors, having inhibition of 25%. NFA-G displaced radioligands from κ-opioid receptors, having Ki value of 1960 nmol/L. NFA-G (10 000 nmol/L) displaced radioligands from μ-opioid receptors, having inhibition of 25%. NFA-G did not displaced radioligand from sigma-opioid receptors (Inhibition at 10 000 nmol/L was 3%). de-CPM-G did not displaced radioligands from κ-, μ- and sigma-opioid receptors (Inhibitions at 10 000 nmol/L were 6, -1 and -8%, respectively). de-CPM or de-CPM·T and NFA-G bound to some opioid receptors with lower affinities than that of nalfurafine hydrochloride.
Pharmacological profiles of the impurity and the main metabolites *in vitro* (pP21 / 1018460)	Human tissue cells. Binding assay. de-CPM 1 – 1000 (nmol/L)	de-CPM·T displaced radioligands from κ-opioid receptors, having the Ki value of 6.74 nmol/L. de-CPM·T (1 000 nmol/L) displaced radioligands from μ-opioid receptors, having inhibition of 78%. de-CPM·T did not displace radioligands from sigma-opioid receptors (Inhibition at 1 000 nmol/L was -2%). de-CPM-G did not have any significant affinity for any of the opioid receptors.
Pharmacological profiles of the impurity and the main	Human tissue cells. Binding assay. NFA-G 1 – 1000 (nmol/L)	NFA-G (1000 nmol/L) displaced radioligands from κ-opioid receptors, having inhibition of 75%. NFA-G (1000 nmol/L) displaced radioligands from μ-opioid receptors, having inhibition of 39%. NFA-G did not displace radioligands from sigma-opioid

metabolites *in vitro* (pP22 / 1018462)		receptors (Inhibition at 1000 nmol/L was 4%). Metabolite activities of NFA-G of nalfurafine hydrochloride was lower than that of nalfurafine hydrochloride.
Pharmacological profiles of the impurity and the main metabolites *in vitro* (pP23 / 1018461)	Human tissue cells. Binding assay. de-CPM-G	de-CPM-G did not displace radioligands from κ-opioid receptors, μ-opioid receptors, and sigma-opioid receptors (Inhibitions at 1000 nmol/L were 4, 4 and 1%, respectively). Metabolite activities of de-CPM-G of nalfurafine hydrochloride was lower than that of nalfurafine hydrochloride.
Pharmacological profiles of the impurity and the main metabolites *in vitro* (pP24 / TG-05007)	Human tissue cells. Forskolin stimulated cAMP accumulation. de-CPM NFA-G de-CPM-G 0.01 – 3000 (nmol/L)	EC50 were not calculated for de-CPM, de-CPM-G and NFA-G for human μ- and sigma -opioid receptors. EC50 of de-CPM and NFA-G against forskolin-stimulated cAMP accumulation were 2.56 sigma 0.14 nmol/L and 43.2 sigma 3.1 nmol/L for human κ-opioid receptors, respectively. EC50 was not calculated for de-CPM-G for human κ-opioid receptors. Agonistic activities of the main metabolites of nalfurafine hydrochloride were lower than that of nalfurafine hydrochloride.
Pharmacological profiles of the impurity and the main metabolites *in vivo* (pP25 / TG-02003)	Mouse, ddY, 8 animals group, 6 groups, Impurity x (0, 0.3, 1, 3, 10 μg/kg s.c.) and 30 min later, a i.d. injection of substance P (250 nmol/body).	Impurity x, administered 30 min before, did not inhibit the substance P-induced scratches. It suggests that Impurity x does not have any antipruritic potential.
Pharmacological profiles of the impurity and the main metabolites *in vivo* (**pP26** /TG-99003)	Mouse, ddY, 8 animals group, 6 groups, de-CPM·T (0, 1, 10, 100, 1000 μg/kg s.c.) and 30 min later, a i.d. injection of substance P (250 nmol/body).	de-CPM·T, administered 30 min before, did not inhibit the substance P-induced scratches. It suggests that de-CPM·T does not have any antipruritic potential.
Pharmacological profiles of the impurity and the main metabolites *in vivo* (**pP27** /TG-99004)	Mouse, ddY, 8 animals group, 6 groups, NFA-G (0, 1, 10, 100, 1000 μg/kg s.c.) and 30 min later, a i.d. injection of substance P (250 nmol/body).	NFA-G, administered 30 min before, did not significantly inhibit the substance P-induced scratches, though inhibition at 1000 μg/kg was 52.0%.

Pharmacological profiles of the impurity and the main metabolites *in vivo* (**pP28** /TG-99006)	Mouse, ddY, 8 animals group, 6 groups, de-CPM-G (0, 1, 10, 100, 1000 µg/kg s.c.) and 30 min later, a i.d. injection of substance P (250 nmol/body).	de-CPM-G, administered 30 min before, did not significantly inhibit the substance P-induced scratches. It suggests that de-CPM-G does not have any antipruritic potential.

Secondary pharmacodynamic studies

There were no secondary pharmacodynamics studies presented. Instead, the Applicant presented data from experiments reported in the literature that describe other pharmacodynamic actions of nalfurafine hydrochloride. In a range of rodent and primate models, nalfurafine hydrochloride showed potent dose-dependent analgesic effects in a range of chemical, thermal or mechanical stimuli and inflammatory, diabetic, herpetic and post-herpetic pain models following various routes of administration including s.c., i.m., intrathecal (i.t.), intracerebroventricular (i.c.v.) or p.o. (Endoh 1999, 2000, 2001, Ohsawa 2005, Suzuki 2004, Takasaki 2004). In rat models of schizophrenia (Yoshikawa 2009) and Parkinson's disease (Ikeda 2009) nalfurafine hydrochloride also demonstrated moderate activity.

Safety pharmacology programme

Central nervous system – general condition

In rats receiving nalfurafine hydrochloride i.m. at 30-300 µg/kg, decreases in alertness and spontaneous motor activity, abnormal gait and blepharophimosis were observed, which recovered almost completely 4-8 hr after administration. In addition to these signs, those animals receiving nalfurafine hydrochloride at 300 µg/kg also displayed slight decreases in position perception, passivity and reactivity, slight low posture, slight hypoactivity of the corneal reflex, slight decrease in ipsilateral flexor reflex, and slight decrease in body temperature that resolved almost completely 8 hr after administration (pP29).

In dogs, slight to moderate sedation, increased muscle tone, ataxia and tremors were observed in animals receiving nalfurafine hydrochloride i.v. at 30-1000 µg/kg, which persisted for 4-6 hr after administration (pP31).

In monkeys, prostration, slow motion, hypoactivity and crouching posture were observed in animals receiving nalfurafine hydrochloride i.v. at 0.5 and 1 µg/kg that persisted for 2-3 hr after administration. Eye-closing was also observed at 1 µg/kg. Furthermore, those animals receiving 2 µg/kg also demonstrated ataxia, jaw dropping and salivation that were still present after 5 hr, but absent 24 hr after administration (pP34).

Central nervous system – spontaneous motor activity

In the rat ANIMEX test, a decrease in spontaneous motor activity was observed in animals receiving nalfurafine hydrochloride i.m. at 10-300 µg/kg for 10-50 min after administration (pP29). An increase in spontaneous motor activity was also observed in animals receiving nalfurafine hydrochloride i.m. at 3 and 10 µg/kg for 50-60 min after administration (pP29).

In the mouse running-wheel test, nalfurafine hydrochloride at 10 and 30 µg/kg, s.c. produced a significant decrease in spontaneous motor activity for a 60-min period 30 min after administration. The ED50 was 7.79 µg/kg (pP35).

In the mouse SUPERMEX test, nalfurafine hydrochloride at 400 µg/kg, p.o. produced a significant decrease in spontaneous motor activity for a 30-min period 30 min after administration. The ED50 was 345 µg/kg (pP36).

In the rotarod test with mice dosed at up to 300 µg/kg of nalfurafine hydrochloride, p.o., there were significant effects 30 and 60 min post-dose. The ED50s were 115 and 72.3 µg/kg, respectively (pP37).

Central nervous system – anaesthetic effect

Nalfurafine hydrochloride at 30-300 µg/kg, s.c. significantly increased pentobarbital-induced sleeping time in mice (pP38).

Central nervous system – EEG

Nalfurafine hydrochloride administered s.c. to conscious and unrestrained rats at dose levels of 3, 10 or 30 µg/kg induced some changes to their spontaneous EEGs. There was no change on the EEG waveform but frequency analysis revealed decreases in the alpha and beta-1 band of the frontal neocortex and the alpha band of the hippocampus. Sleep-wake analysis revealed an increase in the awake state, a decrease in and a prolongation of latency of, slow and fast wave sleep (pP39).

Autonomic nervous system

Nalfurafine hydrochloride administered s.c. to mice at 10-300 µg/kg had no anticonvulsant effects in either the maximum electroshock- or the pentylenetetrazole-induced convulsion studies (pP29).

Nalfurafine hydrochloride at 30-300 µg/kg produced a significant decrease in the body temperature of rats 0.5 hr after i.m. administration and this effect persisted for 2-6 hr after administration (pP29).

Cardiovascular and respiratory systems

In an *in vitro* electrophysiological assay, nalfurafine hydrochloride was shown to exert a significant concentration-related suppression of hERG peak tail current with an IC50 estimated to be 840 nmol/L and a no-effect concentration of 3 nmol/L (pP30). There was a wide safety margin (ratio=7 400) between the IC50 of hERG current suppression and the maximum plasma concentration after a single intravenous injection at 5 µg/body of nalfurafine hydrochloride in haemodialysis patients (58.19 pg/mL). The potential effects of nalfurafine hydrochloride on the cardiovascular system were further investigated *in vitro* using isolated guinea pig papillary muscle (pP40). Nalfurafine hydrochloride significantly delayed repolarisation of the action potentials with APD50 (14.1%) and APD90 (16.4%) at 3000 nmol/L, but not at lower concentrations.

In vivo, nalfurafine hydrochloride at 100 µg/kg or higher, i.m. produced an apparent transient increase (+10% at 100 and 300 µg/kg) and then a decrease (-5% at 100 µg/kg, -6% at 300 µg/kg) in MBP and heart rate in rats (pP29). Nalfurafine hydrochloride at 100 or 1000 µg/kg, i.v. produced a significant increase in SBP and MBP (+11% at 30 µg/kg, +36% at 100 µg/kg, +25% at 300 µg/kg, +35% at 1000 µg/kg in MBP) in conscious dogs (pP31), which was assumed to be due to an indirect effect based on behavioural changes, while nalfurafine hydrochloride at doses up to 30 µg/kg, i.m. produced slight and no significant increase in DBP, SBP and heart rate in conscious dogs (pP32). On the other hand, nalfurafine hydrochloride, p.o. slightly decreased SBP and MBP (-23% at 10 µg/kg, -13% at 100 µg/kg, -11% at 300 µg/kg in MBP) and increased heart rate at 10, 100 and 300 µg/kg, but had no effects on the cardiovascular system at 3 µg/kg or the respiratory system at 300 µg/kg in conscious dogs, including no

significant effects on PR interval, QRS duration, QT interval, or QTc at doses up to 300 µg/kg (pP33). In addition, nalfurafine hydrochloride by i.v. infusion in anaesthetised dogs lowered the SBP, DBP and MBP at 0.1 µg/kg or higher (-23% at 0.1 µg/kg, -39% at 1 µg/kg, -47% at 10 µg/kg in MBP), and it showed a tendency to decrease heart rate (pP41). However, it did not have any effects on ECG parameters at doses up to 10 µg/kg (pP41). In dog repeated-dose toxicity studies which monitored the cardiovascular system, no cardiovascular toxicity was identified (pT22-pT27).

Renal system

Nalfurafine hydrochloride at 1-30 µg/kg, i.m. dose-dependently increased the urine volume and decreased the urinary Na+ and K+ concentration in rats, indicating that nalfurafine hydrochloride had a diuretic effect. However, nalfurafine hydrochloride affected the urinary Na+/K+ ratio only very slightly, and therefore, it was considered unlikely that nalfurafine hydrochloride would exert any adverse effects on the cardiovascular system by inducing blood Na+/K+ imbalance through its potential diuretic effect (pP42).

Nalfurafine hydrochloride had no effect on the ACh-, histamine- or barium chloride-induced contraction of isolated GPI (pP29, pP43) *in vitro*.

Gastrointesinal system

Nalfurafine hydrochloride at 10 µg/kg, s.c. or higher, had an inhibitory effect on gastrointestinal transit in the mouse charcoal meal test (pP44). However, the effect was less marked than that induced by morphine.

Pharmacodynamic drug interactions

Nalfurafine hydrochloride at 10 µg/kg, s.c. did not affect the duration of pentobarbital-induced sleeping time in mice, but significantly increased it at 100 µg/kg.

The effect of nalfurafine hydrochloride administered s.c. on the CNS depressant effect of ketotifen, an antihistamine, was investigated. Concomitant use of nalfurafine hydrochloride at 10 µg/kg, s.c. slightly shortened the ketotifen-induced prolongation of pentobarbital sleeping time. Nalfurafine hydrochloride at 100 µg/kg, s.c. additively prolonged the ketotifen-induced prolongation of pentobarbital sleeping time, although there was no significant difference (pP45).

The effect of nalfurafine hydrochloride administered s.c. on nitrazepam, a sleep inducer was also investigated. Concomitant use of nalfurafine hydrochloride at 1-100 µg/kg dose-dependently increased the nitrazepam-induced prolongation of pentobarbital sleeping time (pP46). Nalfurafine hydrochloride should therefore be used with special care when used with sleep-inducers in clinical situations.

2.3.3. Pharmacokinetics

Methods of analysis

The toxicokinetics, pharmacokinetics, tissue distribution, metabolism and excretion profiles of nalfurafine hydrochloride have been investigated mainly in rats, but also in mice, dogs and primates. Most of the studies were carried out with formulations in 5% mannitol solution using the i.m. and i.v. routes. Also the s.c. and p.o. routes of administrated have been used, the p.o. route is assessed of limited value for this application.

Nalfurafine hydrochloride and its derived metabolite in human plasma, urine and feces were quantitatively measured by electronspray ionization LC/MS/MS using a liquid-liquid extraction method.

The quantification of nalfurafine hydrochloride was feasible in the range of 0.002-0.2 ng/mL for human plasma and 0.05-20 ng/mL for human urine.

Absorption

The plasma concentrations of nalfurafine hydrochloride were determined in male CD-1 mice after single administration via the i.v., s.c. and p.o. routes. After a single i.v. dose of 0.04 mg/kg, plasma nalfurafine hydrochloride concentration decreased to a level less than the lower limit of quantification by 24 hr post-dose. The $t_{1/2}$ was calculated to be 2.16 hr. The PK parameters of nalfurafine hydrochloride following s.c. administration were similar to those from i.v. injection. The bioavailability was 95.8% in mice given a single dose of nalfurafine hydrochloride s.c.. The $t_{1/2}$ observed after p.o. administration (4.51 hr) was about twice that seen after s.c. or i.v. administration, the p.o. bioavailability was 32.2% (pA33).

Similar results were obtained in rats given nalfurafine hydrochloride by the i.v. and i.m. routes. Although following i.v. dosing, a $t_{1/2}$ of 1.60 hr contrasted with 6.86 hr by the i.m. route, the i.m. bioavailability was 102% (pA34).

In dogs, given nalfurafine hydrochloride by the i.v. route, the $t_{1/2}$ was calculated to be 6.75 hr, and that by the i.m. route was 4.69 hr with a bioavailability of 103% (pA36).

In monkeys, by the i.v. route, the $t_{1/2}$ was 5.21 hr (pA38). By the i.m. route, the $t_{1/2}$ was 4.58 hr and comparison of the achieved plasma levels from the 2 routes indicated the bioavailability in this species was also high by the i.m. route (pA39).

After repeated i.m. dosing of [^{3}H]- nalfurafine hydrochloride in the rat, $C_{min,}$ $t_{1/2\alpha}$ and $t_{1/2\beta}$ tended to increase suggesting that there was an accumulation of radioactivity in plasma with increasing duration of dosing (pA40).

Distribution

The PK and tissue distribution of radiolabeled nalfurafine hydrochloride following single i.v. administration was studied in male mice. Nalfurafine hydrochloride was widely distributed, with the highest concentration of radioactivity in the kidney and the least in the brain and testes (pA41). The radioactivity concentrations in tissues decreased in proportion to that in the plasma.

In rats, radioactivity following an i.m. dose of [^{3}H] - nalfurafine hydrochloride was also widely distributed in both sexes and found predominantly in the kidneys. Relatively high concentrations persisted in the kidney, liver and thyroid (pA42). Microautoradiography of the thyroid showed that nalfurafine hydrochloride and/or its metabolites were distributed locally in the follicular colloid but not in the follicular epithelial tissue (pA42). The radioactive materials were shown to cross the placental barrier in rats, be widely distributed in fetal tissues, and appear in the mother's milk.

In a pigmented male rat study, the rate of elimination from the eye was slower than other sampled tissues, suggesting that there may be some affinity to melanin (pA43).

Plasma protein binding and blood cell distribution in rats, mice, dogs, monkeys and humans was investigated *in vitro* (pA44). The binding to plasma proteins ranged from about 58% in mice to 75% in humans. The distribution to blood cells ranged from about 55% in rats to 65% in monkeys and humans. There were no significant sex-dependent difference and the binding was independent of species and dose.

When 3H-nalfurafine hydrochloride was administered p.o. to male dogs at 0.02 mg/kg, the blood cell binding rate was found to be 40.3% 15 min after administration, whereafter it gradually decreased, and was found to be 23.3% 48 hr after administration (pA45). The *in vivo* plasma protein binding rate was

found to be 39.8% 15 min after administration, decreasing to 32.3% 30 min after administration, and then gradually increasing to 83.6% 48 hr after administration (pA45).

Metabolism

Nalfurafine hydrochloride undergoes decyclopropylmethylation and is metabolized to 17-decyclopropylmethylated nalfurafine (de-CPM), which is conjugated with glucuronic acid forming 3-glucoronide of 17-decyclopropylmethylated NAF (de-CPM-G). Nalfurafine hydrochloride is also metabolized to glucuronic acid conjugate forming 3-glucuronide of nalfurafine hydrochloride (NFA-G), and NFA-G in part is metabolized to de-CPM-G. In both rats and dogs, de-CPM was the major metabolite in urine and faeces, suggesting that de-CPM were excreted in faeces via hepatobiliary route.
The distribution of unchanged nalfurafine hydrochloride and its metabolite, NFA-G, de-CPM, de-CPM-G, were determined by HPLC in rats, mice and dogs given a parenteral dose of ^{3}H-labeled compound.

In male mice, nalfurafine hydrochloride was predominant in plasma, with appreciable amount of de-CPM-G, but de-CPM and NFA-G were minor components in plasma. Unknown metabolites, a mixture of polar metabolites, were observed in the liver, whereas nalfurafine hydrochloride was mainly detected in the kidney. Nalfurafine hydrochloride was the primary component found in the brain (pA48).

In the rat, nalfurafine hydrochloride was predominant in plasma, with a relatively large amount of de-CPM-G, but de-CPM and NFA-G were minor components in plasma. In rat tissues, de-CPM was predominant in the liver and nalfurafine hydrochloride was predominant in the kidney. Nalfurafine hydrochloride was the primary component found in the brain. In urine, nalfurafine hydrochloride was predominant, with appreciable amount of de-CPM and NFA-G. In faeces and bile, de-CPM was the major metabolite. The metabolite composition in females was similar to that in males (pA48, pA49).

In the dog, nalfurafine hydrochloride was predominant in plasma, with appreciable amount of NFA-G. de-CPM and de-CPM-G were minor components in plasma. In urine, NFA-G, de-CPM, and de-CPM-G were mainly detected. In faeces, de-CPM was the major excretory metabolite, with small amounts of nalfurafine hydrochloride, NFA-G, and de-CPM-G. No sex-dependent differences were noted in the metabolite composition in plasma and excreta (pA48).

In vitro metabolism

Metabolite profiles were studied using liver microsomes from male and female human, male monkey, male dog, male and female rat, and male mouse. ^{3}H-nalfurafine hydrochloride (0.2 μmol/L) was incubated with various microsomes at 37^{o} C and extracted with acetonitrile. ^{3}H- Nalfurafine hydrochloride and its metabolites were analyzed with HPLC.

The *in vitro* liver microsomal preparations from human, monkey, dog, rat and mouse showed the following metabolites; XO (a mixture of polar metabolites of unknown chemical structure), de-CPM and unchanged nalfurafine hydrochloride were detected in reaction mixtures regardless of the species of microsomal origin. In addition, M2 (an unknown metabolite) was detected in reaction mixtures with liver microsomes from humans and male monkeys. The highest rate of de-CPM formation was observed in male rats, followed in decreasing order by female rats, male dogs, male mice, male monkey, female humans and male humans (pA50). Human microsomal preparations converted nalfurafine hydrochloride to de-CPM through the action of CYP2C8, 2C19 and 3A4 (pA50, pA51).

Drug metabolizing enzymes were not induced in female rats following seven days of repeated i.m. administration of nalfurafine hydrochloride (pA54).

Excretion

The excretion of radioactivity in the urine, feces, expired air and bile, and the enterohepatic recirculation after a single i.m. administration of radiolabeled nalfurafine hydrochloride in rats was examined (pA55). The radioactive material was absorbed and eliminated from the body predominantly in the feces via the hepatobiliary route, with the remainder appearing in the urine. The excreted radioactivity in bile was barely recirculated and there were no sex-dependent differences in the excretory ratios.

Similar results were obtained when 3H-nalfurafine hydrochloride was dosed i.v. to pigmented rats (pA43). In this study, 82.9% of the given dose was excreted in the faeces and 6.9% in the urine. Dosimetry calculations based on the tissue distribution data from this study indicated that an i.v. dose of 6.68 MBq to human volunteers would result in a radiation exposure equivalent to 0.5 mSv.

In male dogs, given a single i.m. injection of radiolabeled nalfurafine hydrochloride, radioactivity was excreted to about 17% in the urine and 54% in the faeces within 48 hours. There was relatively slow elimination over the period remaining in the 168 hr observation. The excretion ration for female dogs was similar to that of male dogs (pA56).

2.3.4. Toxicology

An extensive program has been conducted to characterise the toxicological and toxicokinetic profile of nalfurafine hydrochloride. The studies were conducted according to the GLP standards.

Figure 1. Toxicology programme

Study Type	Route of Administration	Species (duration)
Single-Dose Toxicity	i.v. p.o. i.m	Mouse, Rat Rat, Dog Rat, Dog
Repeated-Dose Toxicity	i.v. infusion p.o. i.m.	Rat (1 month), Dog (2 weeks, 1 month) Mouse (3 months), Rat (2 weeks,1, 3, 6 months), Dog (1, 3, 12 months) Rat (1, 6 months), Dog (1, 6 months), Male Monkey (1 month)
Genotoxicity	*In vitro* ± S9 *In vivo*, i.p.	Ames testChromosomal aberration test for CHL cells Mouse micronucleus
Carcinogenicity	p.o.	Mouse (24 months), Rat (24 months)
Reproduction Toxicity	Fertility p.o. and i.m. Teratology p.o and i.m. Pre- and Post Natal p.o. and i.m.	Rat Rat, Rabbit Rat
Local Tolerance	*In vitro* *In vivo*, i.v., paravenous and intraarterial	Human blood Rabbit ear
Other Studies	Antigenicity Dependency and Abuse Potential Phototoxicity	Guinea Pig ASA and PCA Rat, Monkey Assessment of UV absorption in the range 290 – 700 nm BALB/3T3 cells Rat (single dose)

*In addition, the [redacted]HCl was assessed in a 2-week i.v. repeated dose toxicity study and in 2 *in vitro* genotoxicity assays. All studies indicated in the above table were conducted as GLP compliant studies.

Single dose toxicity

In the toxicology studies single doses of Nalfurafine hydrochloride ranged from 10 to 50 mg/kg (intravenous; mice and rats), from 0.01 to 10.0 mg/kg (intramuscular; dogs), from 189.0 to 320 mg/kg (intramuscular; rats), from 0.003 to 0.3 mg/kg (oral gavage; dogs) and from 651.0 to 1350.0 mg/kg (oral gavage; rats). The proposed clinical dose of Nalfurafine hydrochloride is 10 µg for a patient. The highest dose tested in single dose toxicity studies was 50 mg /kg b.w. (intravenous route). By the clinical route of administration i.e. intravenous (i.v.), a single injection in mice identified the approximate lethal dose as 25 mg/kg in males and 50 mg/kg in females. By the clinical i.v. route in rats, the approximate lethal dose was 25-50 mg/kg, sexes combined. The p.o. approximate lethal dose (rats) was determined to be 651 mg/kg or below for males and 781.3 mg/kg for females. Arrhythmia was noted in one of the six animals treated with Nalfurafine hydrochloride at 937.5 mg/kg. For dogs a single p.o. dose of 0.3 mg/kg was well tolerated. By the i.m. route (dogs), doses up to 0.03 mg/kg were tolerated, but only with

extreme pharmacological symptoms being induced (emesis, salivation and tremors). Doses of 0.1, 1 and 10 mg/kg induced prone position, convulsions, and loss of pupillary reflex. The NOAEL of nalfurafine hydrochloride after a single i.v. injection (clinical route of administration) in rats and mice was 25 mg /kg (F) and 10 mg/kg (M).

Table 4. Overview of the single dose toxicity studies

Species /group size	Dose mg/kg	Route	Major findings	Study ID
CD-1 Mouse (5m+5f)	10, 25, 50	i.v.	≥10 mg/kg: lethargy, ↓ in locomotor activity, staggering, prone position, abnormal respiration, unconsciousness (m). ≥25 mg/kg: unconsciousness and hunched back (f) Minimum Lethal Dose (MLD): 50 mg/kg	96/TAD003 A/0114
CD-1 Mouse (5m+5f)	10, 25, 50	i.v.	≥10 mg/kg: prone posture, decreased locomotor activity, abnormal respiration, staggering, eye close (m), chronic convulsion (m), eye discharge (f), hunched posture (f). All animals were found dead in the highest dose group and 2 males were found dead in the 25 mg/kg dose group. MLD: 50 mg/kg	96/TAD003 B/0115
CD-1 Mouse (5m+5f)	10, 25, 50	i.v.	≥10 mg/kg: ↓ in locomotor activity, prone position MLD: 25 mg/kg (m), 50mg/kg (f)	TLG 96001
Wistar rat (5m+5f)	10, 25, 50	i.v.	≥10 mg/kg: lethargy, ↓in locomotor activity, staggering, prone position, abnormal respiration. ≥25 mg/kg: unconsciousness (f) MLD: 50 mg/kg	96/TAD002 A/0112
Wistar rat (5m+5f)	10, 25, 50	i.v.	≥10 mg/kg: ↓ in locomotor activity, staggering, piloerection, abnormal respiration (f). ≥25 mg/kg: prone position, abnormal respiration (m), purple tail (f), hunched posture (f). ≥50 mg/kg: pallor MLD: 50(m), 25(f)	96/TAD002 B/0113
Wistar rat (5m+ 5f)	10, 25, 50	i.v.	≥10 mg/kg: prone posture decreased locomotor activity. 2 males were found dead in the 25 mg/kg dose group. MLD: 25 mg/kg	TLG-96002
Wistar Rat (6m+6f)	189, 216, 246, 281, 321	i.m.	≥189 mg/kg: decreased body weight (between day 2 to day 4), prone position, decreased locomotor activity, nodule, mass loss of fur and crust formation at the injection site, lacrimation, eye discharge. The CNS effects lasted until day 2 while the effects on the injection site lasted until the end of observation period). In 246, 281 and 320 mg/kg groups, 2males, 1 male and six males and three females were found dead respectively. MLD= 246 in males and 320 in females	TLG 93004
Wistar rat (6m+6f)	651, 781.3, 937, 1125, 1350	Gavage	≥651 mg/kg: stomach and urinary bladder dilation, prone position, decreased locomotor activity, lacrimation, eye discharge, loss of hair, eye close. At 937.5 mg/kg: arrhythmia (1/6); the animal died 4-hour post dose. Irregular respiration in 1/5 animals of 781.3 and 3/6 of 1350 mg/kg groups (these three animals were found dead within several hours). Abnormal gait in 5/5 animals at 651 and 781.3 mg/kg group, and in 2/5 animals of 781.3 mg/kg group. MLD: 651(m), 781(f)	TLG-95002
Beagle dog (2m+2f)	0.003, 0.01,	i.m.	≥0.003 mg/kg: Decrease in locomotor activity, decrease in food consumption, tremor, (F)salivation,	HWA 2157-108

	0.03		(F)sedation, injected sclera, loss of pupillary reflex, abnormal feaces ≥0.01 mg/kg: miosis, prone position, ataxia, (M)salivation, (M) sedation MLD: > 0.03 mg/kg (m), 0.03 mg/kg (f)	
Beagle dog (2m+2f)	0.03, 0.1, 0.3	Gavage	All animals survived to their scheduled termination. ≥0.1 mg/kg: Ataxia, hypoactivity, tremors, salivation, sedation, excessive salivation, dyspnea, discoloration of feces, negative pupillary reflex, miosis. MLD: >0.3 mg/kg	CHV 2157-114 (GLP)

Repeat dose toxicity

Repeated-dose toxicity studies were conducted in mice, rats, dogs and monkeys by i.v., p.o. and i.m. routes. In all the repeated-dose studies, the maximum given doses were limited by under active behaviour and sedation with attendant low respiration rate arising probably by a CNS depressant effect.

In a preliminary 3-month p.o. range-finding toxicity study in mice, doses up to 10 mg/kg/day were given and were well tolerated, with the exception of the initial under active behaviour and decreased body weights and food consumption. There were no sex differences, organ weight changes or macroscopic signs at necropsy. No histopathology was conducted. At this dose level, the Day 1 and Week 13 AUC0-24hr values were 1054 (males) and 995 (females), and 1152 (males) and 1170 (females) ng hr/mL, respectively.

In rats by the continuous i.v. infusion of Nalfurafine hydrochloride (0.07, 0.7, and 7 mg/kg/day) over a 1-month period resulted in hypoactivity in the high-dose groups. Other clinical observations included decreased food consumption and body weight. Serum protein and globulin, and urine Na^+ were increased in the 0.7 and 7 mg/kg/day groups. Mean absolute weights of the seminal vesicles were also significantly decreased compared to the control. At the top dose level, toxicokinetic evaluation indicated that approximately 60 ng/mL Nalfurafine hydrochloride was rapidly achieved and sustained throughout the study. In the control, 0.07 and 7 mg/kg/day groups, a total of six animals died with findings consistent with sepsis.

In the 6-month p.o. toxicity study in rats with toxicokinetic and recovery groups, Nalfurafine hydrochloride was given at 0.5, 5 and 50 mg/kg/day. Decreased activity, food consumption and body weight gain were observed. There were no other remarkable findings. The NOAEL was determined to be less than 0.5 mg/kg/day (Plasma levels: 3.43 and 6.27 ng/mL for males and females, respectively). By the i.m. route of administration rats exposed to Nalfurafine hydrochloride at dose levels of up to 4 mg/kg/day exhibited similar clinical signs to those described in the single-dose study. The body weight suppression and CNS effects limited the determination of NOAEL to 0.004 mg/kg/day. Absolute prostate weight in the 0.04 mg/kg/day or higher groups, and testes weights in the 0.4 and 4 mg/kg/day groups were decreased.

