SCIENTIFIC DISCUSSION

1. Introduction

1.1 Problem statement

Hereditary tyrosinaemia type 1 (HT-1) is a devastating inherited disease, mainly of childhood. It is characterised by severe liver dysfunction, impaired coagulation, painful neurological crises, renal tubular dysfunction and a considerable risk of hepatocellular carcinoma (Weinberg et al. 1976, Halvorsen 1990, Kvittingen 1991, van Spronsen et al. 1994, Mitchell et al. 1995). The condition is caused by an inborn error in the final step of the tyrosine degradation pathway (Lindblad et al. 1977). The incidence of HT-1 in Europe and North America is about one in 100,000 births, although in certain areas the incidence is considerably higher. In the province of Quebec, Canada, it is about one in 20,000 births (Mitchell et al. 1995). The mode of inheritance is autosomal recessive.

The primary enzymatic defect in HT-1 is a reduced activity of fumarylacetoacetate hydrolase (FAH) in the liver, the last enzyme in the tyrosine degradation pathway. As a consequence, fumaylacetoacetate (FAA) and maleylacetoacetate (MAA), upstream of the enzymatic block, accumulate. Both intermediates are highly reactive and unstable and cannot be detected in the serum or urine of affected children. Degradation products of MAA and FAA are succinylacetone (SA) and succinylacetoacetate (SAA) which are (especially SA) toxic, and which are measurable in the serum and urine and are hallmarks of the disease.
SA is also an inhibitor of Porphobilinogen synthase (PBG), leading to an accumulation of 5-aminolevulinate (5-ALA) which is thought to be responsible for the neurologic crises resembling the crises of the porphyrias.

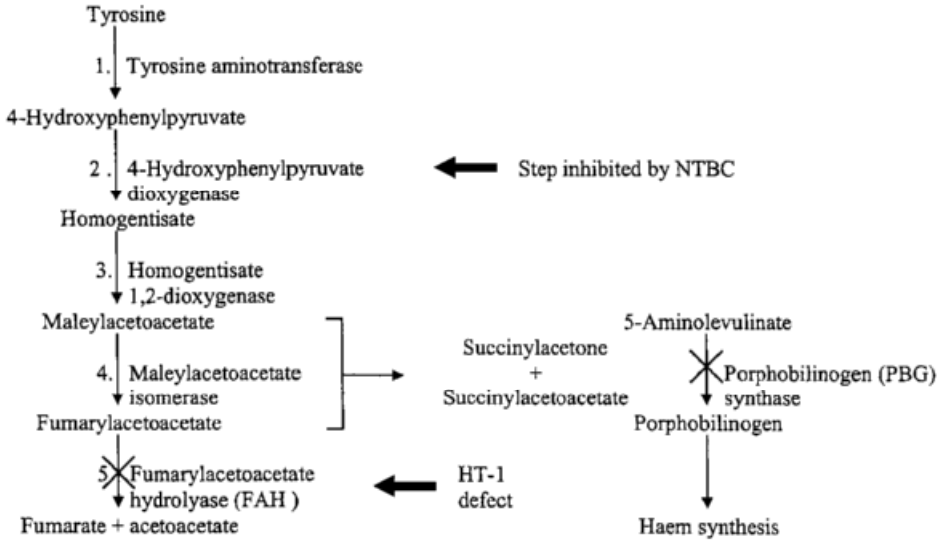


Figure 1.1. The degradation pathway of tyrosine with the HT-1 defect indicated, together with the influence of the accumulated metabolites on the porphobilinogen synthesis.

The accumulation of toxic metabolites starts at birth and the severity of phenotype is reflected in the age of onset of symptoms (Halvorsen 1990, van Spronsen et al. 1994). In the *acute form*, which is the most common, patients develop signs of hepatic failure during the first weeks or months of life. There is failure to thrive, vomiting, diarrhoea and fever. Melena and epistaxis are frequent. If untreated, death from liver failure usually occurs within 2-8 months (Goldsmith and Laberge, 1989). In the *sub-acute form* the clinical course is less rapid than in the acute form, although the symptoms are similar, albeit less severe. This form is characterised by chronic progressive liver disease and renal tubular dysfunction (the Fanconi syndrome) with hypophosphatemia and rickets (Kvittingen 1991). Patients may have recurrent porphyria-like crises with respiratory failure and hepatocellular carcinoma develops in up to 40% of patients (Kvittingen 1991). Both are responsible for death, which usually occurs during the first decade (van Spronsen et al. 1994). In the

chronic form, the progress of the disease is slow with rickets and tubulopathy as the most prominent findings (Halvorsen 1990).