In the 6-month repeated dose i.m. study in rats, doses of 0.004-4 mg/kg/day were given followed by a 1-month recovery. All animals survived throughout the treatment period. There were signs of sedation, but food consumption and body weight gain were decreased in the higher dose groups. An increase in water consumption and urinary excretion was also noted. Absolute prostate weights were decreased in the 0.4 and 4 mg/kg/day groups. Slight infiltration of inflammatory cells into the connective tissue at the injection sites and necrosis of muscle fibres were noted in 1 male in the top dose group. The NOAEL was determined to be 0.004 mg/kg/day.

In dogs Nalfurafine hydrochloride was administered by continuous i.v. infusion for 2 weeks at 0.15 and 0.45 mg/kg/day and for 1 month at 0.01, 0.03 and 0.09 mg/kg/day. In the first study, clinical signs were severe and the dose was immediately reduced by 10-fold. On Day 8, the lower dose was raised to 0.03

mg/kg/day and the higher dose was raised to 0.09 mg/kg/day. All animals survived to termination and there were no significant findings following gross or histopathological examination. In the second study, dogs survived to termination but showed signs of sporadic decreased food consumption, hypoactivity/sedation, tremors and/or convulsions, ataxia, and prostration. Absolute prostate and testes/epididymides weights were decreased and immaturity of the male reproductive organs was observed. There was no other remarkable toxicity or histopathological finding.

In a 12-month p.o. toxicity study, Nalfurafine hydrochloride was administered to beagle dogs at 0, 0.0003, 0.001, and 0.003 mg/kg/day. All dogs survived. No treatment-related findings occurred in females at any dose level (0.0003, 0.001 and 0.003 mg/kg/day) or in males dosed at 0.0003 mg/kg/day. Testosterone concentration was decreased markedly in the 0.001 and 0.003 mg/kg/day males at 1 and 4 hr after dosing on Day 1 and remained less than control values at 8 and 24 hr after dosing. Similarly, decreased testosterone values were seen at 4 hr after dosing during Week 13. At Week 52, the testosterone concentrations were decreased at 4 hr after dosing. The decreased testosterone levels and prostate weights recovered to within the control range following the 1-month non-treatment period. There were no other treatment-related findings in the 0.001 and 0.003 mg/kg/day male dogs. In dogs treated p.o. for 12 months, the NOAELs for nalfurafine hydrochloride were 0.0003 mg/kg/day for males and 0.003 mg/kg/day for females. At Week 52, the C_{max} values were 19.74 and 117.50 pg/mL for males and females, respectively.

In dogs, repeated i.m. dosing with nalfurafine hydrochloride at dose levels of up to 0.01 mg/kg/day for 1 month reflected the single-dose findings. The only significant finding was that maturation of male reproductive organs was reversibly inhibited by Nalfurafine hydrochloride, mediated by suppression of testosterone. The NOAEL was determined to be 0.001 mg/kg/day for females and less than 0.001 mg/kg/day for males based on the changes in serum testosterone, effects on maturation of male reproductive organs, and clinical signs. A 6-month i.m. study at 0.0003-0.01 mg/kg/day with a 1-month recovery period was conducted, which also displayed typical clinical signs of ataxia, tremors and/or convulsions. Two dogs administered at 0.01 mg/kg/day suddenly died or became moribund without explanation on Days 1 and 2.

At the highest dose, profound sedation, tremors and ataxia occurred, and persisted for a number of hours and it was conceivable that life-threatening events occurred. Sexual immaturity in the males was seen, which recovered during the withdrawal period. The NOAEL was determined to be 0.001 mg/kg/day, and the corresponding plasma level of Nalfurafine hydrochloride was approximately 0.2 ng/mL.

In a 1-month i.m. study with male cynomolgus monkeys, there were no significant toxicological findings attributable to Nalfurafine hydrochloride administration, including no effects on spermatogenesis, at dose levels of up to 0.01 mg/kg/day.

Table 5. Overview of the repeated dose toxicity studies

Species /group size	Duration	Dose (mg/kg/day) / Route	Major findings	Study ID
SD Rat (10m+ 10f)	4 weeks	0.07, 0.7 and 7 intravenous infusion	≥0.7 mg/kg: hypoactivity, decreased body weight, decreased food consumption, higher mean values for total protein and globulin values, increased urine sodium, decreased mean absolute weights of seminal vesicle and seminal-vesicle to body weight ratio (only in 7.0 mg/kg)	CHV 6745-103
Wistar Rat (5m+5f)	2 weeks	0.01, 0.1, 1.0, 10 and 100 gavage	≥1 mg/kg: prone position, decreased locomotor activity, decreased food consumption and body weight, lacrimation, eye discharge, increased water consumption	TLG-95005

			and urination, decreased in absolute and relative weights of liver (male and female) and testes and thymus (females) weights and increase in the absolute and relative organ weights of adrenals (males). Decrease in serum ALP, TG and tot cholesterol. NOAEL: 0.1 mg/kg	
Wistar Rat (10m+10f)	4 weeks	0, 0.05, 0.5, 5 and 50 gavage	≥0.5 mg/kg: prone position and decreased locomotor activity, decreased body weight, lacrimation, eye discharge and loss of hair around the eye. ↑ in urine volume, decreased specific gravity and lower PH of urine, increased in urine volume and ↓ excretion of Na^+, K^+ and Cl^- (≥5 mg/kg). ↓ in serum alkaline phosphatase and TG, decrease in total cholesterol (females), ↓ RBC and ↑ MCV, MCH and reticulocyte counts (males) and PLT (females), ↑ total body protein and albumin . ↑ weights of adrenals (males) and thyroid gland (females), ↓ submandibular glands and thymus gland weight (female). NOAEL: 0.05 mg/kg	TLG-95009
SD Rats (20m+20f)	26 weeks	0.5, 5 and 50 gavage	≥ 0.5 mg/kg: ↓ of body weight gain, ↓ food consumption, low seminal vesicle weight (m) NOAEL: <0.5 mg/kg	CHV 6745-105
Wistar Rat (15m+15f)	4 weeks with a 28-days recovery period	0, 0.004, 0.04, 0.4 and 4 i.m.	≥0.04 mg/kg: prone position and decreased locomotor activity, decreased body weight and food consumption. ↑ water consumption and urine volume, ↓ specific gravity and electrolyte concentration of urine. White spots in the anterior ocular segment. The animals returned to normal during the recovery period. NOAEL: 0.004 mg/kg	TLG-93008
Wistar Rat (20-26m+ 20-26f)	6-months with one month recovery	0, 0.004, 0.04, 0.4 and 4 i.m.	≥0.004 mg/kg: sedation, ↑ frequency of urination and lacrimation, decreased body weight gain and food consumption. ↑ water consumption. ≥ 0.04 mg/kg: ↓ absolute organ weight of heart, lung (not at 4 mg/kg), liver, spleen and thymus. ≥ 0.4 mg/kg: ↓ prostate absolute weight, ↑ urine volume, ↓ specific gravity and sodium, potassium and chloride concentrations. ↓ RBC, PLT, Leucocytes and ↑ MCV and MCH (males) ↓ total protein, tot cholesterol, TG and phospholipids in blood. 0.04 and 0.4 mg/kg: ↓hematocrit (males) and ↓ hemoglobin and PLT counts (females), ↓kidney weight (at 0.4 mg/kg only). 4 mg/kg: inflammatory cellular infiltration in connective tissues, epimysium or endomysium of skeletal muscle and necrosis of muscle fiber in injection site upon histopathological examination. NOAEL: 0.004 mg/kg	JBC-94-RMCH-268
Beagle Dog (3m+3f)	2 weeks	Low dose group: 0.15 (day 1), 0.015 (day 1-7), 0.03 (day 8-15) High dose group:	0.015 mg/kg: emesis. 0.03 mg/kg: tremor (f), lacrimation (f). 0.03, 0.09 mg/kg: abnormal feces, emesis (m), tremor (m). 0.015, 0.045 mg/kg: ataxia, ↓in locomotor activity, abnormal feces, sedation (f), tremor (f), red eyes. 0.045 mg/kg: Salivation (m), sedation (m), red eyes (m) 0.09 mg/kg: lacrimation (m). 0.15 mg/kg: ↓ in locomotor activity (m),	CHV 6745-101

		0.45 (day 1) 0.045 (day 1-7) 0.09 (day 8-15) iv. infusion	polypnea (m), emesis (f), abnormal feces (f), mydriasis (f). 0.45 mg/kg: prone position, dyspnea, miosis, red eye (m), partial closure (m). 0.15, 0.45 mg/kg: Ataxia, sedation, tremor, abnormal faeces (m), emesis, salivation, ↓ in locomotor activity, red eye (f), partial closure (f). No NOAELcould be determined	
Beagle Dog (3m+3f)	4 weeks	0.01, 0.03 and 0.09 i.v.	≥0.01 mg/kg: tremor, abnormal faeces, salivation, emesis (f). ≥0.03 mg/kg: ↓in locomotor activity, ataxia(m), emesis(m).0.09 mg/kg: ataxia (f),sedation (f), delayed sexual maturity in both sexes. 0.01and 0.03 mg/kg: small/immature testis and prostate. No NOAELcould be determined	CHV 6745-100
Beagle Dog (3m+3f)	4 weeks	0.01, 0.03 and 0.1 gavage	≥0.01 mg/kg: ↓ in serum testosterone levels, prostate hypoplasia, hypocellularity in testis, absence of normal cyclic activity in female reproductive organs. No NOAELcould be determined	CHV 2157-115
Beagle Dog (4m+4f)	13 weeks	0.01, 0.03 and 0.1 gavage	≥0.01mg/kg: ↓testosterone levels, small/immature testis and prostate, immature uterus. No NOAELcould be determined	Covance 6745-102
Beagle Dog (4m+4f)	13 weeks	0.0003, 0.001, 0.003 and 0.01 gavage	≥0.001 mg/kg: ↓ serum LH , progesterone and estradiol, delay of sexual maturity. ≥0.003: ↓ serum testosterone levels NOAEL: 0.0003 mg/kg	Covance 6745-106
Beagle Dog (4m+4f)	52 weeks	0.0003, 0.001 and 0.003 gavage	All animals survived. No clinical signs in females or in males at 0.0003 mg/kg/day. 0.001 and 0.003 mg/kg/day males: ↓testosterone levels. prostate weights were decreased at terminal sacrifice. Recovered during the 1-month non-treatment period. NOAEL: 0.0003 mg/kg (m); NOAEL: 0.003 mg/kg (f)	Covance 6745-108
Beagle Dog (3m+3f)	4 weeks	0.001, 0.003 and 0.01 i.m.	≥0.003 mg/kg: ↓in body weight gain, ↓in food consumption, low serum testosterone values, ↓of testicular weights, hypospermatogenesis. 0.01mg/kg: ↓ of thymus weight NOAEL: <0.001 mg/kg	HWA 2157-109
Beagle Dog (6m+6f)	26 weeks with 4 weeks recovery period	0.0003. 0.001, 0.003 and 0.01 i.m.	≥0.0003 mg/kg: decrease in serum testosterone levels. ≥0.003 mg/kg: tremor, ataxia, sedation, ↓locomotor activity,↑ serum electrolytes (f), ↓ testis and prostate absolute weights. ↑ serum urea nitrogen (males at 0.003 and females at 0.01 mg/kg). 0.01 mg/kg: immature epididymis and prostate. None of these effects were evident after the 4 weeks recovery period. NOAEL: 0.0003 mg/kg	CHV 2157-112
Cynomolgus Monkey (4m)	4 weeks	0.0003, 0.001, 0.003 and 0.01 i.m.	No effects at any of the tested doses. NOAEL: 0.01 mg/kg	CHV 2157-111

Genotoxicity

The genotoxicity of nalfurafine hydrochloride has been studied with respect to gene mutations in bacteria and mammalian cells and chromosomal aberrations *in vitro*. Also Nalfurafine hydrochloride was evaluated

for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by quantifying micronuclei in polychromatic erythrocyte (PCE) cells in mouse bone marrow. All genotoxicity tests performed were conducted in accordance with GLP requirements. Nalfurafine hydrochloride showed no signs of genotoxicity in these studies.

Table 6. Summary of the genotoxicity studies

Type of test	Test system	Route	Concentrations/Dosages/ Metabolizing system	RESULT
Gene mutation in bacteria	*Salmonella typhimurium* strains TA 1535, TA1537, TA 98 and TA 100	*In vitro*	0.1-5000 μg/plate ±S9	Negative
	Salmonella typhimurium strains TA 1535, TA1537, TA 98 and TA 100	*In vitro*	0.05-5 mg/plate ±S9	Negative
	Escherichia coli WP2urvA	*In vitro*	0.1-5000 μg/plate ±S9	Negative
Chromosome aberration *in vitro*	V79 Chinese hamster cells	*In vitro*	15-81 μg/ml -S9, 57-131 μg/ml +S9	Negative
Bone marrow micronucleus test	CD-1(ICR) mice	*In vivo* i.p., single dose	25, 50, 100 mg/kg	Negative

Carcinogenicity

CD-1 mice received nalfurafine hydrochloride by p.o. at 0, 0.04, 0.2 or 0.5 mg/kg/day over a period of 24 months. Underactive behaviour was observed shortly after dosing on the first day of treatment in a few animals receiving 0.2 or 0.5 mg/kg/day. There were no other signs associated with treatment. Body weight gains were increased in males and females receiving 0.2 or 0.5 mg/kg/day. Haematology investigations at Week 104 revealed minor changes in these two groups. At necropsy, there were no organ weight changes. Masses in the colon for one male which received 0.2 mg/kg/day and one male which received 0.5 mg/kg/day were observed. A high incidence of distended urinary bladder was also observed for males receiving 0.5 mg/kg/day. Adenocarcinomas were identified in the colon of one male given 0.04 mg/kg/day, two males given 0.2 mg/kg/day and two males given 0.5 mg/kg/day but these were considered to be incidental.

There were no non-neoplastic findings attributable to treatment. This is acceptable explanation because adenocarcinomas are not unexpected observation for mice studies.

Han Wistar rats received Nalfurafine hydrochloride by p.o. at 0, 0.04, 0.2 and 0.5 mg/kg/day over a period of 24 months. Underactive behaviour was observed during the first week of treatment in animals receiving 0.5 mg/kg/day and piloerection also seen during Week 1 in several females. Salivation was recorded in animals receiving 0.2 and 0.5 mg/kg/day. Overall body weight gains were decreased in all treated groups of animals with the exception of males receiving 0.04 mg/kg/day. There were minor haematological changes at Week 102 in females receiving 0.2 mg/kg/day and in animals receiving 0.5 mg/kg/day. Urinalysis revealed decreased volumes, decreased pH and increased specific gravity in all treated groups. Urinary protein concentration was increased in all treated male groups and total sodium concentrations were decreased in females receiving 0.2 mg/kg/day and in animals receiving 0.5

mg/kg/day. At necropsy, there were no significant gross findings. There were increased liver weights relative to body weight in all treated groups, with the exception of males given 0.04 mg/kg/day, and decreased seminal vesicle weights in males given 0.5 mg/kg/day. Histopathological examination did not reveal any neoplasms that were related to treatment. The only non-neoplastic treatment-related findings were seen in the kidney, where mineralisation and hyperplasia of the papillary and pelvic epithelium were all decreased in incidence in females given 0.5 mg/kg/day.

Table 7. Summary of the carcinogenicity studies

Study ID /GLP	Dose/Route	Exposure (AUC)	Species/No. of animals	Major findings
TYI 010	0.04, 0.2, 0.5 mg/kg/day p.o.	37.44 ng.hr/ml (males) 43.37 ng.hr/ml (females)	Mouse CD-1 / 54 animals per sex/per group and additional 44 per sex for toxicokinetic monitoring	Survival: average 50% and no significant differences among the study groups Clinical signs: underactive behaviour in a few animals receiving 0.2 or 0.5 mg/kg/day. Necropsy: no organ weight changes, distended urinary bladder 0.5 mg/kg/day (males). For information on tumours see table below. Toxicokinetics: mean C_{24hr} values were below the limit of quantification (0.025 ng/mL) in all dose groups. The dosage of 0.5 mg/kg/day gave rise to an exposure margin of 681 times the human AUC exposure.
TYI 011	0.04, 0.2, 0.5 mg/kg/day p.o.	76.22 ng.hr/ml (males) 34.70 ng.hr/ml (females)	Rat Han Wistar / 54 animals per sex/per group and additional 44 per sex for toxicokinetic monitoring	Survival: average 50% and no significant differences among the study groups Clinical signs: underactive behaviour in animals receiving 0.5 mg/kg/day, salivation and decreased body weight at 0.2 and 0.5 mg/kg/day. Urinalysis: decreased volumes and pH and increased specific gravity in all treated groups. Urinary protein concentration was increased in all treated male groups and total sodium concentrations were decreased in females receiving 0.2 mg/kg/day and in animals receiving 0.5 mg/kg/day. Necropsy: increased liver weights relative to body weight in all treated groups except males treated with 0.04 mg/kg/day. Decreased seminal vesicle weights in males given 0.5 mg/kg/day. Toxicokinetics: mean C_{24hr} values were below the limit of quantification (0.025 ng/mL) in all dose groups. The dosage of 0.5 mg/kg/day gave rise to an exposure margin of 630 times the human AUC exposure.

Reproduction Toxicity

Fertility and early embryonic development

The effects of Nalfurafine hydrochloride on fertility and early embryonic development were evaluated in studies using intramuscular and oral administrations to rats. In a study of the p.o. administration of Nalfurafine hydrochloride to rats before implantation (was given at 0.01, 0.1, and 1 mg/kg/day to M and F rats before and during mating, and continuing with the males after copulation, and to female rats up to implantation (Day 7 of gestation). Results: Decreased food consumption in M and F in the 0.1 mg/kg/day group; decreased locomotor activity, body weights, body weight gains, and food consumption in the 1

mg/kg/day group; and decreased liver weight in M of the 1 mg/kg/day group were observed. Administration of Nalfurafine hydrochloride did not affect copulation rate, fertility, ovulation, implantation, or the viability of embryos or foetuses. Nalfurafine hydrochloride had no adverse effects on M and F rat fertility, mating behaviour, and early embryofoetal development. In the i.m. (dose levels of 0.004, 0.04, and 0.4 mg/kg/day) Nalfurafine hydrochloride were administered to rats before and during the mating period, and continuing with males after copulation and females up to implantation. Results: Sedation, inhibition of body weight gain, and decreased food consumption were observed in M and F receiving 0.04 mg/kg/day or higher. Decreases in liver and spleen weights were observed in M receiving 0.04 mg/kg/day or higher. No effect of Nalfurafine hydrochloride on ovulation was observed, but decreases in the number of oestrus, increases in days of one oestrus cycle, delays in copulation, decreases in fertility and implantation rates, and decreases in the number of live foetuses were observed in the F receiving 0.04 mg/kg/day or higher. Decreases in copulation rate were also observed in the 0.4 mg/kg/day group.

Table 8. Fertility studies overview

Species /group size	Duration	Dose (mg/kg/day)/Route	Major findings	Study ID
SD Rat (24m+ 24f)	Males: 9 weeks Females: 2 weeks prior to mating, though day 7 of gestation	0.002, 0.006, 0.02 i.m.	0.02 mg/kg: suppression of weight gain, ↓ food consumption	JBC-93 RMIM-165
SD Rat (8m+8f)	2 weeks	0.008, 0.04, 0.2, 1 i.m.	≥0.04 mg/kg: sedation, suppression of weight gain. ≥0.08 mg/kg: ↓ food consumption. ≥0.2 mg/kg: ↓spleen weight. 1 mg/kg: suppression of weight gain, ↓food consumption, ↓liver weight	JBC-93 RMIN-165
Wistar Rat (24m+ 24f)	Males: 9 weeks Females: 2 weeks prior to mating, though day 7 of gestation	0.004, 0.04, 0.4 i.m.	≥0.004 mg/kg: ↑water consumption (premating and gestational). ≥ 0.04 mg/kg: ↓body weight and food consumption, ↓in liver and spleen weight, ↓ number of fertile males and pregnant females, ↓ mean number of estrous stage for 15 days, ↓ number of implantation sites, ↓ number of live fetuses. 0.04 mg/kg: ↓ no of spermatozoa.	JBC-94 RMFM-172
Wistar Rat (24m+ 24f)	Males: 9 weeks Females: 2 weeks prior to mating, though day 7 of gestation	0.01, 0.1, 1.0 Gavage	≥0.1 mg/kg: ↓food consumption. 1.0 mg/kg: weight losses or suppression of weight gain, ↓locomotor activity, ↓liver weight (males only)	JBC-95 ROFM-183

Embryo-foetal development

Oral route in rats showed: a decrease in locomotor activity in the 1 mg/kg/day group. Lacrimation, decreased body weights and food consumption in the 0.2 and 1 mg/kg/day groups. No significant differences were detected in the number of implantations, live foetuses, or dead embryo-foetuses. Significantly decreased body weights were noted with the M foetuses in the 1 mg/kg/day group. The incidence of thoracic vertebrae with a centre-split in the 0.2 mg/kg/day group and above was higher than

that in the controls. The incidence of thoracic vertebrae with a dumbbell-shaped centre in the 0.2 mg/kg/day group was also significantly higher. These abnormalities also tended to be higher in the 1 mg/kg/day group, and were considered to be associated with the administration of Nalfurafine hydrochloride. Administration of Nalfurafine hydrochloride (0.01 and/or 0.025 mg/kg/day; i.m.) resulted in increased early embryonic and foetal mortality, decreased ossification in live foetuses and decreases in the number of live foetuses and placental weights. In the rabbit study (p.o. administration): dams exhibited paralytic gait, and decreased stool, body weight gain, and food consumption in the 0.1 mg/kg/day group. Foetal body and placental weights tended to be decreased in the 0.1 mg/kg/day group, but no lethal effects on embryo-foetuses were observed. No potential teratogenicity of Nalfurafine hydrochloride was observed in the external, visceral, or skeletal examinations. In the rabbit study (i.m administration): Nalfurafine hydrochloride at doses up to 0.01 mg/kg/day had no effect on the number of implantations, embryonic or foetal mortality rates, or number of live foetuses. Nalfurafine hydrochloride administered at 0.01 mg/kg/day or lower resulted in decreased food and water intake in rabbit dams with corresponding reductions in body weight and/or body weight gain. At 0.01 mg/kg/day, Nalfurafine hydrochloride caused reversible, slight miosis in dams.

Table 9. Embryo/fetal development overview

Species/group size	Duration	Dose (mg/kg/day)/Route	Major findings	Study ID
NZW Rabbit (7-8 pregnant females)	G6 through G18	0.002, 0.01, 0.05 i.m.	0.01 mg/kg: miosis, 0.05 mg/kg: death (3/8), hypomyotonia, salivation, ↓water consumption, flaccidity , early embryonic and fetal mortality with decreased number of live fetuses, lower fetal body weight	JBC-95 BMTP-185 (GLP)
NZW Rabbit (7-8 pregnant females)	G6 through G18	0.001, 0.003, 0.01 i.m.	All doses: decreased food and water consumption and body weight gain. 0.01 mg/kg: miosis NOAEL < 0.001 and 0.01 mg/kg/day for dams and foetuses, respectively	JBC-95 BMTM-186 (GLP)
Wistar Rat (20-22 pregnant females)	G7 through G17	0.004, 0.01, 0.025 i.m.	≥ 0.004 mg/kg: lower birth weight, ≥0.01 mg/kg: ↑water consumption (G14), increased early embryonic and fetal mortality and decreased ossification in live fetuses, decreased placental weight 0.025 mg/kg: ↑water consumption, all embryos resorption in 7 out of 22 dams, decreased number of live fetuses, lower fetal body weight, decreased ossification in live fetuses, decreased placental weight	JBC-94 RMTM-174 (GLP)
Wistar Rat (16-20 pregnant females)	G7 through G17	0.04, 0.2, 1.0 (Gavage)	≥0.04 mg/kg: increase in water consumption, Polyuria ≥0.2 mg/kg: lacrimation, decrease in body weight gain, decrease in body weight, decrease in food consumption, increase in thoracic vertebra center-split rate, increase in thoracic vertebra dumbbell shaped center rate 1 mg/kg: decrease in locomotor activity, low foetal weight NOAEL= 0.04 (maternal general toxicity and toxic effects on the embryo-foetal development)	TLG-98009 (GLP)
NZW Rabbit (6-7 pregnant females)	G6 through G18	0.001, 0.01, 0.1 (Gavage)	≥ 0.01 mg/kg: ↓ feces, suppression of weight gain. 0.1 mg/kg: paralytic gait, ↓ food consumption	JBC-98 BOTP-230 (GLP)

NZW Rabbit (17-22 pregnant females)	G6 through G18	0.001, 0.01, 0.1 (Gavage)	0.1 mg/kg: paralytic gait, ↓ feces, weight losses or suppression of weight gain, ↓food consumption, slightly low fetal and placental weights Non-toxic dose = 0.01 mg/kg/day	JBC-98 BOTM-231 (GLP)

Prenatal and postnatal development, including maternal function

The effects of Nalfurafine hydrochloride on prenatal and postnatal development, including maternal function, were studied using i.m and oral administration to rats. Nalfurafine hydrochloride was p.o. administered to F0 dams at daily dose levels of 0.01, 0.1 and 1 mg/kg from Day 7 of gestation to Day 21 of lactation. In the dams from the 1 mg/kg/day group, lacrimation, decreased locomotor activity, body weights and food consumption were seen during the gestation period, and after parturition, significantly decreased body weights and little food consumption were seen, which subsequently recovered. The gestation index was significantly decreased in the 1 mg/kg/day group, reflecting abnormal parturition and early embryonic resorption or abortion. Worsening clinical signs, decreases in locomotor activity, lacrimation and depressed body weight gains in dams were considered a cause of early embryonic resorption or abortion. Decreases in nursing behaviour after parturition caused the whole litter loss of three dams during the lactation period in the 1 mg/kg/day group. At necropsy, no abnormality was found, except for changes related to abnormal parturition, early embryonic resorption or abortion, or poor nursing.

In the offspring, the number of deaths from Days 0 to 4 after birth was increased and postnatal survival to Day 4 was significantly lower in the 1 mg/kg/day group due to decreases in nursing behaviour in dams. In male and female pups from the 1 mg/kg/day group, body weights were significantly lower during the lactation period, and females had significantly lower values at Week 3 after birth even after weaning. In the 1 mg/kg/day group, the positive rate of eyelid opening on Day 16 after birth was significantly reduced compared to the control group.

A study by the i.m. route in rats after implantation and in the postnatal period was conducted at 0.004, 0.01 and 0.025 mg/kg/day Nalfurafine hydrochloride. In the dams, slight decreases in locomotor activity, decreased body weight gains and food consumption, and cases of non-delivery in the top dose group, were observed.

There was no effect on nursing behaviour. Three-week-old males and females receiving 0.025 mg/kg/day Nalfurafine hydrochloride also had decreased spleen and kidney weights, respectively. No deleterious effects were seen in the third generation (F2) development or viability.

Table 10. Pre-/postnatal development overview

Species /group size	Duration	Dose (mg/kg/day) /Route	Major findings	Study ID
Wistar Rats	G7-L20	0.004, 0.01, 0.025 i.m.	≥0.01 mg/kg: ↑gestational water consumption (G20) and ↑lactation water consumption (L21, hydrocephaly (1 offspring) 0.025 mg/kg: ↓ male pup weight at weaning, decreased spleen and kidney weights in the offspring, hydrocephaly (2 offsprings), increased litter with stillbirths and resorptions	JBC-94 RMDM-173
Wistar Rats (29-30 pregnant females)	G7-L20	0.004, 0.01, 0.025, 0.05 i.m.	≥0.004 mg/kg: soiled lower abdomen, lacrimation, ≥ 0.01 mg/kg: low female pup weight, ≥0.025 mg/kg: low male pup weight, 0.05 mg/kg: low gestational index	TLG-95006

Wistar Rats (4-5 pregnant females)	G7-L7	0.03, 0.1, 0.3, 1 Oral gavage	Dams: >0.3 mg/kg: decrease in locomotor activity, lacrimation, decrease in body weight gain, reduced implantation Pups: 1 mg/kg: Decrease in number of live pups and birth index	4793
Wistar Rats (21-24 pregnant females)	G7-L21	0, 0.01, 0.1, 1 Oral gavage	Dams: 1 mg/kg: decrease in locomotor activity, lacrimation, decrease in body weight, decrease in food consumption, decrease in the rate of delivery, poor nursing behaviour post parturition Pups: 1 mg/kg: lower body weight, trend toward retarded growth during the lactation period, decrease in postnatal survival to Day 4.	4794

Local tolerance

The initially-conducted safety evaluation studies were designed to support the clinical use of Nalfurafine hydrochloride for parenteral administration. Tolerance studies (blood compatibility, i.v., intra-arterial, perivascular and i.m.) at strengths exceeding those intended for clinical use revealed no evidence of significant irritant potential or incompatibility. Injection site irritation was seen, but it was slight, the effect being ascribed to the vehicle, mannitol. Repeated injection of Nalfurafine hydrochloride in a mannitol formulation has been well tolerated for periods up to six months in rats and dogs. Continuous i.v. infusion in rats and dogs for 4 weeks resulted in some anticipated inflammatory reactions at the infusion sites but these were considered not to be of toxicological significance. This finding strongly suggests that local irritation at the site of injection in humans is unlikely to occur in the clinic.

Table 11. Overview of the local tolerance studies

Species /group size	Duration	Dose (mg/kg/day)/Route	Major findings	Study ID
NZW Rabbit (3m+3f)	Single	1 mL/body i.v. , 0.1 mL/body paravenous, 1 mL/body intraarterial (25 µg/mL)	No local tissue toxicity	96/TAD004/0186 (GLP)
NZW Rabbit (6m)	Single	1 mL/body (50 µg/mL) i.m.	No local tissue toxicity	JBC-93 BMIR-0179 (GLP)
NZW Rabbit (6m)	Single	1 mL/body i.m.	No local tissue toxicity	JBC-94 BMIR-0238 (GLP)

Other toxicity studies

Antigenicity

Nalfurafine hydrochloride was assessed for active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) reactions in guinea pigs. The results maintain that Nalfurafine hydrochloride showed a negative effect for the ASA reaction and a negative effect for the PCA reaction. Special active systemic

anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) antigenicity studies in guinea pigs concluded that Nalfurafine hydrochloride had no potential to produce antibodies.

Table 12. Overview of antigenicity studies

Species /group size	Duration	Dose /Route	Major findings	Study ID
Hartley Guinea pig (5m)	3 weeks (once a week) for sensitization	0.05 mg/kg for sensitization i.m. and s.c. 0.0025 mg/kg for challenge i.v.	Special active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) were negative	JBC-93 GMAG-0180
Hartley Guinea pig (5m)	Once a day during 15 days for sensitization	0.005, 0.05 mg/kg for sensitization 0.005 mg/kg (oral) and 0.0025 mg/kg (i.v.) for challenge	ASA test negative PCA test negative	JBC-95-GOAG-0295

Dependence

The potential of Nalfurafine hydrochloride to induce habituation, dependence or other factors leading to abuse in humans, was assessed in rats and primates. The studies consisted of an assessment of the withdrawal syndrome following twice daily administration at increasing levels up to 0.4 mg/kg/time nalfurafine hydrochloride. A morphine comparator was used (50 mg/kg/time) and naloxone was used to precipitate withdrawal syndrome in one study. It was concluded from the pattern and severity of the effects induced by Nalfurafine hydrochloride in comparison with morphine that slight physical dependency or withdrawal was induced with Nalfurafine hydrochloride. A study in a group of 4 rhesus monkeys self-administering pentazocine (0.25 mg/kg/infusion) was used to assess the reinforcing effects of Nalfurafine hydrochloride at 0.0625, 0.125 and 0.25 µg/kg/infusion. None of the animals showed any reinforcing effects with Nalfurafine hydrochloride. On the basis of these results, the Applicant concluded that Nalfurafine hydrochloride has no abuse potential.

Table 13. Overview of dependence studies

Species /group size	Duration	Dose /Route	Major findings	Study ID
Wistar Rat (8m)	2 times a day for 5 days	Day 1 0.05 mg/kg Day 2 0.1 mg/kg Day 3 0.2 mg/kg Day 4 and 5 0.4 mg/kg i.m.	No naloxone-precipitated withdrawal reaction in rats, whereas morphine treated rats showed signs of withdrawal-related wet-dog-shakes, jumps, writhings or diarrhea upon naloxane precipitation.	V93.704

Wistar rat (8m)	2 times a day for 40 days	0.1, 0.2, 0.4 mg/kg i.m.	The effects seen following the discontinuation of nalfurafine hydrochloride treatment were milder or showed a different time course (e.g. wet-dog-shakes) or were absent (diarrhea, writhing) compared to morphine treated animals.	V94.400
Rhesus Monkey (2 m or f)	Self administration	0.0625, 0.125, 0.25 μg/kg i.v.	No reinforcing effects in monkeys	AT97077

Metabolites

It is evident from PK/PD studies that some non-identified metabolites of nalfurafine hydrochloride exist. An extremely small peak of an unknown metabolite (M2) was uniquely produced by incubation of Nalfurafine hydrochloride with human and monkey liver microsomes, but since this did not appear to be produced in detectable quantities *in vivo*, it was considered unlikely to be of clinical or biological significance.

Studies on impurities

Impurity x has been assessed for potential toxicity and genotoxicity in special studies. The repeated-dose toxicity study was conducted to examine the toxicity of the impurity seen in the Nalfurafine hydrochloride preparation. Dose solutions of Nalfurafine hydrochloride containing Impurity x at an inclusion rate of x% relative to the Nalfurafine hydrochloride content were prepared and a 14-day repeated i.v. dose toxicity study was carried out in rats at 0.005 and 0.5 mg/kg/day. The results were compared to the groups similarly treated with Nalfurafine hydrochloride. No deaths were observed during the administration period and no remarkable differences in the changes caused by Nalfurafine hydrochloride with or without Impurity x were detected. It was concluded that Impurity x -containing Nalfurafine hydrochloride showed no toxicity specifically attributable to the mixture. The mutagenic potential of Impurity x was assessed using *Salmonella typhimurium*, strains TA1535, TA1537, TA98 and TA100, and a tryptophan dependent mutant of *Escherichia coli*, strain WP2*uvr*A/pKM101. No evidence of mutagenic activity was seen. A further study was performed to assess the ability of Impurity x to induce chromosomal aberrations in cultured CHL cells. Cells were incubated with Impurity x in both the absence and presence of S9 mix derived from rat livers. Impurity x showed no evidence of clastogenicity.

Table 14. In vivo and in vitro toxicity studies of impurities

Species / group size	Duration / type of test	Dose / Route	Major findings	Study ID
SD rat (7m+7f)	2 weeks (i.v.)	NAF hydrochloride (0.005, 0.5 mg/kg) including Impurity x hydrochloride as impurity (i.v.)	No difference with or without impurity.	TLG-01004
Bacteria (*in vitro*) *Salmonella typhimurium* strains TA1535, TA 1537, TA 98 and TA 100 and *E coli* strain WP2uvrA/pKM101 (CM891)	Bacterial reverse mutation test	Impurity x 0.05, 0.15, 0.5, 1.5, 5 mg/plate ±S9	No genotoxic potential	TYI012/ 023830

CHL cells	Mammlian chromosome abberation test	Impurity x First test: 0.625, 1.25, 2.5 mmol/L for 3 hrs with and without S9. Second test: 0.1, 0.4, 1 mmol/L for 15 hrs without S9, 1,2, 3 mmol/L for 3 hrs with S9.	No genotoxic effect	TY1013/ 024020

Phototoxic potential

The phototoxic potential of Nalfurafine hydrochloride *in vitro* was evaluated in BALB/3T3 clone A31 cells by means of neutral red uptake (NRU) assay. Nalfurafine hydrochloride produced cytotoxicity in both irradiated and non-irradiated cells with IC50 values of 0.03 and 0.57 mg/mL respectively. The photo-irritation factor was calculated to be 19, indicating that Nalfurafine hydrochloride was phototoxic under the conditions of this study.

The potential for Nalfurafine hydrochloride to elicit phototoxicity *in vivo* was evaluated in a single dose study in male Sprague Dawley rats treated with Nalfurafine hydrochloride p.o. at doses of 3 mg/kg to 40 mg/kg. In this study, there was no evidence of phototoxicity at any dose.

Table 15. Fototoxicity studies

Species / group size	Duration / type of test	Dose / Route	Major findings	Study ID
BALB/3T3 cells	*In Vitro*	NAF hydrochloride + irradiation: 0.0091-0.24 mg/mL–irradiation,: 0.037-1.0 mg/mL Positive control Chlorpromazine + irradiation: 0.016-2.0 mg/mL–irradiation: 0.31-40 mg/	IC50 values: - irradiation; 0.030 mg/mL + irradiation; 0.57 mg/mL PIF: 19 (Phototoxic) PIF positive control group:41.3.	F-08-184
Crl:CD(SD) Rats (5 males)	Oral gavage single dose	NAF hydrochloride+irradiation: 0, 3, 10, 40 mg/kg –irradiation: 40 mg/kg Positive control 8-MOP +irradiation: 10 mg/kg	No phototoxic effects	L-09-004

2.3.5. Ecotoxicity / environmental risk assessment

Summary of main study results

Substance (INN / Invented Name): Nalfurafine hydrochloride			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	-1 – 2.8	Potential PBT (N)

Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default F_{pen})	0.000025	µg/L	> 0.01 threshold (N)

The Predicted Environmental Concentration (PECsw) of nalfurafine hydrochloride expected through use of the product is less than the action limit of 0.01µgL-1 and therefore no Phase II environmental fate and effect analysis was performed. Nalfurafine hydrochloride is not a PBT substance as its logKow does not exceed 4.5.

Considering the above data, nalfurafine hydrochloride is not expected to pose a risk to the environment.

2.3.6. Discussion on non-clinical aspects

Receptor binding affinities of nalfurafine hydrochloride for the recombinant human opioid receptors (µ-, κ- and δ-opioid receptors) demonstrated that nalfurafine hydrochloride preferentially bound to the human κ-opioid receptor. The Ki values of nalfurafine hydrochloride for the κ-opioid receptor were 9 and 17 or 1980 and 3710 times smaller than those for the µ- or δ-opioid receptors, respectively. Thus, the *in vitro* pharmacological profile of nalfurafine hydrochloride was different from those of morphine, buprenorphine and butorphanol.

Nalfurafine hydrochloride showed no detectable affinity when investigated in a general receptor screen, except for a low affinity for the muscarinic M1 receptor.

Substance P is one of the most common mediators of itch sensations in humans. Injection of substance P to mice induces scratching behaviour and can therefore be used as an *in vivo* experimental animal model for itching. Nalfurafine hydrochloride dose-dependently inhibited substance P-induced scratching behaviour following both i.v. (ED50 was 3.77 µg/kg) and s.c. (ED50 was 1.65 µg/kg) administration. Antihistamines did not significantly inhibit substance P-induced scratching behaviour. Furthermore, twice daily administration of nalfurafine hydrochloride (100 µg/kg, p.o.) showed that upon repeated p.o. administration of nalfurafine hydrochloride, tolerance towards its antipruritic effect is probable.

By using a κ-opioid receptor antagonist which dose-dependently antagonised the scratching inhibitory activity of nalfurafine hydrochloride, the Applicant concluded that the antipruritic effect of nalfurafine hydrochloride was mainly mediated by the κ-opioid receptor. The molecular basis of uraemic pruritus has not been fully elucidated which also holds true for the role of κ-opioid receptors in the syndrome. The Applicant proposed several theories connected to the opioid systems which have not been verified scientifically. Overall, the mechanism of nalfurafine hydrochloride in the treatment of uraemic pruritus remains unclear.

Morphine, which is a µ-opioid receptor agonist, also induces itching. The antipruritic effect of nalfurafine hydrochloride was evaluated in the morphine-induced mouse scratching model and compared with that of the antihistamine ketotifen. Nalfurafine hydrochloride dose-dependently inhibited morphine-induced scratching behaviour (ED50 2.34 µg/kg following s.c administration) while ketotifen did not inhibit the scratching behaviour.

The pharmacological profiles of the impurity and the main metabolites of nalfurafine hydrochloride were investigated *in vitro* and *in vivo* in the mouse substance P-induced scratching behaviour model. Neither Impurity x, nor the main metabolites (de-CPM, NFA-G and de-CPM-G) inhibited the substance-P induced scratching behaviour and therefore are not expected to have any antipruritic potential. The *in vitro* data demonstrating in general lower binding affinities than the active compound confirmed this picture.

The toxicokinetics, pharmacokinetics, tissue distribution, metabolism and excretion profiles of nalfurafine hydrochloride were investigated mainly in rats, but also in mice, dogs and primates. Most of the studies were carried out with formulations in 5% mannitol solution using the i.m. and i.v. routes. Also the s.c. and p.o. routes of administrated were used. The p.o. route is considered of limited value for this application.

The absorption of nalfurafine hydrochloride was rapid in mice and rats following i.v. and i.m. routes and the bioavailability was high. In dogs and monkeys, the t1/2 was longer, 6.75h and 5.21 h respectively, and the bioavailability following i.m. administration was high. Accumulation of radioactivity in plasma with increasing duration of dosing was noted after repeated i.m. dosing of [3H]- nalfurafine hydrochloride in the rat.

Nalfurafine hydrochloride was widely distributed following single i.v. administration in male mice, with the highest concentration of radioactivity detected in the kidney and the lowest concentration of radioactivity in the brain and testes. The radioactivity concentrations in tissues decreased proportionally in the plasma. The same pattern of distribution was noted in rats of both sexes, with relatively high concentrations of radioactivity persisted in the kidney, liver and thyroid. The radioactive materials were shown to cross the placental barrier in rats, became widely distributed in foetal tissues, and appeared in the mother's milk. Some affinity to melanin was seen in the eye of the pigmented rat. The binding to plasma proteins *in vitro* ranged from 58% in mice to 75% in humans. The distribution to blood cells ranged from 55% in rats to 65% in monkeys and humans. There were no significant sex-dependent differences and the binding was independent of species and dose. Nalfurafine hydrochloride undergoes decyclopropylmethylation and is metabolized to 17-decyclopropylmethylated NAF (de-CPM), which is conjugated with glucuronic acid forming 3-glucoronide of 17-decyclopropylmethlated NAF (de-CPM-G). Nalfurafine hydrochloride is also metabolized to glucuronic acid conjugate forming 3-glucuronide of NAF (NAF-G), and NAF-G in part is possibly metabolized to de-CPM-G.

The distribution of unchanged nalfurafine hydrochloride and its metabolites, NFA-G, de-CPM, de-CPM-G, were determined by HPLC in rats, mice and dogs given a parenteral dose of 3H-labeled compound. No apparent species variations were noted in the nalfurafine hydrochloride metabolites among animals tested. Hence, nalfurafine hydrochloride was the major component found in the tissues (kidney and brain) and in plasma, de-CPM the major metabolite in bile and faeces. The major metabolites found in the urine were nalfurafine hydrochloride, de-CPM and NFA-g in rats and NFA-G, de-CPM and de-CPM-G in dogs. There are no metabolites that are specific to humans that were not seen in the animal species.

In rats, radiolabeled nalfurafine hydrochloride administered i.v. was eliminated from the body predominantly in the faeces via the hepatobiliary route. Similar results were obtained in the pigmented rat where 82.9% of the given i.v. dose was excreted in the faeces and 6.9% in the urine. In dogs given i.m. injection of radiolabeled nalfurafine hydrochloride, radioactivity was excreted to about 17% in the urine and 54% in the faeces within 48 hours. No sex differences in excretion pattern were noted in either species.

The toxicology program for nalfurafine hydrochloride was performed in mice (single dose toxicity, *in vivo* genotoxicity and carcinogenicity), rats (general toxicity, carcinogenicity and reproductive toxicity), monkeys (general toxicity) and rabbits (developmental toxicity). Pharmacokinetic data have shown that all animal species are relevant models for human safety assessment. However, based on pharmacodynamic data, the dog is the most sensitive species to the CNS depressant action of the compound.

Animal studies of nalfurafine hydrochloride have shown adverse effects of reproductive organs in rats and dogs.

The Predicted Environmental Concentration PECsw of nalfurafine hydrochloride expected through use of the product is much less than the present action limit of 0.01 J-lgL-I. Therefore, the Applicant's opinion

that no Phase II environmental fate and effect analysis needs to be performed is endorsed by the CHMP. Nalfurafine hydrochloride PEC surfacewater value is below the action limit of 0.01 µg/L and is not a PBT substance as log Kow does not exceed 4.5. Therefore nalfurafine hydrochloride is not expected to pose a risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

There are several well known facts in relation to ureamic pruritus. Histamine release is often involved and substance P can induce itching. However, the molecular basis of uraemic pruritus has not been elucidated and the role of substance P and opioid receptors in this syndrome is not clear. However, a strong evidence was demonstrated non-clinically that a κ-opioid receptor antagonist was able to antagonise the scratching inhibitory activity of nalfurafine hydrochloride, the clinical efficacy of nalfurafine hydrochloride is limited.

The molecular basis of uraemic pruritus has not been fully elucidated which also holds true for the role of κ-opioid receptors in the syndrome. The Applicant proposed several theories connected to the opioid systems which have not been verified scientifically. Overall, the mechanism of nalfurafine hydrochloride in the treatment of uraemic pruritus remains unclear.

Only minor differences were noted in pharmacokinetic parameters, thus, no animal species can be considered more relevant to humans than another.

The toxicity of nalfurafine hydrochloride is in general assessed as mild apart from the fairly strong CNS depressant effects. However, findings on reproductive organs were noted in the rat and dog repeated toxicity studies.

Nalfurafine hydrochloride has no genotoxic or carcinogenic potential, no phototoxic or abuse potential, but a weak local irritating effect.

There are no non-clinical issues outstanding. The application for Winfuran is considered acceptable from a non-clinical point of view.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Below, mainly the clinical studies with i.v. administration of nalfurafine hydrochloride are outlined since this is the route of administration sought, whereas studies including oral or intramuscular administration are not included.

Type of Study	Study Identifier	Location of Study Report	Study Objectives	Study Design	Treatments (Dose, Dosage Form, Route)	Number of Subjects/Patients	Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
PK and Safety	178566	5.3.3.1	To investigate the absorption, metabolism and excretion of a single *i.v.* dose of Nalfurafine Toray	Open label, uncontrolled, single-dose	Nalfurafine Toray. Single 4 µg *i.v.* Infusion over 5 minutes	6 subjects	Healthy volunteers	Single dose	Completed Full report
PK and Safety	USTRK-1/01	5.3.3.1	To determine the maximum tolerated single *i.v.* dose of Nalfurafine Toray in healthy volunteers	Double-blind, randomised, placebo-controlled, single-dose, dose-escalation	Nalfurafine Toray. Single *i.v.* Infusion over 5 minutes at doses of 1.25, 2.5, 5, 10, 20, 30 and 40 µg; Placebo. Single *i.v.* Infusion of placebo over 5 minutes	43 subjects (1.25 µg: 4 subjects; 2.5 µg: 4 subjects; 5 µg: 4 subjects; 10 µg: 5 subjects; 20 µg: 4 subjects; 30 µg: 4 subjects; 40 µg: 4 subjects; placebo 14 subjects)	Healthy volunteers	Single dose	Completed Full report
PK and Safety	USTRK-1/02	5.3.3.1	To determine the analgesic dose response from single *i.v.* doses of Nalfurafine Toray compared to morphine sulfate and placebo by measuring pain resulting from the cold pressor test	Double-blind, randomised, active and placebo-controlled, parallel-group, single-dose, dose-response	Nalfurafine Toray. Single *i.v.* Infusion over 5 minutes at doses of 2.5, 10, 20 and 40 µg; Placebo. Single *i.v.* infusion over 5 minutes; Morphine sulphate. Single *i.v.* Infusion of 4 mg morphine over 5 minutes	95 subjects (2.5 µg: 15 subjects; 10 µg: 17 subjects; 20 µg: 16 subjects; 40 µg: 16 subjects; morphine 4 mg: 16 subjects; placebo: 15 subjects)	Healthy volunteers	Single dose	Completed Full report
PK and Safety	820CPC01	5.3.3.3	To examine the PK and safety of *p.o.* nalfurafine hydrochloride in patients with mild Child -Pugh compensatory hepatic cirrhosis	Single-dose, open label, 2-step dose escalation	Nalfurafine hydrochloride *p.o.* 2.5 µg capsule 5 µg capsule	12 patients (2.5 µg: 6 patients; 5 µg: 6 patients)	Patients with compensated hepatic cirrhosis	Single dose	Completed Full report
PK and Safety	AC120-8112	5.3.4.1	To determine the effects of a single dose *i.v.* of Nalfurafine Toray on the duration of cardiac repolarization (QT/QTc) intervals in healthy subjects	Single dose, double-blind, double dummy, placebo controlled, randomised 4 period cross over design	Nalfurafine Toray. *i.v.* Infusion over 5 minutes single doses 5 µg and 20 µg	68 subjects	Healthy volunteers	Single dose	Completed Full report
PK and Safety	LCRC/H/008	5.3.3.3	To determine the PK, safety and tolerability of *i.v.* Nalfurafine Toray in older subjects (45-75 years)	Open label, single-dose, uncontrolled	Nalfurafine Toray. *i.v.* Single 5µg infusion over 5 minutes	8 subjects	Healthy volunteers	Single dose	Completed Full report
PK and Safety	AC120-8111	5.3.3.4	To compare the systemic exposure and safety of *i.v.* Nalfurafine Toray given alone and in combination with *p.o.* ketoconazole	Randomised, open label, 2-period crossover study	Nalfurafine Toray. *i.v.* Single 5µg Infusion over 5 minutes; Nalfurafine Toray. *i.v.* single 5µg Infusion over 5 minutes with multiple doses *p.o.* ketoconazole (400mg)	19 subjects	Healthy volunteers	Single dose	Completed Full report

Type of Study	Study Identifier	Location of Study Report	Study objectives	Study Design; Type of Control	Treatments (Dose, Dosage Form, Route)	Number of Subjects/Patients	Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Efficacy, Safety and PK	LCRC/G/028	5.3.5.1.1	To evaluate Nalfurafine Toray by 1. The PK of a single *i.v.* infusion in haemodialysis patients (SD arm). 2. To evaluate safety and efficacy and tolerability of multiple doses of an *i.v.* in haemodialysis patients compared to placebo MD arm	SD arm: Open label, single-dose MD arm: Multinational, multicentre, double-blind, randomised, placebo-controlled, multi-dose, dose-escalation	SD arm, Nalfurafine Toray. *i.v.* Single 5µg infusion over 5 minutes MD arm Nalfurafine Toray. *i.v.* 5 µg or 10 µg infusion over 5 minutes daily; Placebo *i.v.* Infusion over 5 minutes daily	SD arm: 8 patients MD arm: 17 patients (5 µg: 6 patients; 10 µg: 6 patients; Placebo: 5 patients)	SD arm: Regular haemodialysis MD arm: Regular haemodialysis and severe uraemic pruritus	SD arm: single dose MD arm: 5 days	Completed Full report
Efficacy and Safety	STTOR002	5.3.5.1.1	To compare the effectiveness, safety and tolerability of two *i.v.* dose levels of Nalfurafine Toray versus placebo in haemodialysis patients with severe uraemic pruritus	Multicentre, randomised, double-blind, parallel groups, placebo-controlled study	Nalfurafine Toray. *i.v.* 2.5 µg or 5 µg Infusion over 5 minutes 3 times a week; Placebo Infusion *i.v.* over 5 minutes 3 times a week	79 patients (2.5µg: 28 patients; 5µg: 26 patients; placebo: 25 patients)	Regular haemodialysis and Severe uraemic pruritus	4 weeks	Completed Full report
Efficacy and Safety	STTOR003	5.3.5.1.1	To compare the efficacy and safety of 2 weeks *i.v.* therapy with 5 µg Nalfurafine Toray to placebo in a cross-over design in haemodialysis patients with severe uraemic pruritus	Randomised, double-blind, placebo-controlled, cross-over study	Nalfurafine Toray. *i.v.* 5 µg infusion over 5 minutes 3 times a week; Placebo infusion over 5 minutes 3 times a week	34 patients (*i.v.* Nalfurafine Toray to placebo group: 16 patients; *i.v.* placebo to Nalfurafine Toray group: 18 patients)	Regular haemodialysis and Severe uraemic pruritus	2 weeks active treatment and 2 weeks placebo treatment	Completed Full report

Efficacy, Safety and PK	EU820UPV01	5.3.5.1.1	To evaluate the efficacy, safety and PK of 5 µg *i.v.* Nalfurafine Toray versus *i.v.* placebo for 8 weeks followed by a 4-week re-randomisation period where patients received either *i.v.* Nalfurafine Toray or *i.v.* placebo	Multicentre, Randomised, double-blind, placebo-controlled study	Nalfurafine Toray. *i.v.* 5 µg infusion over 5 minutes 3 times a week; Placebo *i.v.* Infusion over 5 minutes 3 times a week	339 patients (5 µg: 170 patients; placebo: 169 patients). Re-randomisation: 306 patients (5 µg: 153 patients; placebo: 153 patients). Severe UP-75 sub-population (5 µg: 32 patients; placebo: 43 patients)	Regular haemodialysis and Uraemic pruritus (all severities)	8 weeks plus 4 week re-randomised period	Completed Full report
Efficacy and Safety	EU820UPV01 Addendum on the Severe UP-66 sub-population	5.3.5.1.1	To evaluate the efficacy, safety and PK of 5 µg Nalfurafine Toray versus placebo for 8 weeks followed by a 4-week re-randomisation period where patients received either Nalfurafine Toray or placebo	Randomised, double-blind, placebo-controlled	Nalfurafine Toray. *i.v.* 5 µg Infusion over 5 minutes 3 times a week; Placebo *i.v.* Infusion over 5 minutes 3 times a week	66 patients (5 µg: 28 patients; placebo: 38 patients).	Regular haemodialysis and Severe uraemic pruritus	8 weeks plus 4 week re-randomised period	Completed Addendum report
Efficacy, Safety and PK	STTOR004	5.3.5.1.2	To evaluate the long-term safety, efficacy and PK profile of *i.v.* 5 µg Nalfurafine Toray in patients with renal failure on regular dialysis three times a week for 1 year compared to non treated controls	Multicentre long-term safety, open label, non-randomised, controlled Non- treated control	Nalfurafine Toray. *i.v.* 5 µg Infusion over 5 minutes 3 times a week;	227 patients (Nalfurafine Toray: 146 patients; control group: 81 patients)	Active group: regular haemodialysis and Severe uraemic pruritus. Control group: renal failure patients without pruritus.	52 weeks	Completed Full report
Efficacy and Safety	Meta-analysis of STTOR002 and STTOR003	5.3.5.3	To estimate the efficacy with a higher precision based on the combination of the 2 studies than can be achieved by the individual studies alone.	Randomised, double-blind, placebo-controlled	Nalfurafine Toray. *i.v.* 5 µg Infusion over 5 minutes 3 times a week; Placebo *i.v.* Infusion over 5 minutes 3 times a week	116 patients (5 µg: 58 patients; placebo: 58 patients).	Regular haemodialysis and Severe uraemic pruritus	4 weeks (but analysis only done at 2 weeks due to cross-over design in study STTOR003)	Completed Full report
Efficacy and Safety	Meta-analysis of STTOR002, STTOR003 and EU820UPV 01 on sub-populations of Severe UP-75 and UP-66	5.3.5.3	To investigate the efficacy of *i.v.* Nalfurafine Toray 5 µg compared to placebo and to confirm the consistency of results between the three Phase 3 studies at Week 2 and Week 4	Randomised, double-blind, placebo-controlled Placebo	Nalfurafine Toray. *i.v.* 5 µg infusion over 5 minutes 3 times a week; Placebo *i.v.* Infusion over 5 minutes 3 times a week	Severe UP-75 analysis: 106 patients (5 µg: 47 patients; placebo: 59 patients). Severe UP-66 analysis: 96 patients (5 µg: 42 patients; placebo: 54 patients).	Regular haemodialysis and Severe uraemic pruritus	4 weeks	Completed Full report

p.o. = oral, *i.v.*= Intravenous; *i.m.* = Intramuscular; PK = Pharmacokinetics, UP= Uraemic pruritus

2.4.2. Pharmacokinetics

Absorption

- **Bioavailability**

The bioavailability of an oral nalfurafine solution was estimated to be 58% (Study USTRK-1/03). However, this is not relevant for this application since Winfuran is given via intravenous injection only.

PK studies revealed highly overlapping concentrations were observed after dosages 2.5 µg, 5 µg and 10 µg dosages. No information on Cmin or Cave concentrations was collected and thus not available. The variability of those concentrations as well as Cmax concentrations and the impact on safety and efficacy was also missing.

- **Bioequivalence**

No studies on bioequivalence are needed since Winfuran will be administered intravenously over a 5 minute infusion. Further, the formulation used in the clinical trials is the same product that is intended to be marketed.

- **Influence of food**

Not applicable since nalfurafine is given via intravenous injection only.

Distribution

- **Plasma protein binding**

In vitro human plasma protein binding and blood cell distribution of nalfurafine have been investigated in study SBL5BL2-53. Protein binding was determined using the ultrafiltration method. The concentration range covered (1-100 ng/mL) for determination of nalfurafine PPB was above the observed clinically relevant plasma concentrations of nalfurafine of approximately 100 pg/mL (**Table 16**).

Table 16: *In vitro* plasma protein binding of nalfurafine in different species (n=3), study SBL52-53

Plasma concentration of TRK-820 (ng/mL)	Protein binding (%)						
	Rat (male)	Rat (female)	Mouse[1)] (male)	Dog (male)	Monkey (male)	Human (male)	Human (female)
1	68.3 ± 0.1	66.7 ± 1.2	57.6	69.9 ± 3.0	71.4 ± 0.7	74.4 ± 1.2	74.5 ± 1.8
10	67.2 ± 0.5	67.6 ± 1.8	57.4	68.0 ± 1.8	69.5 ± 4.4	76.3 ± 1.8	74.8 ± 2.2
100	68.2 ± 0.7	64.1 ± 2.6	58.5	67.5 ± 2.6	66.5 ± 2.8	73.8 ± 1.1	73.3 ± 1.2

The *in vitro* blood/plasma concentration ratio of nalfurafine was 1.5-1.7 in both male and female blood samples and independent of nalfurafine blood concentration (1-100 ng/mL).

- **Volume of distribution**

Mean volume of distribution (Vd) estimates in healthy volunteers after a single IV dose of 5 µg nalfurafine were 463-487 L throughout studies. In patients receiving an IV dose of 5 µg nalfurafine, the mean V_d was in the range of 471-606 and 344-616 L for single dose and at steady-state, respectively.

In healthy volunteers, the mean V_d at higher doses was 488-524, 519-555, 555 and 541-580 L for single IV doses of 10, 20, 30 and 40 µg, respectively.

Elimination

Based on data at higher doses (20 to 40 µg) where the pharmacokinetic parameters of nalfurafine could be determined with better accuracy, total clearance of nalfurafine was about 50-60 L/h in healthy volunteers. Terminal half-life was about 8 h in healthy volunteers, and 10-15 h in HD patients (study EU820UPV01). The longer t½ in the patient population was expected due to the difference in renal elimination of nalfurafine between healthy subjects and patients.

The metabolism and elimination of nalfurafine were investigated in a mass-balance study in healthy volunteers. The total recovery of radioactivity was 92%, with 36% and 56% excreted in urine and faeces respectively. Drug-related compounds were identified up to the 120 and 216 hour time-point in urine and faeces respectively. In urine samples 87-96% of the radioactivity was extracted and identified and in faeces 62-73%. Consequently, 65-71% of the total dose was identified. The identified drug-related compounds in urine were nalfurafine, de-CPM and NFA-G accounting for 27%, 2.6% and 2.1% of the administered dose respectively. The majority of the excretion in faeces constituted de-CPM (33% of the dose) and to some extent of nalfurafine which was only detected in 3 out of the 6 subjects with a mean of 5.5% in the three subjects with detectable levels. Based on these data the main elimination pathway in healthy volunteers seems to be metabolism of nalfurafine to de-CPM, accounting for 35% of total dose and about 50% based on the identified radioactivity. Glucuronidation seems to be a minor pathway responsible for elimination of less than 5% of the dose. Parent nalfurafine was also eliminated by renal excretion (27.1% of total dose) and a smaller part (<5% of the identified radioactivity) via faecal excretion (or intestinal secretion) in healthy subjects. *In vitro* data suggests that the metabolism of nalfurafine to de-CPM was mainly catalysed by CYP3A4 but with contribution of CYP2C8 and CYP2C19. Renal elimination was very low or absent in the patient population. Thus, nalfurafine elimination will be

even more dependent on metabolism in patients. Nalfurafine was the major identified drug related compound in plasma corresponding to around 30% of the total radioactivity exposure. The metabolites NFA-G, de-CPM and de-CPM-G corresponded to about 8, 6 and <1% of the total radioactivity respectively. Hence, only about 50% of plasma radioactivity was identified.

Dose proportionality and time dependencies

Dose proportionality after a single IV dose of nalfurafine was investigated in study USTRK-1/01 (double-blind, randomized, placebo-controlled, dose-escalation study of seven dose levels of nalfurafine in healthy volunteers). Forty-three subjects were enrolled with 29 subjects receiving nalfurafine. PK samples were taken pre-dose, 5, 15, and 30 minutes, and 1, 2, 4, 6, 8, 12, 18, 24, 36, 48, and 72 hours after dosing.

The results indicate that nalfurafine was dose proportional between 10-40 µg (AUC_{0-inf}/dose: 18.14-21.18). No data for the parameters related to the elimination phase ($t_{½}$, K_e, AUC_{0-inf}, $\underline{CL_{tot}}$, Vd, and CL_R) were available for any of the subjects in the 1.25 or 2.5 µg group because of too many samples being LLOQ to calculate the parameters.

Data on dose proportionality of IV nalfurafine could also be assessed from study USTRK-1/02. The results of this study indicated that nalfurafine was approximately dose proportional between 10-40 µg (AUC_{0-inf}/dose: 16.5-21.1). The percent extrapolated surface in USTRK-1/02 was 29%, 19% and 12% for 10, 20 and 40 µg respectively, which was similar to those in USTRK-1/01. 5 µg was not investigated in this study.

With regards to time-dependency, the primary objective of study EU820UPV01 (multicenter, randomized, double-blind, placebo-controlled study) was to investigate the efficacy of 3 times a week treatment with 5 µg nalfurafine administrated as a 5 minute IV infusion after haemodialysis session. A total of 337 patients were enrolled in the study and PK sampling was performed in 49 of these patients. PK sampling was performed predose, 5, 15 and 30 min, 1, 2, 4, 8, 12, 24 and 44 hours after nalfurafine administration. One patient was excluded since the patient was an outlier with a very high value for nalfurafine on Day 1 for the 4 hours sample.