The treatment strategy of HT-1 includes dietary restriction of phenylalanine and tyrosine and orthotopic liver transplantation (Mitchell et al. 1995). However, the diet does not prevent the progression of the disease. The major concerns about liver transplantation are the peri-operative morbidity and mortality, the potential hazards of chronic immunosuppressive therapy, the psychological stress of the procedure and the difficulties of obtaining a suitable donor liver. In addition, there are particular problems transplanting a child with acute HT-1 in severe liver failure. However, when the transplantation is successful, the disease is cured.

1.2 About the product

Nitisinone or NTBC (2-(2- nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) inhibits the enzyme 4-hydroxy phenylpyruvate dioxygenase (HPPD), i.e. the second step in tyrosine degradation both in vitro and in animals. This has only been indirectly verified in man. HPPD enzyme is located 'upstream' in the tyrosine degradation pathway with respect to the enzyme fumarylacetoacetate hydrolase, the last step in tyrosine degradation which is deficient in patients suffering from HT-1. HPPD inhibition prevents the accumulation of maleylacetoacetate and fumarylacetoacetate, which occurs in HT-1 patients as a consequence of their inherited defect of fumarylacetoacetate hydroxylase activity. Maleylacetoacetate and fumarylacetoacetate are toxic and reactive metabolites and their accumulation may be related to the development of hepatocellular carcinomas seen in HT-1 patients (Endo et al., J Biol Chem 1997, 272:24426-32). They are partially converted to succinylacetone and succinylacetoacetate, which inhibit the porphobilinogen(PBG)-synthase activity in the heme synthesis pathway. This leads to accumulation and increased excretion in the urine of 5-aminolevulinate (5-ALA).
Nitisinone treatment leads to normalised porphyrin metabolism with normal erythrocyte porphobilinogen synthase activity and urine 5-ALA and decreased urinary excretion of succinylacetone.
However, as in animal experiments, plasma tyrosine and phenolic acid excretion increased in all patients treated with nitisinone.Therefore nitisinone is used in combination with a strict low protein diet, supplemented with an amino acid formula deficient in phenylalanine and tyrosine.

1.3 The development programme

Nitisinone was originally developed as a herbicide in the 1980s, but abandoned when it was found to cause eye lesions in rats. Subsequently this was shown to be related to high plasma-levels of the amino acid tyrosine. Most of the non-clinical data in the present submission derive from studies conducted at that time with technical grade material containing 90-92% nitisinone. The proposed drug substance is manufactured by the same route and processes as the would-be herbicide except for a modification of the last purification step to yield a substance with purity ≥ 98%. Since technical grade nitisinone tested positive in some early genotoxicity studies, these tests were repeated with the purified drug substance. When development of nitisinone as a herbicide was discontinued, ongoing animal studies were halted and not reported, although the main findings and conclusions were summarised in interoffice memoranda. The Applicant has subsequently prepared more detailed reports on four repeat-dose toxicity studies based on such memoranda and the available raw data.

Efficacy evaluation is based on a large compassionate use program open to all patients with HT-1.
The promising results of a pioneering study on the first five patients with HT-1 treated with nitisinone were published in 1992. A worldwide study was then started (the NTBC Study), co-ordinated by the team from Sahlgrenska University Hospital (SU), Gothenburg, Sweden, to document the effects of nitisinone treatment. On request, nitisinone was distributed from SU to hospitals all over the world on a compassionate use basis. The physicians/investigators sent blood and urine samples for analysis to SU at regular intervals, according to a protocol designed by the research team at SU, together with results of local laboratory tests and clinical information. The NTBC Study period was from February 1991 to August 1997. The patients continued the treatment after the study on compassionate use basis. Other patients not included in the NTBC study have been also treated with nitisinone. From late 1994 the distribution of nitisinone was gradually shifted from SU to Swedish Orphan AB, Stockholm, Sweden.