The pharmacokinetic data from this study is very variable but indicates that the pharmacokinetics of nalfurafine may be time-dependent. Mean CL, Cmax and Tmax were higher on Day 1 (70.8 L/h, 161.3 pg/mL, and 0.129 h, respectively) compared Day 29 (33.7 L/h, 99.3 pg/mL and 0.079 h, respectively). The Applicant re-evaluated data from study EU820UPV01 and presented a summary report detailing individual concentration-time data, PK parameters and plots (ppk-non-compart-analy). Although the average CL was lower on Day 29 compared to Day 1, this was not considered to represent a time-dependent change in nalfurafine elimination by the Applicant. The Applicant explained that it was more likely to reflect an under-estimation of the Day 1 AUC due to the high proportion of samples that were below the limit of quantitation. Similarly, the higher average Day 1 Cmax value was not considered to be indicative of time dependence in the PK of nalfurafine but rather reflects two anomalously high individual Cmax values for Day 1 (ppk-non-compart-analy). The exclusion of these two values resulted in almost identical average Cmax values for Days 1 and 29. In addition, the population PK model provided a good description of both single and multiple dose data, without the need to incorporate time dependence. The Applicant concluded that nalfurafine does not exhibit time-dependent changes in PK. Similarly, population PK modelling did not show time dependence. These conclusions were agreed by the CHMP.

The Applicant's explanation that the differences in PK parameters on Day 1 vs. Day29 in study EU820UPV01 were due to under-estimation of Day 1 when a high proportion of results was below the limit of quantitation was considered acceptable by the CHMP.

Special populations

- **Impaired renal function**

Since nalfurafine is intended to be used only in patients with ESRD on dialysis, the effect of renal impairment has not been investigated.

- **Impaired hepatic function**

The effect of impaired hepatic function on the pharmacokinetics of IV nalfurafine has not been studied. However, two studies with nalfurafine administered orally included subjects with hepatic impairment. These studies did not include any control group, but was compared to healthy subjects without hepatic impairment from other studies. In study 820CPC01, patients with compensatory hepatic cirrhosis (Child-Pugh A category) showed equivalent to or lower C_{max}, AUC_{0-t}, and AUC_{0-inf} as compared with PK parameters determined in healthy male adult volunteers and in haemodialysis patients in previous studies. However, in study 820HPC02, 5 µg of nalfurafine was orally administered in cirrhosis patients with accompanied itching that were rated as Child-Pugh classification grade B and C, it was demonstrated that the exposure (C_{max} and AUC) was increased and clearance was decreased compared to single oral administration in healthy subjects.

- **Gender**

The effect of gender on the PK parameters of nalfurafine was investigated in studies STTOR004 (patients) and AC120-8111 (healthy). In study STTOR004 some differences in PK parameters between patients of different genders were identified, with the mean value of clearance in males being around 50% higher than in females. In study AC120-8111, these differences were less pronounced with the mean value of clearance being approximately 25% higher in males compared to females.

- **Race**

No specific IV study with nalfurafine was performed to compare different ethnic backgrounds.

- **Weight**

The influence of weight on nalfurafine pharmacokinetics has not been evaluated. The Applicant re-evaluated data from study STTOR004 including weight adjustment to assess the impact of both body weight and gender on the PK of nalfurafine. Individual PK parameters were adjusted for body weight and a linear mixed effects model applied to determine if a gender effect remained after accounting for body weight. Nalfurafine clearance was, on average, 32% (ranging from 57% lower to 6% higher) in female compared to male patients with UP, after adjusting for body weight. The volume of distribution was, on average, 7% higher in female UP patients (ranging from 22% lower to 48% higher). In addition, the potential effect of body weight and gender was also evaluated during the development of a population PK model that incorporated data from studies EU820UPV01 and STTOR004. The weight range for the UP patients included in the population PK database was 47 to 112 kg. Neither body weight nor gender was found to be significant covariate on the PK of nalfurafine.

The Applicant concluded that body weight was not found to be a significant covariate on the PK of NFU which was agreed by the CHMP.

- **Elderly**

An analysis of the data by age in study STTOR004, suggested that the clearance of nalfurafine decreased and its variability increased with increasing age. Mean (±SD) CL_{tot} was 86.9 (±14.4), 45.3 (±21.8) and 22.0 (±19.3) for age groups 20-39, 40-64 and >65, respectively. However, only 2 subjects were over 65 years.

Moreover, study LCRC/H/008 investigated the PK of nalfurafine in 8 healthy volunteers with age range 47-68. The PK parameters of nalfurafine in this study were comparable to those of other studies in healthy volunteers of younger age.

The Applicant analysed PK parameters of NFU from Study PV01 on Days 1 and 29 and calculated from the individual plasma concentration-time data using non-compartmental techniques. Although on both Days 1 and 29 there appeared to be a trend towards lower CL with increasing age, the estimates slope of the regression was -0.3028 (90% CI: -1.7499 – 1.1444) and -0.0959 (90% CI: -0.6019 – 0.4101) for Day 1 and 29, respectively. The 90% CI for the slope estimates for both days contained the null value of 0, indicating no evidence for age-related changes in nalfurafine PK in patients with UP.

This was a population PK model using data from 52 patients (653 concentrations; 25 excluded due to various reasonable reasons) from studies EU820UPV01 (22 patients) and STTOR004 (30 patients) combining concentration-time, dosing and demographics data from studies EU820UPV01 (22 patients) and STTOR004 (30 patients) and investigating covariate-parameter relationships, based on range of covariate values in the dataset, scientific interest, mechanistic plausibility, and exploratory graphics. Further to the analysis of the data above, the Applicant concluded that weight, gender or age didn't appear to significantly influence the PK of NFU. This was agreed by the CHMP.

Liver impairment.

There were 2 to 3 times higher exposures in case of liver cirrhosis. The applicant acknowledged that Winfuran should be contraindicated in patients with severe liver disease.

Pharmacokinetic interaction studies

Nalfurafine has been identified as a substrate of CYP3A4, CYP2C8 and CYP2C19 using multiple *in vitro* methods. However, nalfurafine was not identified as a competitive inhibitor of CYPs or P-gp *in vitro*. Nalfurafine is a P-gp substrate *in vitro* but the submitted mice data did not indicate any effect of the P-gp substrate digoxin and the P-gp inhibitor verapamil on the brain penetration of nalfurafine. However, even given the submitted data it may not be excluded that coadministration of P-gp inhibitors may lead to an increase CNS exposure and subsequently increased risk for adverse events. Therefore, the Applicant is asked to include the risk for drug-drug interactions at P-gp at BBB in the risk management plan (RMP).

An *in vivo* interaction study (AC120-8111) between nalfurafine and ketoconazole (a CYP3A4/P-gp inhibitor) was performed, indicating that there is an interaction between nalfurafine and ketoconzole *in vivo* leading to a moderate increase (52%) in nalfurafine exposure. However, the administration of ketoconazole in this interaction study could not be considered to be "worst case" and the elimination of nalfurafine in the patient population is more dependent of CYP3A4 metabolism compared to elimination in healthy volunteers. Therefore, there is a risk for the interaction between nalfurafine and ketoconazole being more pronounced in the patient population during a "worst-case" scenario.

Nalfurafine contains five chiral carbon atoms. The Applicant presented a theoretical rationale explaining that there is a low risk for *in vivo* inter-conversion of nalfurafine which was considered acceptable by the CHMP.

2.4.3. Pharmacodynamics

Mechanism of action

The mechanism of action of nalfurafine hydrochloride in the proposed indication was elaborated through two *in vitro* studies (TG-05002 and 146924) that revealed that NFU has a higher affinity for the κ- opioid

receptors with Ki values of 0.244 or 0.114 nmol/L and lower affinities for the mu (μ) - and delta (δ)-opioid receptors, with Ki values of 2.21 or 1.9 nmol/L and 484 or 423 nmol/L, respectively.

Further *in vitro* studies (107142-ADD, 107145-ADD, 106115 ADD and 107774-ADD) showed that NFU had low affinities also for muscarine M1, calcitonin gene related protein, adenosine A2A, adrenaline alpha-1, alpha-2, bombesin, dopamine D2, NMDA, 5-HT and sigma-opioid receptors, compared with that for the κ-opioid receptors. It can be concluded that NFU is rather a selective κ- opioid receptor agonist.

Primary and Secondary pharmacology

No specific primary pharmacology studies were performed, except for Study LCRC/G/028, a small study of exploratory character that investigated the effect of i.v. nalfurafine hydrochloride on pruritus in HD patients administered at i.v. doses of 5 and 10 μg once daily. This study is evaluated in the "Dose response studies" section.

Two studies (USTRK-1/01 and USTRK-1/02) assessed safety, tolerability, pharmacokinetics and pharmacodynamics of intravenously administered nalfurafine hydrochloride in healthy volunteers. These studies included doses up to 40 ug and are of interest in determining the therapeutic range of nalfurafine hydrochloride.

In both studies, high doses of nalfurafine hydrochloride were administered and an increasing number of adverse events were reported with administration of increasing doses. The most frequently affected body systems were the central and peripheral nervous system disorders and psychiatric disorders, and the most frequently reported adverse events were dizziness, somnolence, and headache. Temporary decreases in cognitive function were noted in the 30 and 40 ug/body groups. In both studies, decreases in mean serum testosterone concentration as well as decreases in serum TSH concentrations were observed in subjects treated with nalfurafine hydrochloride.

2.4.4. Discussion on clinical pharmacology

The circulating metabolites of nalfurafine have been sufficiently characterised based on the comparison of non-clinical and clinical data and rather low toxicological implications associated because the circulating levels of NFU would be extremely low.

The pharmacokinetic evaluation of nalfurafine is hampered by a large number of samples below LLOQ resulting in an uncertainty in the estimation of PK parameters of nalfurafine, especially at lower doses including the clinical dose 5 μg. The lack of clarity in the bioanalysis of nalfurafine hampered the assessment of the bioanalytical methodology utilised throughout the submitted clinical studies. Therefore, the Applicant was asked to submit a clear overview of all clinical studies and their corresponding bioanalytical method validation and study validation reports. Furthermore, the Applicant was asked to ensure that method validation and in study validation reports for all studies had been submitted. Any deviations from the recommendations in the Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009) were asked to be justified. The Applicant submitted validation methods and reports, which were considered acceptable. The results met the acceptance criteria.

Since nalfurafine is a chiral molecule the Applicant was asked to address whether no inter-conversion of nalfurafine takes place *in vivo*. The Applicant presented an acceptable theoretical rationale explaining that there is a low risk for *in vivo* inter-conversion of nalfurafine.

In order to address the risk of drug-drug interactions at P-gp at the blood brain barrier (BBB), the CHMP requested the Applicant to include it in the RMP.

The proposed nalfurafine dose regimen was three times weekly directly after haemodialysis sessions. Given the half-life and low dose of nalfurafine (approximately 10 hours and 5 μg respectively), the CHMP

was of the opinion that the plasma concentrations of nalfurafine would be very low in the latter part of the dose interval and therefore the pharmacokinetics of nalfurafine do not support the suggested three times weekly dosing regimen. When requested about the rationale for the chosen dose regimen, the Applicant explained that the reason for choosing this schedule is, on the one hand, the lower risk for infections and other complications and, on the other hand, the effect seen (in the UP-75 population in this case). According to the Applicant, the PK/PD relationship based on plasma levels is not relevant. The CHMP agreed that for a substance exerting its pharmacological effect in the CNS, plasma levels may not be closely related to the effect, since effective concentrations are reached later in the effect compartment. However, an equilibrium between plasma and cerebrospinal fluid (CSF) concentrations would be expected and decreasing CSF concentrations are expected to occur in parallel with decreasing plasma concentrations. Mechanistic support, based on effective compartment PK/PD modelling, is missing, and a clear rationale for the chosen posology apart from practical considerations is still missing.

PK studies revealed highly overlapping concentrations after dosages 2.5 µg, 5 µg and 10 µg dosages. No information on Cmin or Cave concentrations was collected and thus not available. The variability of those concentrations as well as Cmax concentrations and the impact on safety and efficacy was also missing. The CHMP was looking for PK/PD analysis based any compartment and not only CNS. Given the awaited difference in pharmacokinetics of nalfurafine between healthy volunteers and the target population, the Applicant was asked to estimate the excretion pattern of nalfurafine in the target population using available data and to provide more information on what constitutes the approximately uncharacterised 50% of plasma radioactivity. The Applicant performed a tritium labelled. The knowledge of the 100% extraction efficiency of nalfurafine and that the extraction efficiency of its metabolites was not determined does not provide any additional information on the uncharacterised 50% of plasma radioactivity. The comparison between human and animal data is of very low value especially as it does not shed any more light on the uncharacterised 50% of plasma radioactivity. Lastly, even though the low clinical dose may indicate a low risk for toxicity of potential uncharacterised metabolites, the pharmacokinetic concern of effects on metabolising enzymes is still relevant. In conclusion, the poor characterisation of plasma radioactivity is unsatisfactory and a mass balance study using 14C-labelled nalfurafine may have provided more informative data. However, the issue is not considered serious enough to warrant a new mass balance study.

The submitted data on special populations and drug-interactions were not adequately presented and investigated. The studies conducted evaluating oral nalfurafine in hepatic impairment subjects did not include any control group but was compared to subjects from other studies. Given the large variability between studies and the large uncertainty in estimated AUC0-inf and clearence in many studies, reliable conclusions regarding the effect of hepatic impairment on nalfurafine exposure cannot be drawn. The Applicant explained that the only contributor to total CL is the liver and that in case of decompensated liver cirrhosis, AUC is 2 -3 times higher as compared to healthy or ESRD populations. The CHMP concluded that Winfuran should be contraindicated in patients with severe liver disease.

In addition, the target range for safe and effective exposure of nalfurafine was poorly defined and the PK profile of nalfurafine i.v could not be considered as comprehensively interpreted and described in the proposed SmPC. Further to the request by the CHMP, the Applicant provided this information using available data (e.g. exposure response relationships for efficacy and safety) and made a proposal to update the drug interaction section of the SmPC accordingly. A clear interaction (70% increase in nalfurafine AUC) was seen between ketoconazole and nalfurafine. The CHMP concluded that there is a risk of lower efficacy of nalfurafine due to induction of CYP3A4.

PK parameters from study EU820UPV01 about NFU were analysed on Days 1 and 29 and calculated from the individual plasma concentration-time data. The 90% CI for the slope estimates for both days contained the null value of 0, indicating no evidence for age-related changes in nalfurafine PK in patients with UP. Analyses of data from studies EU820UPV01 and STTOR004 did not show gender influence on PK.

In addition, body weight was not found to be a significant covariate on the PK of NFU. The CHMP noted that the studies STTOR004 and EU820UPV01 did contain nearly only Caucasian subjects, thus the issue was not further pursued.

2.4.5. Conclusions on clinical pharmacology

The validation methods and reports submitted by the Applicant were considered acceptable and the results met the acceptance criteria.

The Applicant presented an acceptable theoretical rationale explaining that there is a low risk for *in vivo* inter-conversion of nalfurafine.

Regarding the proposed dosing regimen (three times weekly directly after haemodialysis sessions), the CHMP was of the view that the plasma concentrations would be very low in the latter part of the dose interval and therefore the proposed dosing regimen is not supported. Although the CHMP agrees that for a substance exerting its pharmacological effect in the CNS, plasma levels may not be closely related to the effect, since effective concentrations are reached later in the effect compartment, an equilibrium between plasma and cerebrospinal fluid (CSF) concentrations would be expected and decreasing CSF concentrations are expected to occur in parallel with decreasing plasma concentrations. Therefore, mechanistic support, based on effective compartment PK/PD modelling, is missing, and a clear rationale for the chosen posology apart from practical considerations is still missing.

Due to the observed 2 – 3 times higher exposures in case of liver cirrhosis, nalfurafine should contraindicated in patients with severe liver disease.

A clear interaction (70% increase in nalfurafine AUC) was seen between ketoconazole and Nalfurafine resulting in more adverse events. The CHMP concluded that there is a risk for lower efficacy of Nalfurafine due to induction of CYP3A4.

2.5. Clinical efficacy

Five efficacy studies were performed in patients with severe UP undergoing HD. All of these efficacy studies used the i.v. infusion over 5 minutes of nalfurafine hydrochloride. In LCRC/G/028, the study medication was administered daily and in the other studies it was administered 3 times a week.

Table 17. Clinical efficacy study details

Study ID	Design	Study Posology	Subjs by arm entered/compl.	Duration
LCRC/G/028	Multinational, multicentre, double-blind, randomised, placebo-controlled, multi-dose, dose-escalation	 5 μg 10μg placebo	Total 17 6 6 5	5 days
STTOR002	Multicentre, double-blind, randomised, parallel-groups, placebo-controlled	 2.5 μg 5 μg placebo	Total 79 28 26 25	4 weeks
STTOR003	Double-blind, randomised, placebo-controlled, cross-over study	 5 μg Placebo	Total 34 16 18	2 weeks active treatment and

		5 μg placebo	18 16	 2 weeks placebo
EU820UPV01	Multicentre, double-blind, randomised, placebo-controlled	 5 μg Placebo 5 μg placebo	Total 339 / 307 170 / 154 169 / 153 Total 306/293 156 / 147 148 /144	8 weeks and 4 weeks re-randomised period
STTOR004	Multicentre, Open label, non-randomised, non-treated non-pruritic controls	 5 μg controls	Total 227 / 125 146 / 62 81 / 63	52 weeks

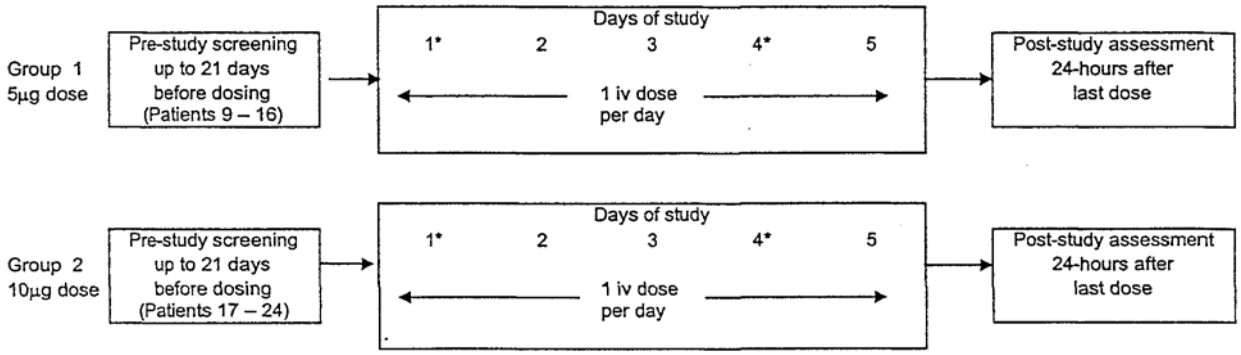
The study EU820UPV01 was considered as the pivotal study for the efficacy assessment as it was the largest placebo-controlled study with longest duration. Both STTOR002 and STTOR003 were considered as relevant clinical phase-3 studies. LCRC/G/028 and STTOR002 were dose-response studies. Open-label study STTOR004 was considered as a supportive, primarily safety study.

2.5.1. Dose response study

LCRC/G/028

The first arm was an open label, single-dose study to determine the pharmacokinetic characteristics of the drug in HD patients. The second arm was a double–blind, placebo-controlled, parallel-group, randomised, multiple-dose, dose escalating study.

Figure 2. Study course of LCRC/G/028



The aim was to investigate the efficacy of 5 μg, 10 μg and 20 μg of nalfurafine hydrochloride vs. placebo in patients undergoing haemodialysis. The primary efficacy variables were the degree of itching assessed by the patient on a visual analogue scale (VAS), a self-rating questionnaire completed by the patient relating to the itching and a questionnaire completed by the investigator recording whether the itching was improving or worsening compared to pre-dose. The study medication was given as an IV infusion for 5 minutes during 5 consecutive days. Due to slow recruitment the study was stopped after the 10 μg dose level and therefore the 20 μg dose level was never investigated. Doses of 10 ug and above were also not considered feasible due to the higher occurrence of AEs.

Of 17 patients randomised to the multiple-dose arm of LCRC/G/028, 15 were included in the efficacy analysis. Baseline and during-treatment variability in itching intensity was high and no clear effect on itching was seen.

The active treatment groups tended to show greater improvement compared with placebo. However, no dose-response was established as the confidence intervals for all dose groups at each time-point were

extremely wide, suggesting a large inter-subject variability. There were more drug related adverse events observed in the 10 μg dose group.

The applicant recommends that Nalfurafine hydrochloride is administered three times weekly as an i.v. infusion immediately after a dialysis session. The half-life of nalfurafine is in the range 8-11 hours in the target population.

2.5.2. Main studies

EU820UPV01: A randomized, double-blind, placebo-controlled study of TRK-820 in haemodialysis patients with uremic pruritus.

Methods

Study Participants

Main Inclusion Criteria

In order to qualify for the 1-week run-in period, the patient had to meet the following criteria at the Screening Visit (Day -20 to Day -6):

• Patient at least 18 years of age.

• Patient had a clinical diagnosis of UP due to ESRD which was uncontrolled by current medication(s) and/or treatment(s).

• Patient on regular HD [or haemofiltration (HF) or haemodiafiltration (HDF)], i.e., 3 times a week with a minimum duration of 12 hours per week and a minimum clearance/volume distribution (Kt/V) of 1.2, for at least 2 months prior to the start of the 1-week run-in period.

In order to qualify for treatment, each patient had to meet all the above criteria and the following criteria at the end of the 1-week run-in period:

• Patient completed ratings of worst itching intensity (VAS) at least 8 times out of 4 VAS assessments during the 1-week run-in period.

• Patient had at least 3 VAS ratings of ⩾ 50 mm during the 1-week run-in period.

• Patient had a mean value of > 25 mm on the worst itching VAS during the 1-week run-in period.

Main Exclusion Criteria

Patients who met any of the following criteria at Screening were not eligible for the study.

• Patient had pruritus other than secondary to ESRD.

• Patient had pruritus only during the dialysis sessions.

• Patient had an abnormal hepatic function based on an overall assessment by the investigator regarding medical history, physical examination and laboratory tests of hepatic function [alanine aminotransferase (ALT) > 3 times the upper limit of normal (ULN), aspartate aminotransferase (AST) > 3 times the ULN].

• Patient had an advanced, severe or unstable disease of any type other than ESRD

• Patient had started any new emollient or oil bath within 1 week prior to the start of the 1-week run-in period.

• Patient who did not agree not to take prohibited concomitant medications: All medication used to treat pruritus was prohibited during the course of the study. During the treatment period, the introduction or re-introduction of a concomitant medication known to prolong the corrected QT interval (QTc) was not

permitted.

Treatments

The study medication, 5 µg nalfurafine hydrochloride, was administered following the end of a HD session, 3 times per week. 0.75 mL of nalfurafine hydrochloride or placebo was diluted to a total volume of 15 mL with normal saline (0.9% saline

Study STTOR002 (described later) was a controlled study with a treatment duration of 4 weeks. These four-week results were used for the power and sample size calculation of study EU820UPV01. According to the Applicant, these considerations justified statistical testing of the primary endpoint at the end of Week 4 and collection of data until Week 8 for testing of secondary endpoints.

Objectives

The primary objective of the study was to compare the efficacy of 3 times a week i.v. treatment with nalfurafine hydrochloride 5 µg versus placebo in HD patients with UP following completion of a 4-week parallel-group treatment period.

The secondary objectives of the study were:

• To investigate the efficacy of i.v. nalfurafine hydrochloride 5 µg over an 8-week treatment period.

• To investigate the maintenance of efficacy of i.v. nalfurafine hydrochloride 5 µg following a re-randomization of the nalfurafine hydrochloride patients to continued i.v. nalfurafine hydrochloride 5 µg or placebo from Week 9 to Week 12.

• To assess the safety of i.v. nalfurafine hydrochloride 5 µg.

Outcomes/endpoints

Primary endpoint

• The change in worst itching recorded on the Visual Analogue Scale (VAS) from baseline to the end of Week 4

Patients were asked to characterize the intensity of their worst itching over the last 12 hours by placing a single vertical mark on a 100 mm VAS where “no itching” is at the left hand end and “worst itching ever” is at the right hand end. Assessments of VAS scores were made twice daily in the diary

Secondary endpoints

The evaluation of the following collected at each week of the eight-week parallel-group treatment period:

• The change in worst itching recorded on the VAS

• The change from baseline in itching intensity recorded on a categorical scale:

1. No itch.

2. Tolerable without scratching.

3. Tolerable with scratching.

4. Itch unrelieved by scratching.

5. Intolerable itching

• The change from baseline in sleep disturbance due to itching on a categorical scale 1-5.

•The percentage and number of nights with sound sleep.

• The change from baseline in the assessment on daytime feeling and activities due to itching by the patient.

• The investigator's global assessment of itching.

The baseline assessment was done by rating the intensity on a categorical scale 1-5 as above. The weekly assessments during the 8-week treatment period were done by choosing 1 of the 3 alternatives, judged relative to the Day 1 rating: Better, Same or Worse.

• The investigator's assessment of excoriations.
Excoriations were recorded by marking affected areas on a body diagram at baseline. The weekly assessments during the 8-week treatment period were done by choosing 1 of the 3 alternatives, judged relative to the Day 1 rating: Better, Same or Worse.

• The response to treatment, where a reduction from baseline of at least 50% in itching VAS is defined as response.

• The evaluation of these parameters at each week of the four-week re-randomised treatment period using week 8 as baseline.

Sample size

Approximately 300 patients were to be randomised into this study, 150 patients in each treatment arm. A VAS score SD of 25 mm, and using a 2-sided significance level of 5% and then a sample size of 133 patients per arm, gave 90% power to detect a difference of 10 mm in mean reduction of worst itching VAS from baseline (1-week run-in period) to Week 4 between the active arm and the placebo arm. This number was increased to 150 patients per arm to allow for 10% of patients who may discontinue prematurely or have major protocol deviations, so as to retain a reasonable power for secondary analyses on the PPS, and/or sensitivity analyses with regards to missing data and premature discontinuations.

Randomisation

Following a 1-week run-in period for baseline assessments, patients were randomly allocated to receive an i.v. administration of 5 µg nalfurafine hydrochloride or placebo given over 5 minutes 3 times a week for a period of 8 weeks immediately following the end of the HD session. At the end of the 8-week parallel-group treatment period, all patients who completed Visit 29 (Day 57) were re-randomised in a ratio of 1:1 to receive either nalfurafine hydrochloride or placebo for a 4-week re-randomised treatment period.

Blinding (masking)

The study design was double-blind. Nalfurafine hydrochloride and placebo were identical in appearance, thus allowing double-blind conditions to be maintained. The investigator obtained randomization codes, and this code could only be broken when it was necessary for the investigator to know which treatment had been assigned to a particular patient in order to manage the patient's medical condition. In case of an emergency it was the responsibility of the investigator to decide whether the emergency required unblinding of an individual patient, and to record in detail on the case report form the date and reason for requesting the code break. Preferably the Medical Monitor or safety staff were to be consulted before the code was broken. Otherwise, unblinding could only occur after database lock, once all protocol deviations had been agreed and after resolution of all outstanding data queries.

No unblinding was performed during the study by clinical staff.

Statistical methods

The change in worst itching VAS values from baseline to Week 4, calculated as change = (mean VAS during run-in period [recorded twice per day over 7 days]) minus (mean VAS during Week 4 in the parallel-group treatment period [recorded twice per day over 7 days]) was the primary efficacy variable. This was the only confirmatory analysis. No alpha adjustment or any other adjustments dealing with multiple testing were applied to the analysis of secondary efficacy parameters, i.e., all other p-values were considered as supportive and descriptive rather than confirmatory results. In case of missing data at Week 4, data from the last recorded week prior to this week were used.

The primary variable was analysed using analysis of covariance (ANCOVA) with treatment and country as fixed effects and baseline as the covariate. The results were presented in terms of p-values, least square means, the estimated treatment difference and the 95% confidence interval (CI) for the estimated difference of nalfurafine hydrochloride minus placebo. The FAS was considered as the primary analysis set and imputation of missing data in this analysis set was performed using the last observation carried forward (LOCF) principle.

The secondary efficacy parameters for the 8-week parallel-group treatment period were analysed using the same model as for the primary endpoint.

Statistical tables were stratified by treatment. The primary efficacy endpoint was also presented by country, gender, age group, duration of kidney disease and time since the start of dialysis. Age groups considered for subgroup analyses were < 65 years versus ⩾ 65 years (elderly).

Statistical analysis system (SAS) datasets were created for all the data that were analysed or summarised.

The sample size calculations were additionally explained and provided together with a justification for the selected drop-out rates in calculations of sample size. Other aspects of statistical analyses were sufficiently explained and justified

Results

Participant flow

The table below shows the patient disposition (All patients screened) for TRK-820 (nalfurafine hydrochloride)

	Randomized		Overall
	TRK-820 N (%)	Placebo N (%)	
Screened	-	-	385
Randomized	170	169	339
Patients completed the first 4 weeks [a]	157 (92.4)	158 (93.5)	315 (92.9)
Patients completed the 8-week treatment [a]	154 (90.6)	153 (90.5)	307 (90.6)
Withdrawals during the 8-week treatment period[a]**:**			
Death	1 (0.6)	4 (2.4)	5 (1.5)
Withdrawal of consent	12 (7.1)	6 (3.6)	18 (5.3)
Intolerable AE	2 (1.2)	5 (3.0)	7 (2.1)
Kidney transplantation	1 (0.6)	0 (0.0)	1 (0.3)
Other	1 (0.6)	1 (0.6)	2 (0.6)
Re-randomized (total)[a]	153 (90)	153 (90.5)	306 (90.3)
Re-randomized: TRK-820[b]	79 (51.6)	77 (50.3)	156 (51.0)
Re-randomized: Placebo [b]	73 (47.7)	75 (49.0)	148 (48.4)
Re-randomized, but not treated	1	-	1
Prolonged treatment without re-randomization: TRK-820[c]	1	-	1
Prolonged treatment without re-randomization: placebo[c]	-	1	1
Completed the study [a, d]	148 (87.1)	145 (85.8)	293 (86.4)
Withdrawals during the re-randomization period (TRK-820)[e]**:**			
Death	0 (0.0)	1 (1.3)	1 (0.6)
Withdrawal of consent	1 (1.3)	0 (0.0)	1 (0.6)
Intolerable AE	1 (1.3)	3 (3.9)	4 (2.6)
Kidney transplantation	1 (1.3)	0 (0.0)	1 (0.6)
Other	1 (1.3)	1 (1.3)	2 (1.3)
Withdrawals during the re-randomization period (placebo)[e]**:**			
Death	0 (0.0)	1 (1.3)	1 (0.7)
Withdrawal of consent	1 (1.4)	2 (2.7)	3 (2.0)

AE: adverse event, N: total number of patients in the group. [a] Percentages based on the number of patients randomised. [b] Percentages based on the number of patients re-randomised. [c] Patients were not re-randomised, but had a prolonged treatment of the first period in error. [d] The number of patients who received TRK-820 or placebo during the 8-week parallel-treatment period and who completed the whole 12-week study period. [e] Percentages based on the number of patients re-randomised to the respective treatment. Counts for number of withdrawals are for the main reasons stated at study termination.

Over 90 % of the patients completed the 8-week treatment in both active treatment and placebo-groups.

Recruitment

The date of the first patient screened was 28 November 2005 and the date of the last patient last visit was 28 March 2007.

Conduct of the study

No changes to the conduct of the study were recorded. Prior to database lock and unblinding, patient data listings of protocol deviations identified through study monitoring visits as well as those programmatically derived from the clinical study database were generated. Major protocol deviations were those that were judged to potentially impair the ability to interpret the study primary endpoint.

Baseline data

	Nalfurafine hydrochloride (N=169)	Placebo (N=168)	Overall (N=337)
Age (years)	57.6 (14.6)	58.2 (13.4)	57.9 (14.0)
Gender, male (%)	64.5	57.1	60.8
Weight (kg)	68.8 (11.7)	70.8 (15.2)	69.8 (13.6)
Ethnic origin (%) Caucasian/ Asian/ Other	98.8/ 0.6/ 0.6	99.4/ 0.6/ 0.0	99.1/ 0.6/ 0.3
Time of onset since renal disease (years)	13.3 (9.8)	12.5 (10.0)	12.9 (9.9)
Time since first dialysis (years)	5.2 (5.1)	4.3 (3.9)	4.7 (4.5)
Time since onset of pruritus (years)	2.3 (2.5)	2.2 (2.7)	2.3 (2.6)
The mean Worst itching VAS	58.9 (15.7)	59.1 (16.4)	59.0

Values are Means (SD) or percentages.

The reported overall medical history by body system was similar between the 2 treatment groups, except that there were slightly more patients (43.5%) in the placebo group who reported gastrointestinal disorders than for the nalfurafine hydrochloride (35.5%).

A total of 17.5% of patients overall reported using pre-study medication for pruritus. The use of pre-study medication was similar between the 2 treatment groups.

Most patients were on HD (92.3% overall). The dialysis details in the nalfurafine hydrochloride group and placebo group were very similar.

Numbers analysed

- Full analysis set (FAS): all randomised patients who received at least 1 dose of study medication and had at least 1 morning and 1 evening worst itching VAS measurement during the treatment period were included in the FAS, and assigned to the treatment as randomised. This was the primary population for efficacy analyses of the 8-week treatment period.

- Per-protocol analysis set (PPS): all patients of the FAS without any major protocol deviation. Patients who dropped out due to AEs or due to lack of efficacy were not excluded from the PPS unless other major protocol deviations were identified. Only the main efficacy summaries were repeated for the PPS.

- Re-randomised nalfurafine hydrochloride and placebo analysis sets: all patients in the FAS, who received nalfurafine hydrochloride or placebo during the 8-week parallel-group treatment period and

completed Visit 29 (Day 57), were re-randomised at this visit, received at least 1 dose of re-randomised study medication and had at least 1 morning and 1 evening itching VAS measurement during the 4-week re-randomised treatment period were included in this analysis. These were the primary populations for efficacy analyses of the 4-week re-randomised treatment period.

Table 18. Actual numbers of subjects in Study EU820UPV01.

			Treatment		
Region	Site	Analysis set	TRK-820	Placebo	Overall
Overall	Overall	Full analysis set	169	168	337
		Safety analysis set [A]	170	167	337
		Per-protocoll analysis set	161	160	321
		PK analysis set	22	27	49
		Re-randomized TRK-820 analysis set	77	71	148
		Re-randomized Placebo analysis set	73	73	146

[A] Patient 511-2, randomized to Placebo, received TRK-820 in the eight weeks parallel group period and is therefor included in the TRK-820 group for the safety analysis.

Outcomes and estimation

Primary endpoint: *Change in worst itching recorded on the Visual Analogue Scale (VAS) from baseline to the end of Week 4*

Table 19. Primary endpoint (TRK-820 = nalfurafine hydrochloride)

	p-value[a]	Estimate[b]	95% CI
Week 4			
ANCOVA parameter [a]			
Treatment	0.9272		
Country	0.4237		
Baseline worst itching VAS	0.0001	-0.316	
Least square means and treatment difference (mm)			
TRK-820		-21.9	[-25.0, -18.8]
Placebo		-21.7	[-24.8, -18.6]
TRK-820 minus placebo		0.2	[-4.2, 4.6]

Note: In case of missing data at Week 4, data from the last recorded week prior to this week were used.
[a] ANCOVA with fixed effects for treatment and country and with baseline worst itching VAS values as covariate. Treatment x country interaction included in the model only if p< 0.05 for such interaction.
[b] Least-squares (type III) estimates derived from the ANCOVA. For the covariate: estimate of the regression coefficient.
ANCOVA: analysis of covariance, FAS: full analysis set, VAS: visual analogue scale, CI:confidence interval.

There was no difference between active treatment and placebo. Both groups improved in the worst itching VAS by approximately 22 mm which is considered clinically significant.

The mean worst itching VAS values and changes from baseline to Week 4 were similar for males and females and similar for both treatment groups. The mean worst itching VAS values was generally similar for both treatment groups within an age group < 65 years, ≥ 65 to < 75 years and ≥ 75 years.

Table 20. Worst itching VAS values and changes from baseline to Week 4, by gender in study EU820UPV01: FAS (N=337)

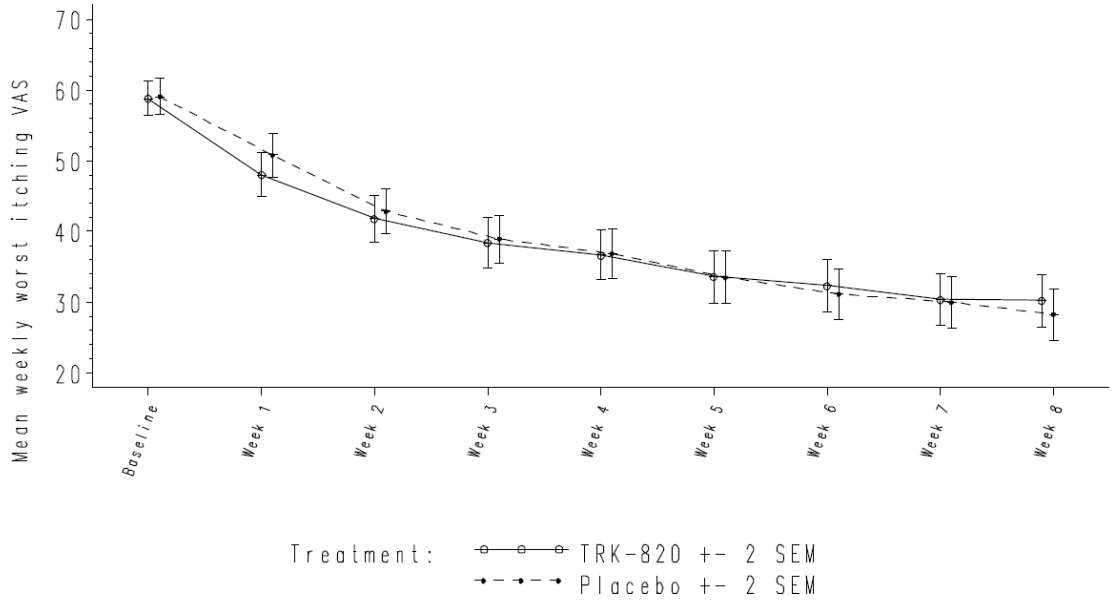
Sex		Values by Visit (mm)		Change from Baseline (mm)	
		TRK-820 N=169	Placebo N=168	TRK-820 N=169	Placebo N=168
Males					
Baseline	n	109	96	-	-
	Mean (SD)	58.29 (15.628)	62.13 (16.366)	-	-
	Range	20.3; 92.2	26.4; 97.8		
Week 4 (LOCF)	n	109	96	109	96
	Mean (SD)	39.42 (22.600)	38.69 (23.342)	-18.87 (19.490)	-23.43 (19.903)
	Range	0.7; 91.16	0.0; 100.0	-62.7; 34.5	-76.2; 42.6
Females					
Baseline	n	60	72	-	-
	Mean (SD)	59.86 (16.099)	55.07 (15.631)	-	-
	Range	28.3; 95.3	33.9; 90.2		
Week 4 (LOCF)	n	60	72	60	72
	Mean (SD)	32.73 (22.848)	35.34 (22.589)	-27.14 (22.555)	-19.73 (22.739)
	Range	0.0; 91.8	0.1; 91.1	-75.5; 14.6	-78.3; 40.9

Source: Section 14, Table 3.1.8.
SD: standard deviation, FAS: full analysis set, VAS: visual analogue scale, LOCF: last observation carried forward, N: total number of patients in the group.
The score ranges from 0 mm = 'no itching' to 100 mm = 'worst itching ever'

Secondary endpoints:

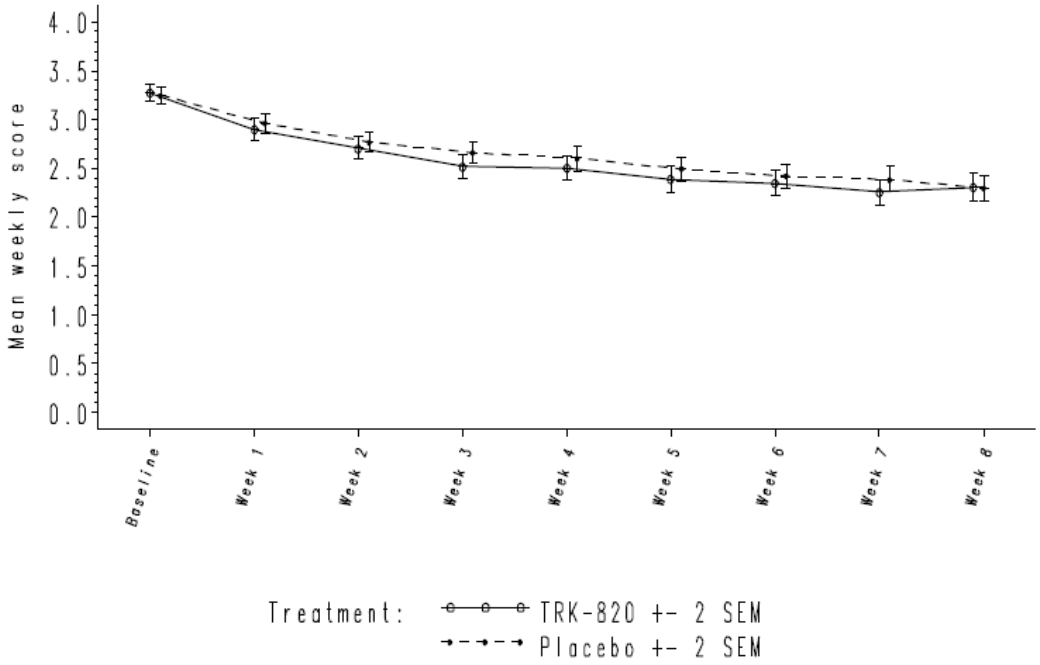
• *Change in worst itching recorded on the VAS*

Figure 3. The mean weekly worst itching VAS values for the FAS (TRK-820 = nalfurafine hydrochloride)



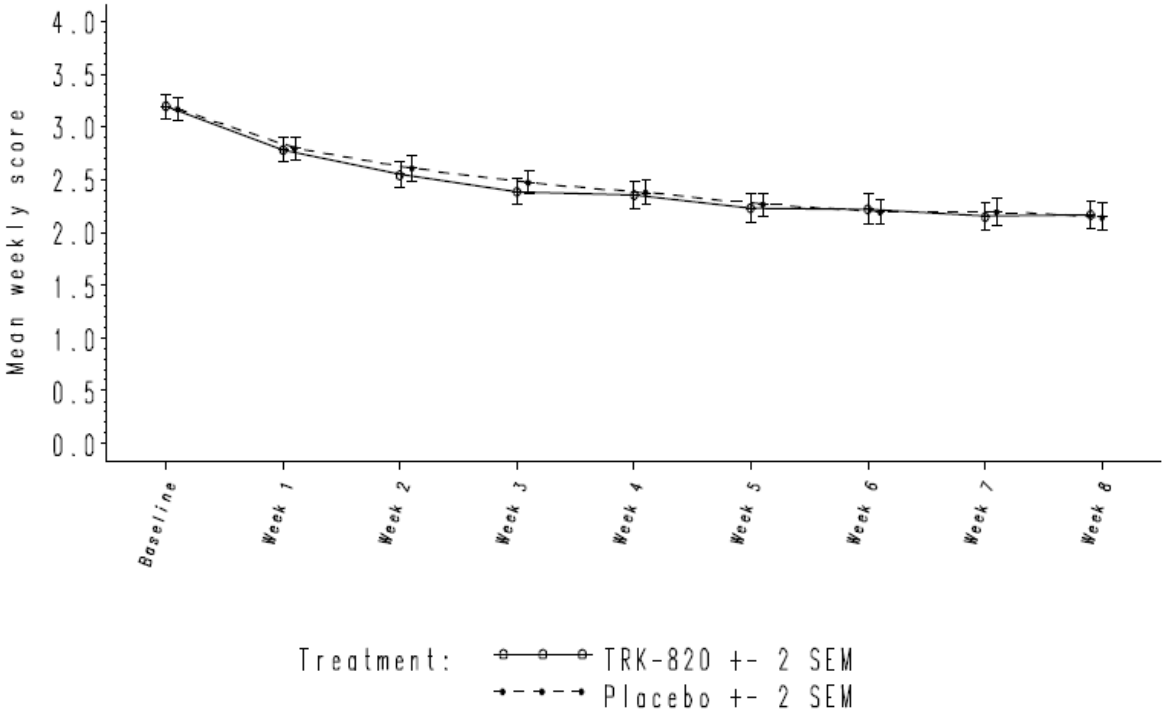
• *Change from baseline in itching intensity recorded on a categorical scale:*

Figure 4. Mean weekly itching intensity scores for the FAS (TRK-820 = nalfurafine hydrochloride)



• *Change from baseline in sleep disturbance due to itching.*

Figure 5. Mean weekly sleep disturbance scores for the FAS (TRK-820 = nalfurafine hydrochloride)

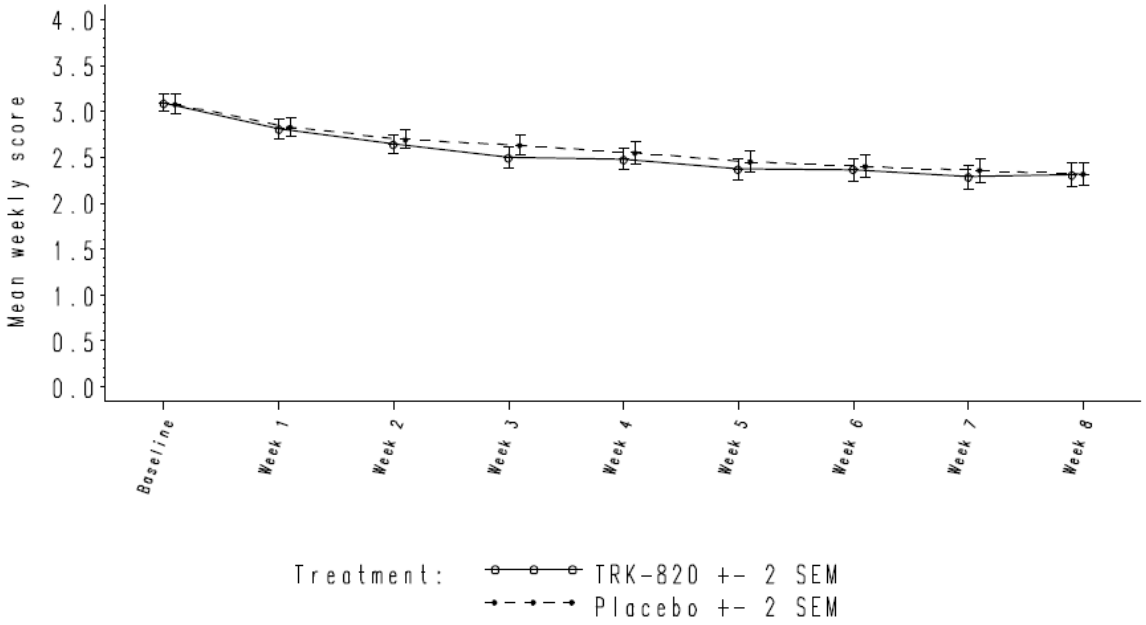


• *Percentage and number of nights with sound sleep.*

The percentage of nights with sound sleep generally increased from baseline to week 8, from 24% to 71% in the nalfurafine hydrochloride group and from 24% to 73% in the placebo group.

• *Change from baseline in the assessment on daytime feeling and activities due to itching by the patient.*

Figure 6. Daytime feeling and activities due to itching (TRK-820 = nalfurafine hydrochloride)



• *The investigator's global assessment of itching.*

Overall, patients' itching improved similarly between treatment groups and improved from Week 1 to 8. By Week 8, investigator's global assessment of itching was "better compared to baseline" in 64% of the nalfurafine hydrochloride group compared to 58% in the placebo group.

• *The investigator's assessment of excoriations.*

Overall, patients' itching improved similarly between treatment groups and improved from Week 1 to 8. By Week 8, investigator's global assessment of excoriations was "better compared to baseline" in 41% of the nalfurafine hydrochloride group and similar 41% in the placebo group.

• *The response to treatment, where a reduction from baseline of at least 50% in itching VAS is defined as response.*

By Week 8, 49.7% of the nalfurafine hydrochloride group and 52.4% of the placebo responded to the treatment.

• *The evaluation of parameters at each week of the four-week re-randomised treatment period using week 8 as baseline:*

There was no difference between active treatment and placebo either in the primary or the secondary end-points in the FAS or during the four-week re-randomised treatment period. Both active treatment and placebo groups improved.

STTOR002: A randomised, double-blind, placebo-controlled, multicentre study of TRK-820 in parallel groups in patients with severe pruritus and who are on regular haemodialysis

Methods

Study participants

In addition to the main inclusion criteria in the pivotal study EU820UPV01, STTOR002 specified that patients should have severe UP. The exclusion criteria and overall baseline characteristics were generally similar to those described for the EU820UPV01 study.

Patients aged >18 years on regular haemodialysis three times weekly, suffering from severe pruritus secondary to End Stage Renal Disease (ESRD), and with available "worst itching" intensity measurements from at least 8 out of 14 times on a visual analogue scale (VAS) during a 1-week run-in period were to be included. In addition, normal hepatic function (based on the Investigator's overall assessment of clinical history, physical examination and laboratory tests of hepatic function) was required as well as ability to understand the nature of the study and to give signed informed consent.

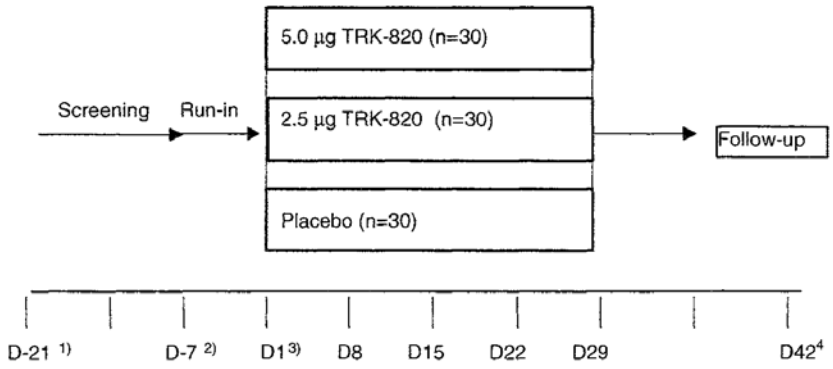
Patients with any associated illness of sufficient severity, clinically relevant abnormalities at screening, less than three individual "worst itching" VAS recordings of > 50 mm or an average of less than 25 mm during the run-in week were excluded from inclusion. Patients who suffered from severe pruritus during the dialysis session only were also excluded.

A total of 93 patients were screened, 79 patients were randomised and 74 patients completed the study. 78 patients were included in the Full Analysis Set Treatment groups: nalfurafine hydrochloride 5 µg n=26 or 2.5 µg n=27, and placebo n=25. One dose of either nalfurafine hydrochloride (5 µg or 2.5 µg) or placebo was administered after the dialysis session on Days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, and 26, respectively.

Treatments

Patients had to keep a record of their visual analogue scale (VAS) for itching and a questionnaire during the 7 days run-in (baseline) period. Only patients, who scored worst itching in at least three (3) individual VAS recordings of 50 mm or more and the average worst VAS value in each patient was 25 mm or more during the run-in period started on treatment. On study Day 1 the patient was randomised, i.e. received a patient number, which in random order allocated the patient to one of the three treatment groups (TRK-820 2.5 µg or 5 µg/body or placebo). After the completion of their regular dialysis session and the pre-dose assessments in the hospital on Study Day 1, the patients received their first dose of TRK-820 (2.5 µg or 5 µg/body) or placebo with an intravenous infusion for exactly 5 minutes. On Day 3, immediately after patients had completed their regular dialysis, the second dose of TRK-820 (2.5 µg or 5 µg) or placebo was given. This procedure was repeated on Study Days 5, 8, 10, 12, 15, 17, 19, 22, 24 and 26. A follow-up visit was performed two weeks after completion of treatment.

Study STTOR002 (described later) was a controlled study with a treatment duration of 4 weeks. These four-week results were used for the power and sample size calculation of study EU820UPV01. According to the Applicant, these considerations justified statistical testing of the primary endpoint at the end of Week 4 and collection of data until Week 8 for testing of secondary endpointsThe figure below shows the study course (planned patient numbers) for TRK-820 = nalfurafine hydrochloride



Objectives

The primary objective was to compare the effect of treatment thrice weekly during four weeks, of two i.v. dose levels, of TRK-820 to the effect of placebo in patients with severe pruritus on regular haemodialysis.

The secondary objective was to determine the safety and tolerance of i.v. TRK-820 versus placebo when given thrice weekly for four weeks in patients with severe pruritus on regular haemodialysis.

Outcomes/endpoints

The primary variable was the maximum of average and worst itching VAS (maximum itching VAS), focusing on reduction from run-in to end of week four, LOCF.

Secondary endpoint variables included reductions in worst itching VAS, average itching VAS, sleep disturbances, patient's assessment of itching intensity, patient's global assessment of pruritus, Investigator's assessment of itching, and Investigator's assessment of excoriation.

Sample size

Ninety (90) patients were to be included in the study, using the ratio (1:1:1) for allocation to the three treatment sequences. When calculating the sample size the standard deviation was estimated to be 20 mm. With 66 eligible patients, with a two-sided test at the 5% level of significance and a power of 90%, the smallest detectable difference would be 20 mm. It was decided to include 90 patients to allow for not eligible patients (transplantations, deaths etc) at a rate of 25%.

Randomisation

Each patient eligible for the study was allocated to one of the three treatment groups according to a computer-generated randomisation list (patient number). A randomisation list was generated for each study centre and forwarded in a sealed envelope to the responsible Pharmacist at each site. When the Investigator had confirmed that all inclusion criteria but none of the exclusion criteria had been met, i.e. on Study Day 1, the patient was assigned a Patient Number in consecutive order, and the Pharmacist issued the treatment according to Patient Numbers.

Blinding (masking)

Infusion solution of TRK-820 or placebo was prepared by the Pharmacist and delivered to the dialysis unit in identically appearing syringes, regardless of content. Unblinding was restricted to emergency situations and was only to be used under circumstances where knowledge of the treatment was necessary for the proper handling of the patient. If the treatment blinding was broken, the reason, time and date were to be recorded and signed by the Investigator. The patient was to be withdrawn if the treatment blinding was broken.

Statistical methods

For each patient, mean values for maximum, worst and average itching VAS scores were calculated for the run-in and treatment period (Week 4 values). In the FAS analyses, missing values were substituted using the last observation carried forward method. The mean scores were analysed using an analysis of covariance (ANCOVA) model, with reduction from baseline (run-in) as the response variable and treatment and baseline value as factors. In addition, the area under the curve (AUC) for VAS was calculated for each week and analysed in the same manner as the mean values. The primary comparison was performed on the reduction in maximum itching VAS at week 4, comparing TRK-820 5 µg with placebo. Sleep disturbance and itching intensity were analysed with the two-sample t-test (pair-wise comparisons), and also with an ANCOVA model corresponding to the model for VAS, and with the normal approximation of the Wilcoxon-Mann-Whitney test. Patient's global assessment, Investigator's global assessment and excoriation were analysed using the normal approximation of the Wilcoxon-Mann-Whitney test. Pair-wise comparisons between treatment groups were performed on the proportion of patients experiencing an adverse event using Fisher's Exact Test.

Results from STTOR002

Table 21. Primary efficacy: mean maximum itching VAS at week 4

Treatment	Mean Score at Run-in ± SD	Mean Score at Week 4 ± SD	Mean Change ± SD	(95% CI) *p*-value*
Nalfurafine hydrochloride 5 µg (n=26)	67.1 ± 15.0	41.4 ± 28.0	25.8 ± 23.6	(-1.1, 24.9) 0.0726
Nalfurafine hydrochloride 2.5 µg (n=27)	62.1 ± 18.1	38.3 ± 26.0	23.8 ± 23.9	(-1.8, 24.3) 0.0892
Nalfurafine hydrochloride pooled (n=53)	64.6 ± 16.7	39.8 ± 26.8	24.8 ± 23.5	(0.3, 22.8) 0.0436
Placebo (n=25)	67.1 ± 14.9	53.2 ± 24.0	13.9 ± 23.2	
*Compared to Placebo CI = Confidence Interval				

Both nalfurafine doses were associated with trends to reduced mean maximum itching VAS, compared with placebo. Pooled nalfurafine treatment group had mean 11mm difference in VAS compared to placebo and borderline statistical significance was achieved in favour of active treatment.

There were no significant differences between treatment groups in secondary endpoint variables.

Participant flow

Recruitment

The first patient was enrolled on 1 September 2000 and the last patient completed on 22 March 2002.

Conduct of the study

There was one amendment to the study protocol as a consequence of comments from an Ethical Committee, which was also approved by the other IECs. In addition, 2 technical amendments were also written.

Baseline data

Table 22. Demographics and baseline characteristics in STTOR002

Characteristic	Nalfurafine hydrochloride 5 µg N=26	Nalfurafine hydrochloride 2.5 µg N=28	Placebo N=34
Mean age in years (SD)	61.8 (13.5)	67.0 (11.8)	53.6 (16.5)
Male (%)	54	81	64
Caucasian (%)	96	100	96
Body weight	68.7 (15.7)	72.8 (12.3)	76.6 (19.1)

A total of 93 patients were screened, 79 patients were randomised and 74 patients completed the study. 78 patients were included in the Full Analysis Set Treatment groups: nalfurafine hydrochloride 5 µg n=26 or 2.5 µg n=27, and placebo n=25. One dose of either nalfurafine hydrochloride (5 µg or 2.5 µg) or

placebo was administered after the dialysis session on Days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, and 26, respectively.

Numbers analysed

	TRK 2.5 ug	TRK 5 ug	Placebo	Total
No. planned	30	30	30	90
No Screened	N.A.	N.A.	N.A.	N.A.
No. randomised and treated	28	26	25	79
No. analysed for efficacy (FAS)	27	26	25	78
Males/females (FAS)	22/5	14/12	16/9	52/26
Mean age (min-max, FAS)	67(43-84)	62(25-80)	54(19-79)	61(19-84)
No. analysed for safety	28	26	25	79
No. completed	25	25	24	74

Outcomes and estimation

The baseline characteristics are similar in the three treatment groups, thus, it can be concluded that the treatment groups were comparable.

The primary efficacy variable, maximum itching in the FAS, was on average reduced by 25.8 mm from baseline to week 4 in the TRK-820 5 ug group, by 23.8 mm in the TRK-820 2.5 ug group, and by 13.9 mm in the placebo group. Although the reduction on active drug was more pronounced than on placebo, there was no statistical significance versus placebo, i.e. the p-values were 0.0726 (TRK-820 5 ug) and 0.0892 (TRK-820 2.5 ug).

Also the initial primary variable, worst itching in the FAS, had a more marked reduction for active drug (on average 25.0 mm for TRK-820 5 ug, 23.5 mm for TRK-820 2.5 ug, compared to 12.8 mm in the placebo group). However, differences versus placebo did not reach statistical significance; 0.0649 (TRK-820 5 ug) and 0.0713 (TRK-820 2.5 ug).

A similar pattern of more pronounced but not statistically significant improvements on active drug was observed in several of the secondary variables. One secondary variable, however, the patient's assessment of itching reached significance p = 0.0076 (TRK-820 5 ug vs. placebo) and p=0.0212 (TRK-820 2.5 ug vs. placebo).

STTOR003: A randomised, double-blind, placebo-controlled, multicentre, cross-over study of TRK-820 in patients with severe pruritus and who are on regular haemodialysis

Methods

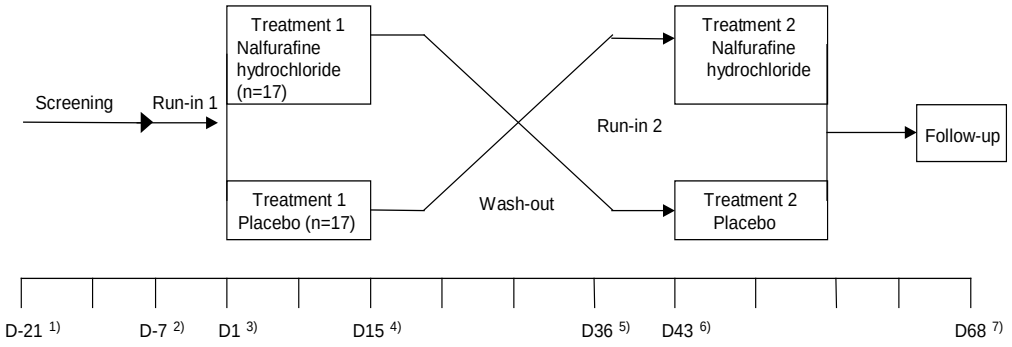
Study participants

In addition to main inclusion criteria in the pivotal study EU820UPV01, STTOR003 specified that patients should have severe UP. The exclusion criteria and overall baseline characteristics were generally similar to those described for the EU820UPV01 study. Patients aged >18 years on regular haemodialysis three times weekly, suffering from severe pruritus secondary to End Stage Renal Disease (ESRD), and with available "worst itching" intensity measurements from at least 8 of 14 times on a visual analogue scale (VAS) during a 1-week run-in period were to be included. In addition, normal hepatic function (based on the Investigator's overall assessment of clinical history, physical examination and laboratory tests of hepatic function) was required as well as the ability to understand the nature of the study and to give signed informed consent.

Patients with any associated illness of sufficient severity, clinically relevant abnormalities at screening, less than three individual "worst itching" VAS recordings of > 50 mm or an average of less than 25 mm during the first 1-week run-in period were excluded from inclusion. Patients who suffered from severe pruritus during the dialysis session oniy were also excluded.

Treatments

The figure below shows the study course (planned patient numbers) for TRK-820 (nalfurafine hydrochloride). Nalfurafine hydrochloride 5 µg or placebo was administered IV three times a week during the treatment periods. Thirty-four (34) patients were randomised to study treatment and 31 completed the study.



Objectives

The primary objective was to compare the effect of two weeks' intravenous (i.v.) therapy with 5 ug of TRK- 820 administered three times weekly to placebo in a cross-over design in patients with severe pruritus on regular haemodialysis. The secondary objective was to determine the safety of 5 ug TRK-820 in these patients.

Outcomes/endpoints

The primary efficacy analysis was reduction from run-in to Week 2 in mean worst itching VAS – for each patient and time-point (i.e. twice daily).

Secondary analyses included patient's rating of average itching intensity VAS, patient's assessment of different aspects of itching (including sleep disturbances, itching intensity, global itching, and self-rating questionnaire), Investigator's assessment of pruritus, Investigator's assessment of excoriation and itching.

Sample size

Thirty-four (34) patients were to be included in the study, using the ratio 1:1 for allocation to the two treatment sequences. If there were 22 eligible patients, it would be possible to detect a difference of 15 mm between TRK-820 and placebo, if the standard deviation was 20 mm for the difference between treatments, using a two-sided test with significance level a=5% and a power of 90%. To allow for ineligible patients at a rate of 35%, the total number of patients included should have been 34. Using a formula, an approximation of the total number of patients (n) needed was estimated to be 20. The critical value for a t-test with n-2 degrees of freedom was calculated, as well as the non-centrality parameter. A power of at least 90% was reached for n=22.

Randomisation

Each patient eligible for the study was allocated to one of the two cross-over treatment sequences according to a computer-generated randomisation code list. A randomisation list was generated for each study centre. When the Investigator had confirmed that all inclusion criteria and none of the exclusion criteria had been met, the Pharmacist issued treatments according to patient numbers in the order the patients entered the study on Day 1.

Blinding (masking)

The blinding for study STTOR003 was similar than for study STTOR002.

Statistical methods

For each patient, mean values for worst and average itching VAS scores were calculated for the run-in and treatment periods (Week 2 values). In the FAS analyses, missing values were substituted using the last observation carried forward method. The mean scores were analysed using an analysis of covariance (ANCOVA) model, with reduction from baseline (run-in 1 and run-in 2 periods) as the response variable and patient, period, treatment and baseline mean VAS (run-in 1 and 2, respectively) as effects. In addition, the area under the curve (AUC) for VAS was calculated for each week and analysed in the same manner as the mean values. Sleep disturbance and itching intensity were analysed with Student's paired t-test, and also with an ANCOVA model corresponding to the model for VAS. Patient's global assessment, Investigator's global assessment and excoriation were analysed using a marginal homogeneity test.

Results

Recruitment

The first patient was enrolled on 11 December 2000 and the last patient completed on 4 August 2001.

Conduct of the study

There was one amendment to the study protocol which was also approved by the IEC.

Baseline data

Demographics and baseline characteristics in STTOR003

Characteristic (N=34)	
Mean age in years (SD)	51.1 (11.8)
Male (%)	56
Caucasian (%)	100
Body weight	65.8 (14.1)

Numbers analysed

	TRK-820→Placebo	Placebo→TRK-820	Total
No. planned	17	17	34
No. randomised and treated	16	18	34
Males/females (FAS*)	11/5	8/10	19/15
Mean age (range, FAS*)	48 (25-67)	53 (31-73)	51 (25-73)
No. analysed for efficacy (FAS*)	16	18	34
No. analysed for efficacy (FAS**)	15	17	32
No. analysed for safety (FAS*)	16	18	34
No. analysed for safety (FAS**)	15	17	32
No. completed*:	15	16	31

FAS: Full Analysis Set, *all centres included, **Centre 7 excluded

Outcomes and estimation

For the primary variable, the mean value of VAS recordings of worst itching, there was a reduction of 17.9 mm from baseline (56.3 mm at baseline) after 2-week treatment with TRK-820. The corresponding value for placebo was 11.9 mm (58.5 mm at baseline). The p-value for the difference between the treatments was 0.0863. For the difference in reduction of AUC for worst itching (VAS) from baseline, the p value was 0.0718. Similar results were obtained for the secondary efficacy variable, average itching on VAS. For the secondary efficacy variable Investigator's assessment of itching, itching was rated as better in 74% of the patients after TRK-820 treatment vs. 48% after placebo, p=0.0781. Excoriation was assessed by the Investigator as better in 83% of the patients after TRK-820 treatment compared with 63% after placebo, p=0.1685. Virtually no differences in improvement between TRK-820 and placebo were observed for three of the other secondary efficacy variables, sleep disturbances, patient's assessment of itching intensity and patient's assessment of global pruritus.

The primary analysis outcome is summarised below:

Table 23. Summary of reduction from baseline to Week 2 in worst itching VAS (mm) (FAS).

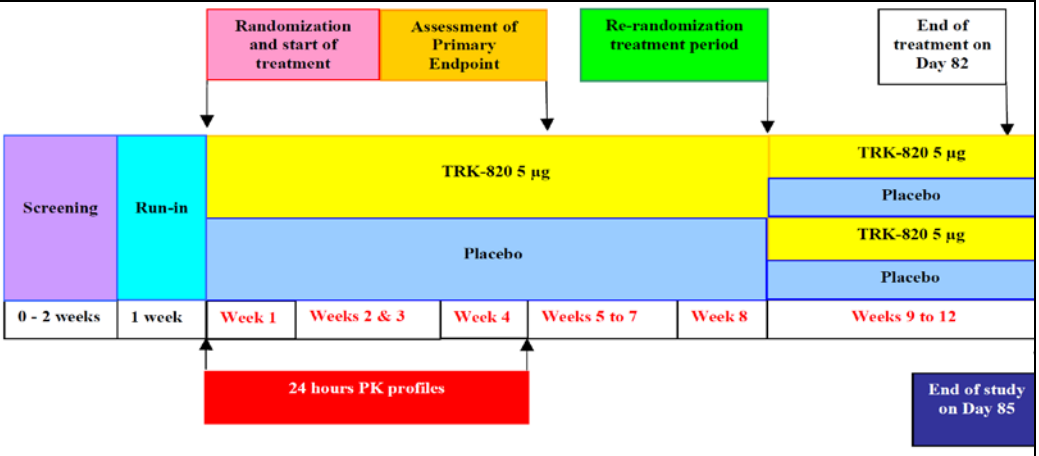
	n	Mean	SD	Min	Q1	Median	Q3	Max
Nalfurafine hydrochloride	32	17.9	19.6	–16	3.5	15.8	34.3	62
Placebo	33	11.9	17.2	–18	–1.6	8.9	22.1	56
Nalfurafine hydrochloride minus Placebo	31	5.3	25.5	–47	–18.5	8.5	27.6	54

No significant effect of nalfurafine was seen in reduction of worst itching VAS (95% CI for difference –1.06; 14.9, p=0.08). Period and order effects were examined and found non-significant. The primary efficacy variable was additionally tested for ratio of week 2 value divided by baseline value, responder rate (>50% improvement), and AUC for worst itching. For all analyses, non-significant trends were identified in favour of nalfurafine. In the responder analysis, 5 (31%) of patients treated with nalfurafine had >50% improvement vs. 2 (11%) on PLA, p=0.21. No significant effect of nalfurafine could be identified for sleep disturbances, or patient's global assessment of pruritus. The study was relatively small.

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 24. Summary of Efficacy for trial EU820UPV01

Title: A randomized, double-blind, placebo-controlled study of TRK-820 in haemodialysis patients with uremic pruritus		
Study identifier	**EU820UPV01**	
Design		
	Duration of main phase:	8 weeks + 4 weeks re-randomised withdrawal phases
	Duration of Run-in phase:	1 week
	Duration of Extension phase:	not applicable

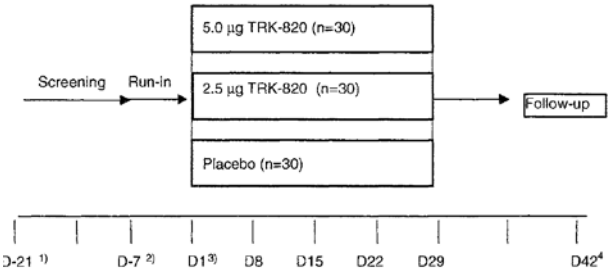
Hypothesis	Superiority		
Treatments groups	Nalfurafine 5 μg		Nalfurafine 5 μg 5-minute i.v. infusion 3 times weekly; n=170 in 8-week period and n=153 in 4-week period
	Placebo		5% mannitol 5-minute i.v. infusion 3 times weekly; n=169 in 8-week period and n=153 in 4-week period
Endpoints and definitions	Primary endpoint	Efficacy	To compare the efficacy of 3 times a week i.v. treatment with TRK-820 5 μg vs placebo in HD patients with UP following completion of a 4-week parallel-group treatment period.
	Secondary	Efficacy	To investigate efficacy of i.v. TRK-820 5 μg over 8-week treatment period
	Secondary	Efficacy	To investigate the maintenance of efficacy of i.v. TRK-820 5 μg following a re-randomization of the TRK-820 patients to continued i.v. TRK-820 5 μg or placebo from Week 9 to Week 12.
	Secondary	Safety	To determine nalfurafine safety
	Secondary	PK	To determine PK characteristics of i.v. TRK-820 5 μg in approximately 30 patients with UP on regular HD.
Database lock	14 May 2007. The SAP was finalized before disclosure of the randomization codes. Unblinding occurred on 14-May-2007.		

Results and Analysis

Analysis description	**Primary Analysis: Worst itching VAS values and change from baseline to Week 4**				
Analysis population	Full analysis set				
	Worst itching VAS values and change from baseline to Week 4*				
Descriptive statistics and estimate variability		**Values by visit (mm)**		**Change from baseline (mm)**	
	Treatment group	Nalfurafine	Placebo	Nalfurafine	Placebo
	Number of subject	169	168	169	168
	Mean Score at Baseline ± SD	**58.9 ± 15.8**	**59.1 ± 16.4**		
	Mean Score at Week 4 (LOCF) ± SD	37.0 ± 22.8	37.3 ± 23.0	-21.8 ± 20.9	-21.8 ± 21.2
	ANCOVA of change in worst itching VAS values from baseline to Week 4**				
	Estimate[a]			**0.2**	
	P value[b]			0.9272	
	95% CI			-4.2, 4.6	

Notes	VAS=Visual Analogue Scale; SD=standard deviation; CI=Confidence Intervals; LOCF=last observation carried forward; FAS=full analysis set; ANCOVA=analysis of covariance If missing data at Week 4, data from the last recorded week prior to this week were used. [a] Least-squares (type III) estimates derived from the ANCOVA. For the covariate estimate of the regression coefficient was used. [b] ANCOVA with fixed effects for treatment and country and with baseline worst itching VAS values as covariate. Treatment x country interaction included in the model only if $p<0.05$ for such interaction. *Data from Table 11 in page 75 of Study protocol. ** Data from Table 10 in page 74 of Study protocol.

Table 25. Summary of efficacy for trial STTOR002.

Title: A randomised, double-blind, placebo-controlled, multicentre study of TRK-820 in parallel groups in patients with severe pruritus and who are on regular haemodialysis		
Study identifier	**STTOR002**	
Design	*Parallel-group design*  1) Eligibility check within 8-21 days before start of treatment, i.e. Study Day1. 2) Run-in period: Assessments on Days -7, -5 and -3. 3) Study treatment period: Assessments and administration of TRK-820 (5 µg or 2.5 µg) or Placebo dosing on Days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24 and 26, respectively 4) Follow-up visit was performed 2 weeks after completion of the treatment period, i.e. on Study Day 42.	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	4 weeks 7 days not applicable
Hypothesis	Superiority	
Treatments groups	Nalfurafine 2.5 µg	Nalfurafine 2.5 µg 5-minute i.v. infusion 3 times weekly for 4 weeks, n=28
	Nalfurafine 5 µg	Nalfurafine 5 µg 5-minute i.v. infusion 3 times weekly for 4 weeks, n=26
	Placebo	5% mannitol 5-minute i.v. infusion 3 times weekly for 4 weeks, n=25

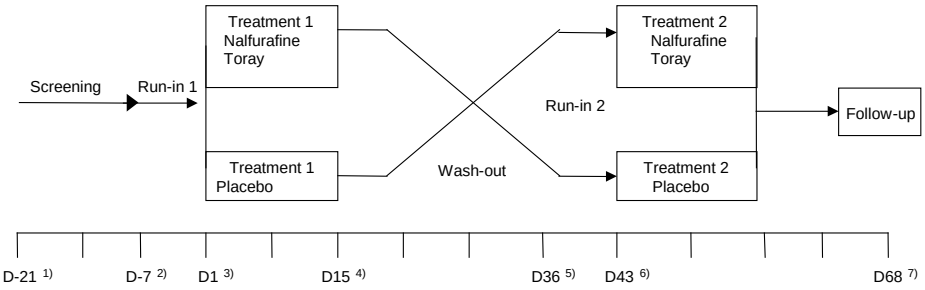
Endpoints and definitions	Primary endpoint	Efficacy	To compare efficacy of 2 i.v. dose levels of nalfurafine vs placebo using thrice weekly treatment over 4 weeks in patients with severe pruritus on regular haemodialysis.
	Secondary	Safety	To determine safety and tolerance of nalfurafine vs placebo.
Database lock	07 May 2002		

Results and Analysis

Analysis description	**Primary Analysis**				
Analysis population	Full analysis set				
	Mean maximum itching VAS*				
Descriptive statistics and estimate variability	**Treatment group**	**Nalfurafine 5 μg**	**Nalfurafine 2.5 μg**	**Nalfurafine pooled**	**Placebo**
	Number of subject	26	27	53	25
	Mean Score at Run-in ± SD	**67.1 ± 15.0**	62.1 ± 18.1	64.6 ± 16.7	**67.1 ± 14.9**
	Mean Score at Week 4 ± SD	41.4 ± 28.0	38.3 ± 26.0	39.8 ± 26.8	53.2 ± 24.0
	Mean Change ± SD	**25.8 ± 23.6**	23.8 ± 23.9	24.8 ± 23.5	**13.9 ± 23.2**
	p-value	0.0726	0.0892	0.0436	___
	95% CI	(-1.1, 24.9)	(-1.8, 24.3)	(0.3, 22.8)	___
	Mean worst itching VAS**				
	Mean Score at Run-in ± SD	**65.3 ± 15.2*****			**65.3 ± 15.0*****
	Mean Change ± SD	**20.4 ± 22.6 or 25.0 ± 23.6*****			**9.8 ± 19.8 or 12.2 ± 23.1*****
	Difference of mean change	**10.6**			
	p-value	0.0572			
	95% CI	-0.3, 21.5			
Notes	VAS=Visual Analogue Scale; SD=standard deviation; CI=Confidence Intervals *Data from Table 35 in page 64 of Module 2.7.3 (if otherwise not given); ** Data from Table 6 in page 23 of Module 2.5 *** Data from Tables 22 and 23 in page 64 of Study 002 (and in Appendix Table 2 in page 106 of Module 2.7.3)				

Table 26. Summary of efficacy for trial STTOR003

Title: A randomised, double-blind, placebo-controlled, multicentre, cross-over study of TRK-820 in patients with severe pruritus and who are on regular haemodialysis.

Study identifier	STTOR003		
Design	*Cross-over design*  1. Eligibility check within 8-21 days before start of treatment 1. 2. Run-in period 1: Assessments on Days -7, -5 and -3 (in this figure "–" denotes before treatment). 3. Study treatment period 1: Assessments and Nalfurafine or placebo dosing on Days 1, 3, 5, 8, 10 and 12.4. 3-week wash-out period. 5. Run-in period 2: Assessments on Days 36, 38 and 40. 6. Study treatment period 2: Assessments and Nalfurafine or placebo dosing on Days 43, 45, 47, 50, 52 and 54. 7. A follow-up visit was performed 2 weeks after completion of the second treatment.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		2 weeks active treatment + 2 weeks placebo treatment 7 days not applicable
Hypothesis	Superiority		
Treatments groups	Nalfurafine 5 μg		Nalfurafine 5 μg 5-minute i.v. infusion 3 times weekly for 2 weeks, all subjects (n=34)
	Placebo		5% mannitol 5-minute i.v. infusion 3 times weekly for 2 weeks, all subjects (n=34)
Treatments sequences	Nalfurafine –> Placebo (S1)		n=16
	Placebo –> Nalfurafine (S2)		n=18
Endpoints and definitions	Primary endpoint	Efficacy	To compare the effect of 2 weeks' i.v. therapy with 5 ug of TRK- 820 administered 3 times weekly to placebo in a cross-over design in patients with severe pruritus on regular HD.
	Secondary	Safety	To determine nalfurafine safety
Database lock	15 November 2001		

Results and Analysis

Analysis description	**Primary Analysis: reduction from baseline to Week 2 in mean worst itching VAS**				
Analysis population	Full analysis set				
Descriptive statistics and estimate variability	**Treatment group**	**Nalfurafine 5 μg**		**Placebo**		**Nalfurafine minus placebo**
	Sequence	**S1**	**S2**	**S1**	**S2**	
	Number of subject	16	16	15	18	
	Mean Score at Run-in ± SD*	**63.6±10.9**	**48.9 ± 18.8**	**54.5 ± 19.3**	**61.9 ± 12.6**	

	Mean Score at Week 2 ± SD	**41.5 ± 20.5**	**35.2 ± 19.8**	**44.6 ± 19.5**	**48.4 ± 19.1**	
	Mean Change ± SD	**17.9 ± 19.6**		11.9 ± 17.2		**5.3 ± 25.5* or 6.9****
	p-value	N/A		N/A		0.0863
	95% CI	N/A		N/A		-1.1; 14.9
Notes	VAS=Visual Analogue Scale; SD=standard deviation; CI=Confidence Intervals; S1=Sequence 1 (Nalfurafine –> Placebo); S2= Sequence 2 (Placebo –> Nalfurafine) *Data from Table 48 in page 74 of Module 2.7.3. ** Data from Table 6 in page 23 of Module 2.5.					

Analysis performed across trials (pooled analyses and meta-analysis)

- **Meta-analyses of STTOR002, STORR003 and EU820UPV01**

Since all 3 studies STTOR002, STORR003 and EU820UPV01 evaluated 3 times a week 5-minute i.v. administration of nalfurafine hydrochloride in randomised, double-blind, placebo-controlled studies and the principal features with respect to design and analytical issues were the same, the applicant considered appropriate to pool the data in order to further investigate the efficacy of nalfurafine hydrochloride in patients with severe UP.

In order to use comparable data from the 3 studies, only the run-in, Week 2 and Week 4 results were used in the meta-analyses, i.e. the Week 8 data from Study EU820UPV01 was not included. For the Week 4 analysis, only data from studies STTOR002 and EU820UPV01 were included. For the STTOR003 study, only the first period was used for this analysis because of the problems with the cross-over study design. In addition, only data from the treatments common to all 3 studies, i.e. nalfurafine hydrochloride 5 µg and placebo, are included in the analyses.

This meta-analysis was based on two sub-populations of patients from the efficacy studies STTOR002, STTOR003 and EU820UPV01 who met i) the Severe UP-75 criteria and ii) the Severe UP-66 criteria.

Table 27. Numbers analysed in meta-analyses.

Study Identifier	Severe UP-75		Severe UP-66	
	Nalfurafine Toray 5 µg	Placebo	Nalfurafine Toray 5 µg	Placebo
STTOR002	9	11	8	11
Period 1 STTOR003	6	5	6	5
EU820UPV01	32	43	28	38
Total	47	59	42	54

UP-75 sub-population (defined after unblinding the data):

After unblinding of the data, a sub-population (UP-75 sub-population) with more “severe” symptoms during the run-in period was defined and requested by the sponsor for post-hoc analyses on patients after unblinding of the data: VAS assessment ≥ 60 mm (patient’s assessment) and intolerable itching intensity

of grade 5 (patient's assessment) or unrelieved by scratching as grade 4 (as assessed by the investigator during the run-in period).

Primary endpoint in UP-75:

Table 28. ANCOVA of change in worst itching VAS values at Week 4 for the sub-population (TRK-820=nalfurafine hydrochloride)

	p-value[a]	Estimate[b]	95% CI
Week 4			
ANCOVA parameter [a]			
Treatment	0.1708		
Country	0.7195		
Baseline worst itching VAS	0.7982	-0.080	
Least square means and treatment difference (mm)			
TRK-820		-33.6	[-41.7, -25.5]
Placebo		-26.1	[-33.0, -19.1]
TRK-820 minus placebo		7.5	[-3.3, 18.4]

Note: In case of missing data at Week 4 data from the last recorded week prior to this week were used.
[a] ANCOVA with fixed effects for treatment and country and with baseline worst itching VAS values as covariate. Treatment x country interaction included in the model only if $p < 0.05$ for such interaction.
[b] Least-squares (type III) estimates derived from the ANCOVA. For the covariate: estimate of the regression coefficient.
ANCOVA: analysis of covariance, VAS: visual analogue scale, CI: confidence interval.

Secondary endpoints in UP-75

The secondary endpoints in the UP-75 population were sleep disturbances, nights with sound sleep, patient's assessment of itching intensity and responder rates at Weeks 2 and 4.

No relevant differences in this sub-population were seen compared to the results that were observed in the FAS.

UP-66 sub-population (defined after unblinding the data):

From the Severe UP-75 analysis it was observed that patients with baseline worst itching VAS scores of 90 – 100 mm had very small to no changes following treatment. As such a further post-hoc analysis was carried out on a sub-population of the Severe UP-75 sub-population that had VAS < 90 mm (ranging from 60 to 90 mm and itching grade of 4 or 5) in addition to the UP-75 criteria. Sixty-six patients were included in the Severe UP-66 sub-population analysis, 28 patients in the nalfurafine hydrochloride group and 38 patients in the placebo group.

Primary endpoint in UP-66:

Mean worst itching VAS values at baseline were similar for the nalfurafine hydrochloride and placebo groups (73.0 mm and 75.3 mm, respectively).

Table 29. Primary endpoint in the UP-66 sub-population (Nalfurafine = nalfurafine hydrochloride)

Change in Worst Itching VAS Values at Week 4 (LOCF)	p-value[a]	Estimate[b]	95% CI
ANCOVA parameter			
Treatment	0.0279		
Least-square means and treatment difference			
Nalfurafine Toray minus placebo		13.4	[1.5, 25.2]

If missing data at Week 4, data from the last recorded week prior to this week were used.
a ANCOVA with fixed effects for treatment and country and with baseline worst itching
VAS values as covariate. Treatment x country interaction included in the model only if p<0.05 for such interaction.
b Least-squares (type III) estimates derived from the ANCOVA. For the covariate: estimate of the regression coefficient.
FAS=full analysis set; VAS=Visual Analogue Scale; CI=confidence interval; ANCOVA=analysis of covariance

Mean LOCF worst itching VAS values at Week 4 were 36.7 mm for the nalfurafine hydrochloride group and 50.4 mm for the placebo group, corresponding to mean decreases of 36.3 mm and 24.9 mm, respectively.

Secondary endpoints in UP-66:

• *The change in worst itching recorded on the VAS*

There were statistically significant differences between treatment groups up to week 4 in favour of nalfurafine hydrochloride treatment (Week 2 p=0.003, Week 4 p=0.007) but not at Weeks 5 to 8. Mean LOFC worst itching VAS values at week 8 were 33.2 mm for the nalfurafine hydrochloride group and 42.1 mm for the placebo group, corresponding to mean decreases of 39.8 mm and 33.3 mm, respectively.

• *The change from baseline in itching intensity recorded on a categorical scale:*

There was a statistically significant difference in itching intensity on a categorical scale (1-5) at Week 4 in favour of nalfurafine hydrochloride (mean difference 0.5 points, p=0.04) but not in any other weeks.

• *The change from baseline in sleep disturbance due to itching on a categorical scale:*

There was a significant difference in sleep disturbance on a categorical scale (1-5) at weeks 2-3 in favour of nalfurafine hydrochloride (mean difference 0.5 points, p<0.05). There were no statistically significant differences due to the effects of treatment at Weeks 1 or 4-8.

• *The number of nights with sound sleep.*

At baseline, the mean number of nights of sound sleep was 0.3 nights for the nalfurafine hydrochloride group and 0.6 nights for the placebo group. The mean number of nights with sound sleep increased up to Week 8 (LOCF) (4.8 nights in the TRK-820 group and 4.4 nights in the placebo group) and was similar between nalfurafine hydrochloride and placebo at all time points.

• *The change from baseline in the assessment on daytime feeling and activities due to itching by the patient.*

No significant differences between treatment groups.

• *The investigator's assessment of excoriations.*

No significant differences between treatment groups.

• *The response to treatment, where a reduction from baseline of at least 50% in itching VAS is defined as response.*

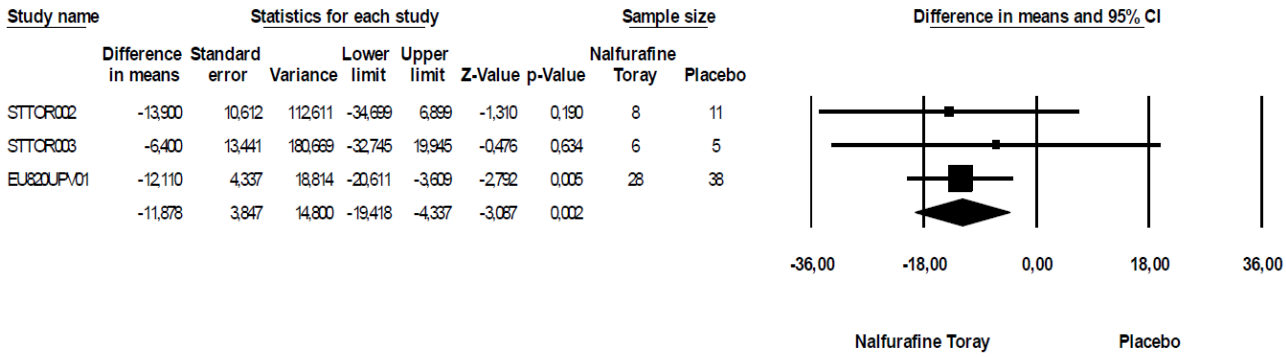
No significant differences between treatment groups.

- **Results from meta-analyses in subpopulations UP-75 and UP-66**

In the Severe UP-75 sub-population, a statistically significant improvement was seen in worst itching VAS for patients treated with nalfurafine hydrochloride 5 µg compared to placebo at Week 2 (mean VAS difference 8.9 mm, p=0.014), and at Week 4 statistical significance was almost achieved (mean VAS difference 8.9 mm, p=0.055). In the Severe UP-66 sub-population statistical significance was achieved at both time-points (Week 2 mean VAS difference 11.9 mm, p=0.002; Week 4 mean VAS difference 13.6 mm, p=0.006).

In the post-hoc UP-75 subpopulation of the pivotal study, the difference in VAS was 7 mm in favour of Winfuran.

Figure 7. Comparison of nalfurafine hydrochloride and placebo in mean worst itching VAS; Severe UP-66 Sub-population at Week 2



Clinical studies in special populations

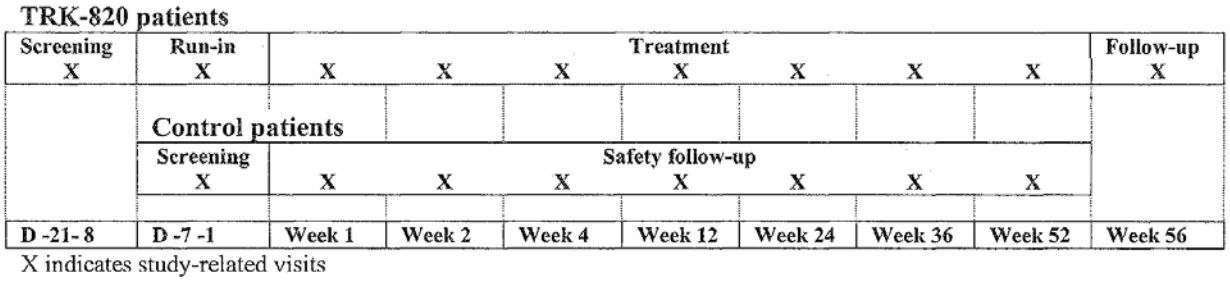
Nalfurafine hydrochloride has not been studied in children. The target population is patients with renal impairment on HD. There are studies on p.o. but not i.v. formulation of nalfurafine hydrochloride in patients with liver disease.

Supportive studies

STTOR004: A long-term safety, open, multi-centre study of TRK-820 in patients with renal failure on regular haemodialysis, who are experiencing severe pruritus, in comparison with renal failure patients without severe pruritus.

This was a multicentre, non-randomised, open label, control design study comparing ESRD patients on routine HD with severe UP to ESRD patients on routine HD without UP

Figure 8. Study course in STTOR004.



X indicates study-related visits

In this study, patients assigned to Nalfurafine received 5 µg 3 times a week by i.v. infusion for up to 52 weeks. For each patient, the mean of the worst itching VAS scores was calculated for the run-in period and for each treatment week. The non-treatment control patients did not receive any study medication following each HD session. Due to the design of this trial, efficacy evaluation was only performed for the Nalfurafine group.

Results from STTOR004

In total, 62 patients (42%) in the TRK-820 group completed the 52-week treatment period. In the control group 63 patients (78%) completed the study period. Adverse events (38 patients) and consent withdrawal (30 patients) accounted for the majority of the cases in the TRK-820 group, while treatment failure accounted for 8 patients. The worst itching VAS was similar at baseline in both the total TRK-820 population and in the cohort completing the 52-week treatment period.

Table 30. Summary of Patient's Assessment of Worst Itching VAS Score in Study STTOR004

	Worst Itching VAS (mm)							
	n	Mean	SD	Min	Q1	Median	Q3	Max
Run-in	146	66.4	15.5	27	55.4	67.5	77.2	100
Week 1	142	57.4	19.2	6	46.6	56.9	72.1	100
Week 2	138	49.2	23.1	1	34.2	52.3	67.3	96
Week 4	123	43.8	24.0	0	24.9	43.9	62.6	93
Week 12	100	37.7	26.2	0	14.9	34.0	58.9	90
Week 24	86	31.5	26.3	0	8.1	22.3	54.3	88
Week 36	72	30.7	26.2	0	5.5	25.5	52.5	90
Week 52	62	27.2	26.8	0	5.4	18.9	52.7	86

VAS=Visual Analogue Scale; SD=standard deviation; min=minimum; max=maximum; Q1=first quartile; Q3=third quartile
Data Source: STTOR004 clinical study report Table 10

In the long-term open-label study STTOR004, itching intensity was markedly reduced during the study period. However, only 42% of the patients were analyzed at the end of the study at week 52. It was not possible to distinguish between placebo-effect, a natural course of pruritus over time and an effect of Nalfurafine hydrochloride. Consequently, only limited conclusions can be drawn from the data.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The clinical program of nalfurafine hydrochloride was designed to support the registration of the product in the following indication: "*Treatment of severe uraemic pruritus (UP) in patients of 18 years of age or older, with end stage renal disease undergoing regular haemodialysis, haemodiafiltration or haemofiltration (dialysis). Severe UP is defined as intolerable itching or itching unrelieved by scratching*".

There is currently no medicinal product authorized for the treatment for severe uraemic pruritus (UP). The exact pathophysiology of the condition remains elusive and no mechanism of nalfurafine hydrochloride specific to uraemic pruritus has been proven.

Five efficacy studies were performed in patients with severe UP undergoing HD. The study EU820UPV01 was the largest (N=339) multicentre, double-blind, randomised study, with a duration of 8 weeks and considered as the pivotal study. The design of this study is considered adequate.

The supportive studies included 2 smaller studies, STTOR002 and STTOR003 with a duration of 2 and 4 weeks and one long-term open-label study STTOR004 of 52 weeks. In the long-term study, the control group did not have pruritus. In a dose-response study LCRC/G/028, the medication was given daily instead of 3 times a week as in other studies.

In the pivotal study EU820UPV01, the Applicant presented results in the middle of the treatment period, after 4 weeks, as the primary endpoint. The main inclusion and exclusion criteria in the study were considered acceptable. The VAS scale was used to assess itching severity in several previous studies and proved to be a valid patient-reported outcome measure for UP. The original study populations included adult patients with UP and a minimum 3 mean worst itching VAS ≥ 50mm, as well as a mean value > 25mm during the run-in period which is considered acceptable.

Overall, the baseline data in the pivotal study is considered to be balanced between the active treatment and placebo-groups.

A four week run-in period in study EU820UV01 would have the advantage to properly identify the patients with severe non-cyclic itch instead of identifying the individuals who have a period of intense itch and spontaneously improve. This would be a useful approach if this group with severe non-cyclic itch was the group that is most likely to benefit from the drug. The Applicant argued that a four week run-in is not ethical. This is not supported by the CHMP as all patients were allowed to have any standard of care treatment for UP during this period as during the whole study period.

Post-hoc analyses including studies STTOR002, STORR003 and EU820UPV01 were performed in subpopulations with different baseline VAS –limits (UP-75 population and UP-66 population) with the objective of supporting the claimed indication.

In the "severe UP-75" post-hoc meta-analysis of EU820UPV01 and STTOR002 at 4 weeks, a subpopulation of 95 patients out of 315 (24%) was used. The UP-75 population was defined as worst itching intensity (VAS) ≥ 60 mm by the patient and itching intensity that was grade 4 (itching unrelieved by scratching) or grade 5 (intolerable itching) by the investigator during the one-week run-in period.

From the Severe UP-75 analysis it was observed that patients with baseline worst itching VAS scores of 90 – 100 mm had very small to no changes following treatment. Therefore, in order to exclude these patients, a further post-hoc analysis was carried out on a subpopulation of the Severe UP-75 subpopulation with baseline worst itching VAS ranging from 60 to 90 mm and itching grade of 4 or 5 was analysed (Severe UP-66 sub-population) and the Applicant provided the relevant post-hoc analyses. However, the UP-66 subpopulation was not considered by the CHMP as distinctly different from the total

population. In addition in clinical practice these patients would not be easy to distinguish. Therefore the UP-66 population was not considered as clinically meaningful by the CHMP.

The CHMP was also of the opinion that the complex definitions of subpopulations used in post-hoc analyses are not considered applicable in clinical settings to identify patients who would potentially benefit from the treatment. The data for patients who are not included in the chosen subpopulations should have been analysed and presented separately.

Efficacy data and additional analyses

The main efficacy study failed to demonstrate a benefit of nalfurafine therapy over placebo (p= 0.93 for the primary endpoint). In the main study EU820UPV01 there was no demonstrable benefit of NFU compared to placebo against uraemic pruritus. Both placebo and treatment with NFU 5 µg i.v. 3 times a week equally reduced worst itching intensity (VAS) by approximately 22 mm. Moreover, in the main analysis at week 4 there were no significant differences between placebo and active treatment in important secondary endpoints such as sleep disturbances and excoriations, either.

The supportive clinical trials (STTOR002 and STTOR003) also failed to demonstrate a statistically significant difference to placebo in the primary endpoint (placebo responses of 12.4 mm and 13.6 mm respectively).

In the long-term open-label study STTOR004 (52 weeks study), itching intensity was markedly reduced during the study period. However, only 42% of the patients were analysed at the end of the study at week 52. It was not possible to distinguish between placebo-effect, a natural course of pruritus over time and an effect of Nalfurafine hydrochloride. Consequently, only limited conclusions can be drawn from the data.

In the post-hoc UP-75 subpopulation of the pivotal study, the difference in VAS was 7.5 mm in favour of Winfuran which is considered modest and the clinical relevance of this finding is questioned

In the further post-hoc analyses provided by the Applicant, i.e. a meta-analysis of patients in clinical studies EU820UPV01, STTOR002 and STTOR003 who fulfilled the subgroup UP-75 criteria, a ≥20 mm improvement from baseline was seen at 4 weeks both in the active treatment and placebo groups. The mean difference between active treatment and placebo was 8.9 mm and of borderline statistical significance (p=0.055). The exploratory post-hoc analyses in a selected subgroup and meta-analysis of subgroups across different studies are considered at best hypothesis generating and are not considered to support neither a full nor a conditional marketing authorisation.

The mean difference in UP-66 population between active treatment and placebo groups at week 2 was 11.9 mm in VAS. VAS changes ≥20 mm are deemed by experts in UP as clinically relevant and therefore the difference was not considered relevant by the CHMP.

No differences in the response rates between placebo and active treatment were seen in the pivotal study. There was no difference in the absolute number of patients who responded to the treatment in the severe UP-75 population. As the proportion of patients who received placebo compared to active treatment was higher in the UP-75 population, the percentage of responders was numerically somewhat higher. The small number of patients in the sub-population limits the value of these results. The responder rate analyses do not give support for a significant effect of nalfurafine hydrochloride.

All placebo-controlled studies demonstrated a substantial beneficial placebo-effect. The results may be affected by the nature of uraemic pruritus, a chronic condition that may have a cyclic course and vary greatly in intensity at individual level. The placebo-effect could partly be explained by VAS-value regression to the mean, as only the patients with highest VAS ratings in the short run-in period were included.

Additional efficacy data needed in the context of a conditional MA

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of the above mentioned Regulation based on several claims (see section 1.1 'Conditional Marketing Authorisation'), including the provision of comprehensive clinical data. In particular, the Applicant proposed a post-approval study to assess the temporality of the benefit of nalfurafine. However, given the negative benefit/risk balance at the time of this CHMP Opinion, details of this proposal were not further assessed.

2.5.4. Conclusions on the clinical efficacy

There was no demonstrable benefit of Nalfurafine hydrochloride compared to placebo against uraemic pruritus in the pivotal EU820UPV01 study population with severe pruritus. Both placebo and treatment with NFU 5 µg i.v. 3 times a week equally reduced worst itching intensity (VAS) by approximately 22 mm at 4 weeks (p= 0.93). The mean difference between active treatment and placebo was 0.2mm and not statistically significant (95% CI -4.2, 4.6)) There were no significant differences between placebo and active treatment in any important secondary endpoints such as sleep disturbances and excoriations, either. Responder analyses did not reveal beneficial effects compared to placebo.

The supportive clinical trials in the i.v. NFU program, STTOR002 and STTOR003 also failed to demonstrate a statistically significant difference to placebo in the primary endpoint.

The Applicant provided further post-hoc analyses in subgroups of patients with more severe UP after unblinding of the data and proposed to focus on the UP-75 population only. The adequacy of performing exploratory post-hoc analyses in a selected subgroup considering that no effect was shown in the overall population in the pivotal study is not justified. Furthermore, the adequacy of applying post-hoc subgroup analyses across different studies in a meta-analysis after unblinding is questionable from a methodological point of view. In addition, it was only possible to analyse the data from all three studies (STTOR002, STORR003 and EU820UPV01) up to two weeks. The majority of patients were from the pivotal study EU820UPV01, thus, the results are expected to mirror the first two weeks of that study.

The CHMP concluded that results from the post-hoc meta-analyses in selected subpopulations are considered hypothesis-generating and not sufficient for marketing authorization.

Moreover, all placebo-controlled studies demonstrated a substantial beneficial placebo-effect. The results may be affected by the nature of uraemic pruritus, a chronic condition that may have a cyclic course and vary greatly in intensity at individual level. The placebo-effect could partly be explained by VAS-value regression to the mean, as only the patients with highest VAS ratings in the short run-in period were included.

Taking all of the above into account, the CHMP is of the opinion that the efficacy of Nalfurafine hydrochloride is not properly or sufficiently demonstrated.

2.6. Clinical safety

A total of 865 subjects have been exposed to ≥ 1 dose of i.v. nalfurafine in the safety population and a total of 497 HD patients have been exposed to at least one dose of i.v. nalfurafine hydrochloride.

The long-term study STTOR004 was not placebo-controlled but had an open-label design with only non-treatment non-pruritic HD patients as controls, which is a limitation of the safety database. For this

reason, safety results from STTOR004 are presented separately from the placebo-controlled studies throughout this AR. The safety data from studies with healthy volunteers is also presented separately.

Patient exposure

The safety review includes data on patients/healthy volunteers that were exposed to at least one dose of nalfurafine hydrochloride in 19 studies worldwide by the intravenous (i.v.), intramuscular (i.m.) route of administration. In addition, 14 studies were conducted using an oral formulation of nalfurafine hydrochloride.

Table 31. Patient exposure

	Treatment		**Total**
	Nalfurafine	**Placebo / Non-treatment controls**	
Administration iv/im			
Healthy volunteers	308	135	443
Other patients	60	7	67
HD patients total	497	385	882
HD Longterm data (STTOR004)	146	81	227
Total for iv/im studies	865	527	1392
Oral administration			
Healthy volunteers	107	20	127
Liver disease patients	134	35	169
HD patients	611	171	782
Total oral studies	852	226	1078
Total iv/im/oral	1717	753	2470

The dose of exposure in the safety study pool for proposed indication was >2.5 µg - ≤5 µg in 92.5% of the patients.

Adverse events

CNS and gastrointestinal adverse reactions are the most frequent adverse reactions expected of an opioid receptor agonist.

The analysis of adverse events (AEs) was based on the assessment of Treatment Emergent Adverse Events (TEAE). These are defined as AEs that occurred during the study period or were conditions present at baseline but worsened during the study.

Healthy volunteers:

In a total of 308 healthy volunteers who were exposed to i.v. or i.m. nalfurafine, there were no serious TEAEs and no deaths. The majority of the studies were single dose and the majority of subjects had a supratherapeutic dose of exposure <5 µg ≤40 µg.

Placebo-controlled HD populations:

Table 32. Overall summary of TEAEs Reported in HD Patients Receiving i.v.Nalfurafine in placebo controlled studies with i.v. Long-Term Exposure to Nalfurafine hydrochloride

	Placebo controlled studies		**Long-term study STTOR004**	
Category	**Nalfurafine (N=351)**	**Placebo (N=304)**	**Nalfurafine (N=146)**	**Non-Pruritic control (N=81)**
TEAEs	195 (55.6%)	148 (48.7%)	139 (95.2%)	76 (93.8%)
Severe TEAEs	23 (6.6%)	26 (8.6%)	55 (37.7%)	16 (19.8%)
Serious TEAEs	29 (8.3%)	28 (9.2%)	78 (53.4%)	27 (33.3%)
Deaths	1 (0.3%)	6 (2.0%)	14 (9.6%)	7 (8.6%)

TEAEs leading to withdrawal	11 (3.1%)	12 (3.9%)	40 (27.4%)	0
Related TEAEs	73 (20.8%)	36 (11.8%)	56 (38.4%)	10 (12.3%)
Related serious TEAEs	1 (0.3%)	4 (1.3%)	5 (3.4%)	5 (6.2%)

- Common adverse events:

Healthy volunteers:

The most common TEAEs were dizziness (24.0%) in the Nalfurafine treated group compared to 2.2% of controls and somnolence 21.4% in the Nalfurafine treated group compared to 6.7% of controls.

In addition, the following PTs occurred at a frequency of ≥2% of subjects: Headache, disturbance in attention, fatigue, thirst, nausea, insomnia and vision blurred. There were also laboratory changes in testosterone, prolactin and thyroid hormones. The occurrence of these PTs in the Nalfurafine group exceeded the control group by greater than 2%, suggesting a possible causal relationship.

Placebo controlled HD populations:

Table 33. TEAEs with a frequency of ≥5% of subjects in the following system organ classes (SOC) and PT reached a frequency level of >2% in the Nalfurafine treated group. Placebo controlled HD trials with Nalfurafine i.v.

SOC/PT	Nalfurafine treated group (%, N=351)	Placebo (%, N=304)
Gastrointestinal disorders	**15.1**	**11.8**
Diarrhoea	4.3	3.0
Nausea	3.1	3.9
Vomiting	4.6	1.3
Nervous system disorders	**15.1**	**10.9**
Headache	9.4	6.9
Dizziness	2.3	1.3
Vascular disorders	**10.8**	**15.5**
Hypotension	5.1	7.6
Hypertension	3.4	6.3
Musculoskeletal and connective tissue disorders	**11.1**	**10.9**
Muscle spasms	4.0	3.9
Back pain	2.3	1.0
Injury, poisoning and procedural complications	**8.5**	**10.5**
Hypotension	3.4	4.3
Arteriovenous fistula thrombosis	2.3	1.0
Metabolism and nutrition disorders	**9.1**	**4.6**
Hypercalcaemia	2.3	0.3
Psychiatric disorders	**6.8**	**4.3**
Insomnia	4.0	1.6
Respiratory thoracic, and mediastinal disorders	**5.1**	**3.0**
Cough	2.6	1.3
Infections and infestations	**11.7**	**15.5**
Cardiac disorders	**6.6**	**6.9**
Investigations	**6.6**	**7.9**
General disorders and administration site Conditions	**8.3**	**8.6**

Long-term open label study STTOR004:

Table 34. TEAEs with a frequency of ≥5% of subjects in the following system organ classes (SOC) and PTs reached a frequency level of >5% in the Nalfurafine treated group. Long-term open label study STTOR004.

SOC/PT	Nalfurafine treated group (%, N=146)	Non-UP HD (%, N=81)
Infections and infestations	**48.6**	**48.1**
Bronchitis	8.2	18.5
Pneumonia	8.2	3.7
Gastrointestinal disorders	**34.2**	**28.4**
Diarrhoea	11.6	8.6
Nausea	6.8	3.7
Vomiting	8.2	4.9
Abdominal pain	5.5	2.5
Nervous system disorders	**32.2**	**18.5**
Headache	17.1	6.2
Vascular disorders	**34.2**	**29.6**
Hypotension	12.3	7.4
Hypertension	13.0	4.9
Musculoskeletal and connective tissue disorders	**28.8**	**28.4**
Muscle spasms	12.3	8.6
Pain in extremity	8.2	8.6
Cardiac disorders	**26.0**	**25.9**
Atrial fibrillation	6.2	6.2
Injury, poisoning and procedural complications	**29.5**	**17.3**
Arteriovenous fistula thrombosis	6.8	4.9
Metabolism and nutrition disorders	**17.8**	**24.7**
Psychiatric disorders	**21.2**	**12.3**
Insomnia	8.9	6.2
Respiratory thoracic, and mediastinal disorders	**23.3**	**14.8**
Dyspnoea	8.9	4.9
Blood and lymphatic system disorders	**13.7**	**9.9**
Anaemia	10.3	6.2
Investigations	**18.5**	**8.6**
Ear and labyrinth disorders	**8.9**	**3.7**
Vertigo	5.5	1.2
Skin and subcutaneous tissue disorders	**22.6**	**13.6**
Pruritus	6.2	4.9
Skin ulcer	5.5	1.2
General disorders and administration site Conditions	**26.0**	**12.3**

Serious adverse event/deaths/other significant events

- **I.V. formulation of Nalfurafine Hydrochloride**

The numbers of deaths are summarized in table 32. None of the 15 deaths in any HD i.v. Nalfurafine treatment groups was judged to be Nalfurafine -related. 14 deaths occurred in the STTOR004 and one in EU820UPV01. The majority of the deaths were associated with cardiovascular SAEs; cerebral stroke, cardiac arrest, myocardial infarction, and sudden death. Occasionally the death event was accompanied by other SAEs such as tumour, hepatomegaly, and hyperpotassemia.

Regarding serious adverse events (SAEs), in the HD UP placebo-controlled studies with HD patients the most commonly reported SAEs (with an incidence of ≥2%) in the Nalfurafine population were Infections (3%); Injury Poisoning and procedural Complications (2.8%), Vascular disorders (2%).

6 SAEs were said to be possibly or probably related to the treatment; these are:

- Study EU820UPV01 –cardiac failure (possibly related)

- Study STTOR004 –chest pain (possibly related)
- Study STTOR004 -vomiting (possibly related)
- Study STTOR004 –urticarial rash (probably related)
- Study STTOR004 –confusional state (possibly related)
- Study STTOR004 –cardiac failure congestive (possibly related).

The relationship with Nalfurafine is unclear, as these SAEs are not clearly explained by the toxicology results and have other possible explanations such as concurrent disease and medications.

- **ORAL formulation of Nalfurafine Hydrochloride**

Healthy volunteers

A total of 6 studies were carried out. There was no information on deaths or SAEs in this group.

HD populations:

A total of 6 studies were carried out in Japan for the oral formulation. There were 4 deaths and 12 severe adverse events in the treatment group compared to none in the placebo group. 2 of the SAEs were considered related to the treatment.

Liver Disease populations:

The applicant conducted 3 trials of oral nalfurafine hydrochloride in patients with liver disease. There were no deaths in these studies and one serious adverse event. This SAE was considered related to the treatment.

The modes of administration exhibit similar patterns of TEAE with respect to Headache, Insomnia and Vomiting. There were differences with respect to urinary (output increased) and gastrointestinal disorders (constipation). In terms of the urinary TEAE, most of the exposure took place in patients undergoing HD, and so the urine effects would not be seen as clearly as in the liver disease non-HD patients. The gastrointestinal TEAE differences are likely accounted for by the mode of administration.

Significant adverse events

- **Related adverse events**

Healthy volunteers:

All common adverse events (dizziness, somnolence, headache, disturbance in attention, fatigue, thirst, nausea, insomnia and vision blurred, laboratory changes in testosterone, prolactin and thyroid hormones) were considered related to the treatment.

Placebo-controlled HD populations:

The following related events PT were more frequent in the nalfurafine hydrochloride treated group and reached a frequency level of >2%:

- Vomiting 8/351 (2.3%) in the Nalfurafine treated group, compared to 1/304 (0.3%) in placebo.
- Headache 12/351 (3.4%) in the Nalfurafine treated group, compared to 6/304 (2.0%) in placebo.
- Dizziness 8/351 (2.3%) in the Nalfurafine treated group, compared to 2/304(0.7%) in placebo.
- Insomnia 10/351 (2.8%) in the Nalfurafine treated group, compared to 3/304 (1.0%) in placebo.

Long-term open-label study STTOR004:

The following related events PTs showed a substantial difference (>2% greater incidence between patients on active treatment and control) between patients receiving nalfurafine hydrochloride *i.v.* and no-UP HD control patients:

- Vomiting 3/146 (2.1%) in the Nalfurafine treated group, compared to 0 of controls.

- Nausea 3/146 (2.1%) in the Nalfurafine treated group, compared to 0 of controls.

- Headache 10/146 (6.8%) in the Nalfurafine treated group, compared to 2/81(2.5%) of controls.

- Fatigue 5/146 (3.4%) in the Nalfurafine treated group, compared to 0 of controls

- Pruritus 5/146 (3.4%) in the Nalfurafine treated group, compared to 0 of controls.

- Insomnia 7/146 (4.8%) in the Nalfurafine treated group, compared to 0 of controls.

- **Vomiting**

In the HD patients placebo-controlled studies a total of 20 TEAE episodes of vomiting were recorded. Sixteen of these were in patients taking Nalfurafine (incidence rate 4.6 per 100 patients studied). In the placebo population 4 were recorded (incidence 1.3 per 100 patients studied). Vomiting was also recorded in the long-term study, where 12/146 (8.2%) patients receiving nalfurafine hydrochloride exhibited vomiting, compared to 4/81 (4.9%) of the non-treatment controls. The symptoms of vomiting were recorded as an SAE in one patient and caused the withdrawal of two other patients in STTOR004.

- **Insomnia**

In the HD patients placebo-controlled studies a total of 19 episodes of insomnia were recorded. Fourteen of these were in patients taking nalfurafine hydrochloride (incidence rate 4.0 per 100 patients studied as AEs). In the placebo population 5 were recorded (incidence 1.6 per 100 patients studied). In the open-label study (STTOR004), insomnia was recorded 13 of the patients receiving nalfurafine hydrochloride (incidence rate 8.9 per 100 patients) and 5 of the non-treatment controls (incidence 6.2 per 100 patients).

- **Headache**

In healthy volunteers headache was more common in the nalfurafine hydrochloride treated group (30/308 (9.7%)), compared to controls (7/135(5.2%)).

In placebo controlled trials enrolling HD patients with UP headache was more common in nalfurafine hydrochloride treated patients (33/351 (9.4%)) compared to placebo (21/304(6.9%)).

In the long-term study headache was more frequent in the nalfurafine hydrochloride treated group (25/146 (17.1%)) than in the control group (5/81(6.2%)).

The association seems therefore to be constant and likely to be a real effect. There were no SAE or patient discontinuations associated with headache.

- **Infections**

Chronic renal insufficiency (patients have an impaired functioning of their immune systems and are at high risk for infectious complications. Pruritus may create excoriations and this may increases the chance of infection. In placebo-controlled i.v. HD studies, there were more AEs and SAEs of Infections and infestations (SOC) in the placebo-group compared to treatment group. In STTOR004, AE frequency of Infections and infestations (SOC) were similar in the treatment group and controls.

- **Injury, poisoning and procedural complications**

In the placebo-controlled studies, there were 8 cases (2.3%) of arteriovenous fistula thrombosis in nalfurafine hydrochloride group compared to 3 cases (1.0%) in the placebo group.

Table 35. Examples of TEAEs in SOC Injury, poisoning and procedural complications in long-term study STTOR004.

	Nalfurafine hydrochloride (N=146)	Control (N=81)
Injury, poisoning and procedural complications	43 (29.5%)	14 (17.3%)
Arteriovenous fistula thrombosis	10 (6.8)	4 (4.9)
Shunt occlusion	5 (3.4)	0
Any fracture	5 (3.4)	1 (1.2)
Fall, contusion, injury or accident	10 (6.8)	4 (4.9)

- **Potential withdrawal events**

The events classified as withdrawal symptoms with an incidence of >2%:

Healthy volunteers

Dizziness 74/308 (24.0%) of nalfurafine hydrochloride group versus 3/135 (2.2%) of controls)

Nausea 22/308 (7.1%) of nalfurafine hydrochloride versus 2/135 (1.5%) of controls)

Vision blurred 7/308 (2.3%) of nalfurafine hydrochloride versus 0 in the controls)

Placebo-controlled HD populations:

Nausea 11/351 (3.1%) in nalfurafine hydrochloride group, compared to 12/308 (3.9%) with placebo

Vomiting 16/351 (4.6%) in nalfurafine hydrochloride group, compared to 0 with placebo

Dizziness 8/351 (3.1%) in nalfurafine hydrochloride group, compared to 4/308(1.3%) with placebo

Long-term open-label study STTOR004:

Nausea 10/146 (6.8%) in nalfurafine hydrochloride group, compared to 3/8 (3.7%) in controls

Vomiting 12/146 (8.2%) in nalfurafine hydrochloride group, compared to 4/81(4.9%) in controls

Dizziness 5/146 (3.4 %) in nalfurafine hydrochloride group, compared to 1/8 (1.3%) in controls

Laboratory findings

Healthy volunteers:

The following PTs occurred at a frequency of ≥2%:

Blood testosterone free decreased, Blood prolactin increased, Blood testosterone decreased, Thyroxine free decreased, Tri-iodothyronine free decreased.

The occurrence of these PTs in the Nalfurafine group exceeded the control group by greater than 2%, suggesting a possible causal relationship.

Placebo controlled HD populations:

The following laboratory parameters were determined in the pivotal EU820UPV01 study:

- Haematology: Red blood cell (RBC) count, haemoglobin (Hb), haematocrit (Hct), white blood cell (WBC) count with differential (%) and platelet counts,lymphocytes, monocytes, neutrophils, eosinophils, basophils, platelets, mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV).

- Biochemistry: sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, glucose, serum creatinine, urea, AST, ALT, gamma-glutamyl-transpeptidase (GGT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), total bilirubin, albumin, blood urea nitrogen (BUN) and total cholesterol.

- Hormones: Testosterone, prolactin and parathyroid hormones were studied in LCRC/G/028. In the active treatment group (N=12), one patient had a clinically significant high values for prolactin and no patients had clinically significant low testosterone levels.

Long-term open-label study STTOR004:

The laboratory investigations in included:

- Serum clinical chemistrv: S-Sodium, S-Potassium, SMagnesium, S-Chloride, S-Urea, S-Calcium (adjusted), Inorganic Phosphate, S-Creatinine, SAlbumin, Alkaline Phosphatase, S-Aspartate Transaminase (S-ASAT, S-GOT), S-Alanine Transaminase (S-ALAT, SGPT), Total bilirubin, S-Lactate Dehydrogenase (S-LDH), Total Cholesterol and Random Glucose.

- Hormones: S-Parathormone (TRK-820 patients, normal lab routine), S-Prolactin and S-Testosterone.

- Haematology: B-Haemoglobin (B-Hb), B-Red Blood Cells (B-RBC), Haematocrit, B-Platelets, B-White Blood Cells (B-WBC), Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes.

According to the Applicant, the change in mean value over time was similar comparing nalfurafine hydrochloride to control group patients in all laboratory parameters. A majority of the clinically relevant chemistry abnormalities was probably caused by renal failure/insufficient dialysis. Other coexisting diseases or conditions (e.g. diabetes mellitus, chronic hepatitis, hyperparathyroidism) explained the remaining cases. The clinically relevant haematology abnormalities were associated with anaemia caused by renal failure (except for one patient treated with antibiotics).

The following PT had an incidence of >2% in the nalfurafine hydrochloride group relating to clinical laboratory tests:

- Anaemia 15/146 (10.3%) in the Nalfurafine group, compared to 5/81 (6.2%) of controls

- Hyperkalaemia 7 (4.8%) in the Nalfurafine group compared to 3 (3.7%) of controls

- Hypercalcaemia 6 (4.1%) in the Nalfurafine group compared to 10 (12.3%) of controls

- Haemoglobin decreased 3/146 (2.1%) in the Nalfurafine group, compared to 0 in the controls.

- Blood parathyroid hormone increased 3/146 (2.1%) in the Nalfurafine group, compared to 1/81 (1.2%) of controls.

Vital signs

Healthy volunteers:

Among the healthy volunteers in the clinical studies there were no clinically important changes in vital signs recorded.

Placebo controlled HD populations:

In the HD patients enrolled into placebo-controlled studies with Nalfurafine, pyrexia was recorded 7/351 (2.0%) in the Nalfurafine treated group, compared to 4/304 (1.3%) of controls. Hypotension and hypertension were more frequent in the placebo group.

Long-term open-label study STTOR004:

The following PTs had an incidence of >2% in the Nalfurafine treated group:

- Hypotension 18/146 (12.3%) in the Nalfurafine treated group, 6/81 (7.4%) of controls

- Hypertension 19/146 (13.0%) in the Nalfurafine treated group, 4/81 (4.9%) of controls

- Pyrexia 7/146 (4.8%) in the Nalfurafine treated group, 3/81 (3.7%) of controls

ECG changes

In the preclinical program it was noted that Nalfurafine had some potential for repolarisation in the hERG assay. This may be a concern as ESRD patients are on multiple drugs and have a propensity to develop cardiac disease and cardiac arrhythmias. A thorough QT/QTc study was conducted in healthy volunteers. This study enrolled 68 healthy male volunteers in a 4 period cross over design. Subjects randomly received 4 treatments (Nalfurafine 5µg, Nalfurafine 20µg, moxifloxacin 400 mg and placebo) in 4 treatment periods.

For the 5 µg Nalfurafine dose, the largest mean time-matched baseline-corrected difference from placebo in QTcF interval was 2.62 msec, occurring 1 hour after dosing. The upper limit of the 2-sided 90% CT was 5.80 msec. For the 20 µg Nalfurafine dose, the largest mean time-matched baseline-corrected difference from placebo in QTcF interval was 6.06 msec, occurring 2 hours after dosing. The upper limit of the 2-sided 90% CI was 9.51 msec. In summary, for both doses, the upper limit of the 2-sided 90% CI around the largest mean baseline-corrected QTcF difference from time-matched placebo was less than 10 msec.

Results of the categorical analysis showed that 1 of 63 subjects (1.6%) had a QTcF interval greater than 450 msec after dosing with 20 µg of Nalfurafine. One of 62 subjects (1.6%) had a QTcF prolongation from baseline greater than 30 msec after dosing with 5 µg of Nalfurafine. No subjects had QTcF changes greater than 60 msec after dosing with Nalfurafine.

In summary, the effects of therapeutic (5 µg) and supra-therapeutic doses (20 µg) of Nalfurafine in healthy volunteers were below the levels of regulatory concern for inducing QTcF changes.

In the HD population the following 12-lead ECG parameters are evaluated by treatment and over time (Weeks 4 and 12 for the Pooled Studies EU820UPV01 and STTOR004 [pooled studies population]). The mean ECG parameters (HR, PR, RR, QRS, QT, QTcF, QTc1, and QTcB) in the pooled studies population were similar for both the Nalfurafine and placebo groups.

In categorical analysis, a notable difference was observed in the percentage of patients with QTcF prolongation at Week 4 pre-dose post-dialysis and post-dose (> 450-480 msec) between the Nalfurafine (11.5% and 11.5%, respectively) and placebo (3.7% and 5.4%, respectively) groups. The reason behind this finding is unknown.

Table 36. QTc Categorical Analysis IV UP Studies – Pooled Studies EU820UPV01 and STTOR004 QT Correction Method:

Time Point		Nalfurafine Toray 5µg (N=391)	Placebo (N=241)	Non-UP Controls[a]
Week 4 (Day 29) Pre-Dialysis	QTc (msec)	n=61	n=19	
	>450- 480	46 (11.8%)	17 (7.1%)	15 (18.5%)
	>480- 500	11 (2.8%)	0	3 (3.7%)
	>500	4 (1.0%)	2 (0.8%)	0
	Change from Baseline in QTc (msec)	n=24	n=14	
	>30- 60	18 (4.6%)	12 (5.0%)	NA
	>60	6 (1.5%)	2 (0.8%)	NA
Week 4 (Day 29) Pre-Dose Post Dialysis	QTc (msec)	n=62	n=11	
	>450- 480	45 (11.5%)	9 (3.7%)	11 (13.6%)
	>480- 500	12 (3.1%)	1 (0.4%)	6 (7.4%)
	>500	5 (1.3%)	1 (0.4%)	1 (1.2%)
	Change from Baseline in QTc (msec)	n=15	n=7	
	>30- 60	12 (3.1%)	6 (2.5%)	NA
	>60	3 (0.8%)	1 (0.4%)	NA

Week 4 (Day 29) 30 Minutes Post-Dose	QTc (msec)	n=64	n=15	
	>450- 480	45 (11.5%)	13 (5.4%)	NA
	>480- 500	13 (3.3%)	1 (0.4%)	NA
	>500	6 (1.5%)	1 (0.4%)	NA
	Change from Baseline in QTc (msec)	n=21	n=11	
	>30- 60	18 (4.6%)	10 (4.1%)	NA
	>60	3 (0.8%)	1 (0.4%)	NA

[a] For non-pruritic control arm, only Week 4 data are available on pre-/post dialysis due to no study drug administration and there is no baseline data due to no Run-in period.

Safety in special populations

Impaired hepatic function: A study with nalfurafine hydrochloride in patients with compensatory hepatic cirrhosis (in the mild Child-Pugh category) has not led to increased exposure or changes in the AE profile. In patients with pruritus associated with liver disease oral nalfurafine hydrochloride was not associated with new adverse events that could not be explained by the mode of administration.

Gender: 63% of the i.v. nalfurafine hydrochloride safety population were male, 27% were female. No gender differences are reported in the safety data.

Race: >98% of the i.v. studies safety population were Caucasian. In healthy volunteers, the changes in testosterone and thyroxine were only seen in the Japanese studies. (High dose i.m. treatment)

Children: Patients <18 years were not included in the studies.

Pregnancy and lactation: There are no data from the use of Nalfurafine Hydrochloride in pregnant women. Studies in animals have shown reproductive toxicity. It is unknown whether Nalfurafine Hydrochloride/metabolites are excreted in human milk.

Elderly: The HD i.v. studies safety population includes patients up to 88 years of age (mean age 57 *years).*

Safety related to drug-drug interactions and other interactions

Common concomitant medications (>10%) in the studies included antianaemic preparations, calcium, Heparin, Vitamin D and analogues, Iron, Platelet aggregation inhibitors, ACE inhibitors, Beta blocking agents, Folic acid, Proton pump inhibitors, Electrolyte solutions, Organic nitrates, Imidazole receptor agonists, Drugs for treatment of hyperkalemia and hyperphosphatemia, Sufonamides, Pyrazolones, H2-receptor antagonists and Vitamin B-complex.

There is the potential for a drug-drug interactions with CYP3A4 inhibitors, e.g. ketoconazole. Patients receiving both ketoconazole and Nalfurafine should be monitored for signs of Nalfurafine toxicity. Two drug-drug interaction studies have been performed, and while no safety issues were raised, a PK drug interaction between Nalfurafine and the CYP3A4 inhibitor, ketoconazole, was found. This study is discussed further in the PK section. In the clinical studies, no AEs anticipated to be due to drug-drug interactions were reported.

Pharmacodynamic interactions may be expected in the target population with heavy background medication, including analgesics, sedatives and antihypertensives.

Discontinuation due to adverse events

No healthy volunteers withdrew from the studies.

Placebo controlled HD populations:

Nalfurafine treated UP HD patients had the study drug discontinued due to adverse events less frequent than placebo treated. The Nalfurafine possible/probably/definitely related to i.v. Nalfurafine non-serious events leading to study drug discontinuation were:

Study EU820UPV01 –insomnia (possible)

Study EU820UPV01 –hallucination/feeling abnormal – 2 separate events both definitely related

Study EU820UPV01 –nightmare (probable)

Study EU820UPV01 –diarrhoea (definite)

Study EU820UPV01 –drug eruption (probable)

Study STTOR002 -dizziness/ hypoaesthesia – 2 separate events both definitely related

Study STTOR002 -vomiting / nausea two separate events – both probable

Long-term open-label study STTOR004:

In the long-term study, 27% of patients (40/146) discontinued study drug administration due to adverse events. In total, 58% of patients (62/146) discontinued. The results of the discontinuation analysis in STTOR004 are difficult to interpret due to the open-label nature and lack of comparability in the control group in the long-term study. The Nalfurafine possible/probably/definitely related non-serious events leading to withdrawal were:

- electrocardiogram QRS Complex Prolonged (possible)

- myasthenia gravis (possible)

- lacrimation Increased, Muscle Spasms (probable)

- hyperhidrosis (probable)

- somnolence (possible)

- paraesthesia (definite)

- headache (definite)

- insomnia (definite)

- insomnia (probable)

- nightmare (probable)

- insomnia (probable)

- vomiting (possible)

- fatigue (definite)

Post-marketing experience

No marketing applications have been approved for *i.m.* or *i.v.* of Nalfurafine Hydrochloride and therefore no data are available.

Oral Nalfurafine Hydrochloride is marketed in Japan under the trade name *Remitch*. As of March 2011, 266 patients with HD UP have been enrolled in a specific use-result survey for Remitch Capsules 2.5 µg. In the survey, the incidence of ADRs in the population was 7.14% (19/266)

2.6.1. Discussion on clinical safety

In placebo controlled studies, the differences between nalfurafine hydrochloride and placebo in frequencies of severe TEAEs or deaths were very small. The treatment-related TEAEs were more common in nalfurafine hydrochloride-treated patients compared to placebo. Vomiting, headache, dizziness, back pain, arteriovenous fistula thrombosis, hypercalcaemia, insomnia and cough were all more common in the nalfurafine hydrochloride treated group, whereas infections and hypo/ hypertension were more common in the placebo treated group. The common withdrawal symptoms were nausea, vomiting, dizziness and blurred vision.

The long-term study STTOR004 had a duration of 52 weeks. Of the 146 patients with UP, 62 (42%) completed the study compared to 78% of the controls. The limited number of HD individuals that were exposed over a longer period hampers the evaluation of possible safety concerns during chronic treatment and issues such as for example the possibility of developing tolerance and potential risk for habituation. The long-term study was not placebo-controlled but made use of a control population of non-pruritic dialysis patients. This is a limitation of the safety database. However, this approach may be conservative, as it is unlikely that the control population is less severely affected by co-morbid conditions. In general, there were substantial differences in TEAEs and serious TEAEs between the groups in the long-term open label study. The differences are likely not only related to the treatment but also to the different populations UP and non-UP.

For nalfurafine hydrochloride, the most common safety issues were gastrointestinal disorders (15.1% vs 11.8% for placebo) and nervous system disorders (15.1% vs 10.9% for placebo) which confirms the findings from the placebo-controlled trials.

The increased frequencies of headache and vomiting persisted during long-term use and 58 % of the patients did not complete the open-label long-term study vs. 22% of controls.

In “severe UP-75” post-hoc meta-analysis of EU820UPV01 and STTOR002 at 4 weeks, statistically significant improvements in sleeping disturbance at week 2 and less reported AEs of infections were reported in this group compared to placebo; however, these were not pre-defined secondary endpoints. Safety in this limited subpopulation has not been addressed separately and is of limited value due to the very small number of patients in this subpopulation.

Nalfurafine hydrochloride increases the prevalence of mild to moderate headache. The overall reporting of dizziness and fatigue was low in clinical studies, probably due to underreporting in both placebo and treatment groups. The statement by the Applicant that these events are not known to alter patient quality of life is not supported by the CHMP.

An increase in insomnia (4.0% of nalfurafine vs 1.6% of placebo treated patients) and other sleep disorders was a consistent finding with the placebo-controlled studies. Sleep disturbances were also the most common AEs that resulted in discontinuation in the nalfurafine hydrochloride treated populations. This finding is contradictory to the potential beneficial effect on sleeping disturbance associated with UP. The Applicant maintained that only vomiting (4.6% of nalfurafine vs 1.3% of placebo treated patients) and nausea have the potential to be a burden on HRQoL. The CHMP acknowledged that the potential impact of adverse events on Quality of Life varies between patients. However, the CHMP concluded that all the common adverse events (especially vomiting), should be considered undesirable in a patient population already heavily burdened by symptomatology of similar kind that affects quality of daily life.

Opioids are known to cause hormonal changes due to depression of hypothalamus/ pituitary function. Animal studies of nalfurafine hydrochloride have shown adverse effects of reproductive organs in rats and dogs. In human studies of healthy volunteers, blood testosterone, thyroxine and tri-iodothyronine decreased. No hormones were measured in the human pivotal study. The hormonal effects of nalfurafine

hydrochloride in haemodialysis patients were not investigated and is considered as a limitation in the main study.

Smaller increases in the frequencies of arteriovenous fistula thrombosis, pyrexia and peripheral oedema were reported in the Nalfurafine treated populations both in long-term and placebo-controlled studies. This is a concern as it may be related to the drug i.v. administration. In both the long-term and placebo controlled studies the incidence of AV fistula thrombosis is approximately twice that of the placebo/control groups (18 vs. 7 cases), making the chance finding an explanation unlikely. The i.v. infusion of study medications was given into a distant catheter attached to the patient's fistula since any intervention to hemodialysis patient's fistula may potentially increase the risk of fistula-related complications. This is a serious complication for haemodialysis patients.

In the long-term study adverse events, there was a substantial increase in the SOC Injury and procedural complications, 29.5% in the nalfurafine hydrochloride group compared to 17.3% in controls. This was mainly due to different arteriovenous fistula and other shunt complications (approximately 7% more common in the treatment group) and different trauma such as fractures (approximately 6% more common in the treatment group). Both of these serious AE may be treatment-related. The potential local effects on blood vessels of nalfurafine hydrochloride are not known.

The common CNS adverse events such as dizziness, vertigo and somnolence may increase the risk of trauma in long-term use of nalfurafine hydrochloride as they are known to increase the risk of falls.

In healthy volunteers, blood testosterone, thyroxine and tri-iodothyronine decreased. This is in line with animal studies which identified an issue in the reproduction studies. No hormones were studied in the pivotal study and the results from the long-term study were not summarised. The hormonal effects of nalfurafine hydrochloride on haemodialysis patients are, thus, unclear. Therefore, the CHMP concluded that hormonal effects are a potential risk for nalfurafine hydrochloride.

Further to the review of the deaths reported in the long-term study STTOR004, the CHMP agreed that the deaths were unlikely to be treatment-related.

Narratives of the 6 treatment related SAEs in the i.v. formulation of Nalfurafine Hydrochloride were reviewed by the CHMP. The relationship of these 6 SAEs with Nalfurafine is unclear as these SAEs are not clearly explained by the toxicology results and have other possible explanations such as concurrent disease and medications. It is agreed that they were all possibly but not definitely related to the treatment. Two of these were associated with hypercalcaemia.

A total of 611 HD patients were exposed to at least one dose of p.o. Nalfurafine hydrochloride. The Applicant was asked to summarise the causes of death and types of SAEs in the Japanese ORAL formulation of nalfurafine Hydrochloride treated populations. The narratives for deaths and related SAEs were provided and the analysis of these data did not show any major concern related to NFU.

A thorough QT/QTc study of i.v. nalfurafine hydrochloride in healthy volunteers did not raise concerns. In HD patients QTcF prolongation at Week 4 was observed. However, a notable difference in the percentage of haemodialysis patients with QTcF prolongation >450 ms was observed in the Nalfurafine-treated group (16%) compared to placebo (5%) at week 4. The reason behind this finding is unknown. Cardiac disorders or deaths were not overrepresented in the nalfurafine hydrochloride-treated patients. The prolongation was observed both pre- and post-dose. The possibility that nalfurafine hydrochloride has cardiac QTcF prolongation effects that increase with treatment duration in HD patients is acknowledged by the CHMP.

A total of 611 HD patients were exposed to at least one dose of p.o. Nalfurafine hydrochloride. The Applicant was asked to summarise the causes of death and types of SAEs in the Japanese ORAL formulation of nalfurafine Hydrochloride treated populations. The narratives for deaths and related SAEs were provided and the analysis of these data did not show any major concern related to NFU.

Pharmacodynamic interactions can occur when nalfurafine hydrochloride is administered with other sedatives.

2.6.2. Conclusions on the clinical safety

For nalfurafine hydrochloride, the most common safety issues were gastrointestinal disorders (15.1% vs. 11.8% for placebo) and nervous system disorders (15.1% vs. 10.9% for placebo). These adverse events are those expected from the pharmacological class and include insomnia, dizziness, fatigue, somnolence, headache, vertigo, nausea, upper abdominal pain and vomiting. In addition, both hypo- and hypertension may occur. The increased frequencies of headache and vomiting persisted during long-term use and 58 % of the patients did not complete the open-label long-term study vs. 22% of controls. Nalfurafine hydrochloride also increases the prevalence of mild to moderate headache. In addition, the overall low reporting of dizziness and fatigue in clinical studies is probably due to underreporting in both placebo and treatment groups. The increase of insomnia with Nalfurafine (4.0% of nalfurafine vs. 1.6% of placebo treated patients) is contradictory to the potential beneficial effect on sleeping disturbance associated with UP.

CNS adverse events may explain the increased frequency of fractures in nalfurafine hydrochloride treated patients compared to controls in the long-term study.

In both the long-term and placebo controlled studies the incidence of AV fistula thrombosis in NFU-treated patients was approximately twice that of the placebo/control groups (18 vs. 7 cases). The i.v. infusion of study medications were given into a distant catheter attached to the patient's fistula.

A thorough QT/QTc study in healthy volunteers did not raise safety concerns. However, a notable difference in the percentage of hemodialysis patients with QTcF prolongation >450 ms was observed in the Nalfurafine-treated group compared to placebo at week 4. The reason behind this finding is unknown..

Opioids are known to cause hormonal changes due to depression of hypothalamus/ pituitary function. Animal studies of nalfurafine hydrochloride have shown adverse effects of reproductive organs in rats and dogs. In human studies of healthy volunteers, blood testosterone, thyroxine and tri-iodothyronine decreased. No hormones were measured in the human pivotal study.

These adverse reactions must be considered undesirable in a patient population already heavily burdened by symptomatology of similar kind that affects quality of daily life.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

PRAC Advice

Based on the PRAC review of the Risk Management Plan version 3.0, the PRAC considers by consensus

that the risk management system for Nalfurafine hydrochloride (Winfuran) for the treatment of severe uraemic pruritus (UP) in adults, with end stage renal disease undergoing regular haemodialysis, haemodiafiltration or haemofiltration (dialysis) could be acceptable provided an updated risk management plan and satisfactory responses to the following List of Questions is submitted:

1. Use in elderly should be added to the RMP as missing information.
2. The Elements of Public summary need further revision: the 5th paragraph of section VI.2.1 should be moved to the section VI.2.2; sections VI.2.2 – VI.2.4 should be filled in; section VI.2.VI should not refer to Table 43 in other part of the RMP but include List of studies in post authorisation development plan.

This advice is based on the following content of the Risk Management Plan:

- **Safety concerns**

Summary of safety concerns	
Important identified risks	• Sleep disorders including insomnia, somnolence, poor quality sleep and nightmares • Nausea and vomiting • Interaction with CYP3A4 inhibitors and inducers; interaction with substrates mediated by MDR1
Important potential risks	• QTc Prolongation • AV Fistula thrombosis • Fractures • Emotional Disorders • Alteration of plasma hormones: Prolactin increased, testosterone decreased, thyroid hormones decreased • Hyperkalaemia • Interaction with sedatives • Off-label use • Potential for overdose / Medication errors
Missing information	• Safety data in paediatric patients • Safety in patients with moderate or severe hepatic impairment • Safety data in pregnancy and lactation

Abbreviations: AV =Arteriovenous; CYP = cytochrome P450; MDR1= multi-drug resistance 1.

The PRAC considered that use in the elderly should also be considered as missing information.

- **Pharmacovigilance plans**

Activity / Study title	Objectives	Safety concerns addressed	Status (planned)	Date for submission of Interim or final Reports (planned)
A two-week multicentre, randomised, double-blind, placebo-controlled, parallel group clinical efficacy, safety and PK study of Winfuran in ESRD patients on regular haemodialysis (three times per week)	To assess the clinical efficacy and PK of Winfuran in ESRD patients on regular haemodialysis (three times	Safety in a nonclinical trial setting	Protocol Synopsis available To be finalised during marketing authority review	Estimated:

with severe UP, will be performed as a post approval commitment subsequent to granting of the Conditional MA	per week) with severe UP		No DSMB planned, therefore no interim data review	

Abbreviations: DSMB= Data Safety Monitoring Board; ESRD=end stage renal disease;MA=Marketing Authorisation, PK=pharmacokinetic; UP=uraemic pruritus.

The PRAC, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

- **Risk minimisation measures**

Table 2.4: Summary table of Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Important identified risks		
Sleep disorders including insomnia, somnolence, poor quality sleep and nightmares	In the SmPC Section 4.8 Undesirable Effects, insomnia and somnolence are listed as a common side effects occurring in 1/10 to 1/100 patients. In the SmPC Section 4.8 Undesirable Effects, nightmares, sleep disorder and poor quality sleep are listed as an uncommon side effects occurring in >1/1000 to <1/100 patients. Since insomnia can also lead to daytime sleeping Section 4.7 of the SmPC also warns of possible effects on the ability to drive or operate machinery: “Winfuran has a moderate influence on the ability to drive and use machines. Dizziness and somnolence are common adverse drug reactions in subjects taking Winfuran, therefore patients should be informed that Winfuran may affect their ability to drive or use machines”	None
Nausea and vomiting	In the SmPC Section 4.8 Undesirable Effects, nausea and vomiting are listed as common side effects occurring in 1/10 to 1/100 patients.	None
Interaction with CYP3A4 inhibitors and inducers; interaction with substrates mediated by MDR1	SmPC Section 4.5 (Interaction with other Medicinal Products and Other forms of Interactions) provides elaboration and guidance on potential interactions with Winfuran, including interactions with CYP3A4 inhibitors (ketoconazole) and inducers, and MDR1 substrates.	None
Important potential risks		
QTc Prolongation	In the SmPC Section 4.8 Undesirable Effects, Electrocardiogram QT prolonged is listed as uncommon side effects occurring in 1/100 to 1/1000 patients.	None

	In the Winfuran SmPC Section 4.8 Undesirable Effects, other cardiac disorders including *palpitations, angina pectoris, bundle branch block right, cardiac failure, cardiac failure congestive and tachycardia* are also listed as uncommon side effects occurring in >1/1000 to <1/100 patients.	
AV Fistula thrombosis	SmPC Section 6.6 gives detailed instructions on the appropriate administration of Winfuran.	Education for patients not to compress the fistula after Winfuran administration
Fractures	None	None
Emotional Disorders	'emotional disorder, hallucination', is listed as an uncommon side effect occurring in ≥ 1/1000 to ≤1/100 patients in the SmPC Section 4.8 (Undesirable Effects).	None
Alteration of plasma hormones: Prolactin increased, testosterone decreased, thyroid hormones decreased	None	None
Hyperkalaemia	None	None
Interaction with sedatives	Section 4.5 (Interaction with other Medicinal Products and Other forms of Interactions) provides elaboration and guidance on potential interactions with Winfuran, including interactions with CYP3A4 inhibitors (Ketoconazole) and inducers.	None
Off label use	Section 4.1 (Therapeutic Indications) clearly states that Winfuran is indicated for the treatment of severe uraemic pruritus (UP) in adults, with end stage renal disease undergoing regular haemodialysis, haemodiafiltration or haemofiltration (dialysis). Severe UP is defined as intolerable itching or itching unrelieved by scratching.	None
Potential for overdose / Medication errors	Section 6.6 of the Winfuran SmPC contains clear instructions on how to prepare and administer the product appropriately. No cases of overdose with Winfuran have been reported, however, Section 4.9 (Overdose) of the Winfuran SmPC contains guidance on how to manage the patient in the event of an overdose by consideration of additional dialysis.	None
Missing information		
Safety data in paediatric patients	An appropriate statement has been added to Section 4.2 (Posology and Method of Administration) stating that the safety and efficacy of Winfuran in children aged 0-17 years has not yet been established. No data are available.	None
Safety in patients with moderate or severe hepatic impairment	•Section 4.4 (Special Warnings and Precautions for Use) of the Winfuran SmPC contains the following statement: *"Hepatic impairment Analysis of pharmacokinetics (PK) in patients with mild hepatic impairment*	None

	(Child-Pugh grade A-B) showed no significant change in PK. This finding suggests that Winfuran is safe to administer to patients with mild hepatic impairment. Caution should be exercised when treating patients with moderate to severe hepatic disease due to limited clinical experience in this population". Section 4.2 (Posology and Method of Administration) also includes the following statement: *"Hepatic impairment "No dose adjustment is necessary in patients with mild hepatic impairment"*	
Safety data in pregnancy and lactation	An appropriate statement has been added in Section 4.6 of the Winfuran SmPC Pregnancy There are no data from the use of nalfurafine hydrochloride in pregnant women. Studies in animals have shown reproductive toxicity (see Section 5.3). Winfuran is not recommended during pregnancy. Women of childbearing potential There are no data from the use of nalfurafine hydrochloride in women of childbearing potential not using contraception, even studies in animals have not shown teratogenicity (see Section 5.3). Women of childbearing potential have to use effective contraception during treatment. Breast-feeding A radiolabel study in pregnant rats showed that nalfurafine hydrochloride was excreted in breast milk. It is unknown whether nalfurafine hydrochloride or its metabolites are excreted in human milk. A risk to the suckling child cannot be excluded. A decision must be made whether to discontinue breast-feeding or to discontinue/abstain from Winfuran therapy taking into account the benefit of breast-feeding for the child and the benefit of therapy for the woman. Fertility There are no data on the effect of nalfurafine hydrochloride on human fertility. Studies in animals have shown a decrease in fertility and implantation rates (see Section 5.3).	None

Abbreviations: AV = arteriovenous; CYP = cytochrome P450; MDR1 = multi-drug resistance; PK = pharmacokinetic; SmPC = Summary of Product Characteristics; UP = uraemic pruritus.

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

The CHMP, having considered the data submitted in the application was of the opinion that it was not appropriate to consider risk minimisation activities at this time.

2.9. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

In the post-hoc UP-75 subpopulation of the pivotal study, the difference in VAS was 7.5 mm in favour of Winfuran which is considered modest and the clinical relevance of this finding is questioned.

In the post-hoc meta-analysis of patients in clinical studies EU820UPV01, STTOR002 and STTOR003 who fulfilled the subgroup UP-75 criteria, a ≥20 mm improvement from baseline was seen at 4 weeks both in the active treatment and placebo groups. The mean difference between active treatment and placebo was 8.9 mm and of borderline statistical significance (p=0.055).

In "severe UP-75" post-hoc meta-analysis of EU820UPV01 and STTOR002 at 4 weeks, statistically significant improvements in sleeping disturbance at week 2 and less reported AEs of infections were reported in this group compared to placebo; however, these were not pre-defined secondary endpoints.

Uncertainty in the knowledge about the beneficial effects

The primary endpoint in the pivotal study of 8 weeks was the change in worst itching intensity (VAS) from baseline to week 4. Both placebo and treatment with nalfurafine hydrochloride 5 µg i.v. 3 times a week equally reduced worst itching intensity (VAS) by approximately 22 mm at 4 weeks. The mean difference between active treatment and placebo was 7.5 mm and not statistically significant (p=0.08). There were no significant differences between placebo and active treatment in important secondary endpoints such as sleep disturbances and excoriations, either. Responder analyses did not reveal beneficial effects compared to placebo.

The other smaller and shorter duration clinical trials in the i.v. Nalfurafine program, STTOR002 and STTOR003 also failed to demonstrate a statistically significant difference to placebo in the primary endpoint.

The Applicant provided post-hoc analyses in subgroups of patients with more severe UP after unblinding of the data (UP-66 and UP-75 population). Further to the CHMP request to justify the clinical meaningfulness of the presented UP-66 population (VAS ≥ 60 mm but ≤ 90 and itching intensity that was grade 4 or grade 5, the Applicant proposed to focus on the UP-75 population only.

In the pivotal study (EU820UPV01), a 20 mm improvement from baseline was seen at 4 weeks both in the active treatment and placebo groups. The mean difference between active treatment and placebo was 7.5 mm and not statistically significant (p=0.08). A significant treatment effect was not supported by any of the pre-defined secondary endpoints in the main analysis at week 4. From the Severe UP-75 analysis it was observed that patients with baseline worst itching VAS scores of 90 – 100 mm had very small or no effects following treatment.

The Applicant provided further post-hoc analyses, i.e. a meta-analysis of patients in clinical studies EU820UPV01, STTOR002 and STTOR003 who fulfilled the subgroup UP-75 criteria. In these analyses, a ≥ 20 mm improvement from baseline was seen at 4 weeks both in the active treatment and placebo groups. The mean difference between active treatment and placebo was 8.9 mm and of borderline statistical

significance (p=0.055). The relevance of the size of the borderline significant effect, 8.9 mm difference in VAS compared to placebo, is unclear, as there are no formal guidelines defining a minimum clinically important difference in patients with UP at a group level. After the first 4 treatment weeks, no differences between nalfurafine hydrochloride and placebo were demonstrated in any subpopulations. The results may be affected by the nature of uraemic pruritus, a chronic condition that may have a cyclic course and vary greatly in intensity at individual level. In addition, all placebo-controlled studies demonstrated a substantial beneficial placebo-effect. This could be explained by VAS-value regression to the mean, as only the patients with highest VAS ratings in the short run-in period were included. However, taking all of the above into account, the CHMP is of the opinion that the efficacy of Nalfurafine hydrochloride is not properly or sufficiently demonstrated.

Regarding the UP-66 population, which encompassed just the severe UP patients by excluding very severe pruritus patients (baseline Worst Itching VAS value of ≥ 90 mm) from the UP-75 since these patients were identified not to benefit from the treatment, this was not considered as clinically meaningful by the CHMP as this population was not distinctly different from the total population.

The CHMP was of the opinion that the adequacy of performing exploratory post-hoc analyses in a selected subgroup (24%) considering that no effect was shown in the overall population in the pivotal study, have not been adequately justified. The results are considered at best hypothesis generating. The efficacy results are of limited value at this stage and are not sufficient to support a Marketing Authorisation.

Risks

Unfavourable effects

For nalfurafine hydrochloride, the most common safety issues were gastrointestinal disorders (15.1% vs. 11.8% for placebo) and nervous system disorders (15.1% vs. 10.9% for placebo). These adverse events include insomnia, dizziness, fatigue, somnolence, headache, vertigo, nausea, upper abdominal pain and vomiting. In addition, both hypo- and hypertension may occur. The increased frequencies of headache and vomiting persisted during long-term use and 58 % of the patients did not complete the open-label long-term study vs. 22% of controls.

Uncertainty in the knowledge about the unfavourable effects

In both the long-term and placebo controlled studies the incidence of AV fistula thrombosis in nalfurafine hydrochloride treated patients is approximately twice that of the placebo/control groups (18 vs. 7 cases). The i.v. infusion of study medications were given into a distant catheter attached to the patient's fistula. Shunt related events were also overrepresented in placebo-controlled oral nalfurafine hydrochloride studies but the reason is unknown.

There was a numerical increase in fractures in nalfurafine hydrochloride treated patients compared to controls in the long-term study.

A thorough QT/QTc study in healthy volunteers did not raise concerns. However, a notable difference in the percentage of hemodialysis patients with QTcF prolongation >450 ms was observed in nalfurafine treated group (16%) compared to placebo (5%) at week 4. The reason behind this finding is unknown. Cardiac disorders or deaths were not overrepresented in nalfurafine hydrochloride treated patients.

Opioids are known to cause hormonal changes due to depression of hypothalamus/ pituitary function. Animal studies of nalfurafine hydrochloride have shown adverse effects of reproductive organs in rats and dogs. In human studies of healthy volunteers, blood testosterone, thyroxine and tri-iodothyronine decreased. No hormones were measured in the human pivotal study. The hormonal effects of nalfurafine hydrochloride in hemodialysis patients were not investigated in the main study.

The safety in UP-75 subpopulation has not been addressed separately but is of limited value due to the very small number of patients in this subpopulation.

Benefit-risk balance

Importance of favourable and unfavourable effects

Nalfurafine hydrochloride had no favourable effects on itching intensity, sleep disturbances, daytime feeling or excoriations compared to placebo in the full analysis set of the pivotal study. The complex definitions of sub-populations used in ad-hoc analyses are not considered sufficient due to post-hoc nature of the findings.

Common adverse effects such as dizziness, headache, nausea and vomiting must be considered as undesirable in a patient population already heavily burdened by symptomatology of similar kind. Further, insomnia is especially undesirable as sleep disturbances are a major problem in UP patients. A potential increase in arterious fistula thrombosis is a concern for patients on haemodialysis.

Benefit-risk balance

Discussion on the benefit-risk balance

There is an unmet need for new treatments of uraemic pruritus. However, nalfurafine hydrochloride did not relieve itching in the primary analyses of the clinical studies. Results from meta-analysis in narrow sub-populations defined after unblinding the data are not accepted.

The main efficacy studies failed to demonstrate a benefit of nalfurafine therapy over placebo (p= 0.93 for the primary endpoint).

In the long-term open-label study STTOR004 (52 weeks study), itching intensity was markedly reduced during the study period. However, only 42% of the patients were analysed at the end of the study at week 52. It was not possible to distinguish between placebo-effect, a natural course of pruritus over time and an effect of Nalfurafine hydrochloride. Consequently, only limited conclusions can be drawn from the data.

The exploratory post-hoc analyses in a selected subgroup (24%) and meta-analysis of subgroups across different studies are considered at best hypothesis generating and is not considered to support either a full nor a conditional marketing authorisation.

The treatment effect compared to placebo with respect to the primary endpoint in the post hoc subgroup analysis (UP75 population) is modest and of borderline statistical significance. The clinical relevance of the results has not been demonstrated and should be further justified.

The negative benefit-risk balance is based on almost total absence of demonstrable benefit in controlled trials. In this light, any side effects are unacceptable.

4. Recommendations

Outcome

New Active Substance Status

In light of the negative recommendation, CHMP is of the opinion that it is not appropriate to conclude on the new active substance status at this time.

Paediatric Data

N/A

Based on the CHMP review of data on quality, safety and efficacy for Winfuran in the treatment of severe uraemic pruritus (UP) in patients of 18 years of age or older, with end stage renal disease undergoing regular haemodialysis, haemodiafiltration or haemofiltration (dialysis), the CHMP considers by majority decision that the efficacy of the above mentioned medicinal product is not sufficiently demonstrated:

- The main efficacy studies failed to demonstrate a benefit of nalfurafine therapy over placebo (p= 0.93 for the primary endpoint). The exploratory post-hoc analyses in a selected subgroup (24%) and meta-analysis of subgroups across different studies are considered at best hypothesis generating and is not considered to support either a full nor a conditional marketing authorisation.
- The treatment effect compared to placebo with respect to the primary endpoint in the post hoc subgroup analysis (UP75 population) is modest and of borderline statistical significance. The clinical relevance of the results has not been demonstrated and should be further justified.

The CHMP therefore considers by majority decision that the benefit-risk balance is negative and, therefore recommends the refusal of the granting of the conditional Marketing Authorisation for the above mentioned medicinal product.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan and follow-up measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

A divergent position to the majority recommendation is appended to this report.

Divergent Positions

The undersigned members of the CHMP did not agree with the CHMP's opinion recommending the refusal of the granting of a Marketing Authorisation for Winfuran.

The reasons for divergent opinion were as follows:

Whereas

- Despite the fact that the main efficacy studies failed to demonstrate a benefit of nalfurafine therapy over placebo, the exclusively high placebo effect in this study on the one hand (diminishing the difference over placebo effect due to the inclusion of ineffective population with non-severe pruritus severity into the analysis) and the repeatedly constant positive trends in verum effect in rather small study populations on the other hand note the constant activity shown in clinical studies.
- The post-hoc analysis in a subgroup of severe and very severe patients population (named as UP-75) that is defined as Worst Itching VAS>60mm together with itching score 4 (unrelieved by scratching) or 5 (intolerable) showed clinically relevant difference in effect size over placebo of ~9 mm in changing worst itching VAS from baseline. That confirms the findings observed already previously in a comparable severity patient population (N=337) in study 820UPC04 with a comparable effect size over placebo (8.3 mm).
- The observed effect size (difference over placebo) in severe and very severe UP-75 population of 8.92 mm seems marginal although not unwelcome in the circumstances of unmet medical need.
- The safety profile is rather benign.
- The manageability of the risk of ineffective treatment can be easily managed in clinical practice through routine medical care.
- The long term (52 weeks) effects in a UP-75 population show clear tendencies of better efficacy in changing worst itching VAS from baseline as compared to non UP-75 population where no clear difference is observed between comparative arms. This could be seen as supporting the efficacy shown in UP-75 population during short term (4 weeks) period.
- Further clarification of the efficacy over placebo should be performed in a prospective randomised study to be performed as a post-authorisation commitment under conditional marketing authorization, specifying the study design to be agreed by the CHMP, including stopping criteria for either futility or efficacy reasons.

Therefore the undersigned are of the opinion that pursuant to Article 12 of Regulation (EC) No 726/2004, the efficacy of the above mentioned medicinal product is sufficiently demonstrated to grant a conditional marketing authorization for Winfuran.

London, 19 December 2013

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Romaldas Mačiulaitis Natalja Karpova