The clinical documentation in the present application consists mainly of the analysis of the results of the NTBC Study. The safety analysis also includes those patients given nitisinone on compassionate use basis but not participating in the NTBC Study. The cut off date was December 2003. In addition, one pharmacokinetic study on 10 healthy adult volunteers has been completed.

The pharmaceutical processes and facilities for both drug manufacturing and finished product comply with GMP rules.

With the exception of a comprehensive battery of genotoxicity tests and reproduction toxicity tests, none of the safety studies of nitisinone meet statutory GLP provisions (Directive 2001/83/EC, Annex I, and Council Directives 87/18/EEC and 88/320/EEC). However, in view of the rare occurrence and seriousness of the disease, the lack of therapeutic alternatives and the obvious clinical efficacy, the CHMP was of the opinion that the limited non-clinical investigations should not preclude a recommendation for a marketing authorisation under exceptional circumstances.

The NTBC study was not performed according to the GCP rules, was never monitored and the reporting was inconsistent and decreased over time.

2. Quality aspects

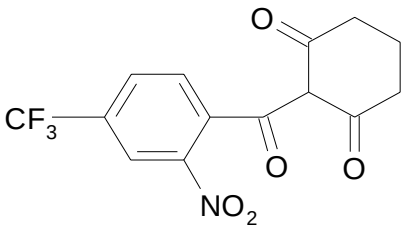
Introduction

Orfadin is presented as hard capsules containing 2mg, 5mg and 10mg of nitisinone as active substance. Other ingredients are pregelatinised starch (maize)**,** and the capsules contain gelatine and colouring agents.

The capsules are packed in HDPE bottle sealed with a LDPE cap.

Drug Substance

Nitisinone is a white to yellowish-white crystalline powder poorly soluble in water. The active substance is a weak acid and it is highly soluble in the pH range 4.5-7.2 in phosphate buffer solutions. Nitisinone has the chemical name 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione. It does not show polymorphism.



- Manufacture

Nitisinone is synthesized in two main steps followed by isolation and purification of the active substance. Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents, have been presented. A reprocessing procedure is performed in case of non-compliance of the product.

Batch analysis data are provided for 6 batches produced with the proposed synthetic route. Such data show that the active substance can be manufactured reproducibly.

- Specification

The active substance specification includes tests for appearance, identity (IR, melting point DSC), assay (98.5-101.0%, HPLC), related substances (HPLC), loss on drying, sulphated ash, heavy metals, residual solvents (GC), chlorides, and microbiological contamination.

The specifications reflect all relevant quality attributes of the active substance. The analytical methods used in the routine controls are suitably described. The validation studies are in accordance with the ICH Guidelines. Impurity limits in the specification are justified by toxicology studies.

- Stability

Stability studies (long term 25°C ± 2°C/60% ± 5% RH; accelerated 40°C ± 2°C/75% ± 5% RH) have been performed in three production batches of the active substance. Such studies, performed in accordance with current ICH guidelines, were conducted on the drug substance proposed packaging for storage and distribution of the active substance.
Tested parameters, particularly those relative to "related substances" (HPLC) and "assay" (HPLC) are suitable to detect significant changes in the quality of the product. Analytical methods were those described for the control at release.
A photostability study, performed according to ICH guidelines, has also been presented; tests and analytical methods are those used in the stability studies.
The data presented suggest that nitisinone is a stable and not light-sensitive compound.
Therefore, the data provided are sufficient to confirm the proposed re-test period.

Drug Product

- Pharmaceutical Development

The intrinsic physico-chemical properties of the active substance were taken into account for the development of an oral solid formulation.

During the development of the product different excipients were used. A sufficiently stable preparation was obtained using pregelatinised starch as single excipient (diluent).

Some batches used for the clinical trials contain other excipient instead of pregelatinised starch. The dissolution profiles of both formulations showed that it is not likely that the change in excipient would have any influence on bioavailability.

Pregelatinised starch is the only excipient for the powder mixtures. Gelatin and titanium dioxide (E 171) are used for the capsules shell, and antifoam, iron oxide black (E 172), shellac, soya lecithin are used in the imprint. All the excipients comply with the Ph. Eur. The bovine gelatin used is in compliance with the European Commission Decision 2001/2/EC regulating the use of material presenting risks as regards transmissible spongiform encephalopathy. The components of the ink have been properly specified. The excipients have been chosen based on their function and on their compatibility with the active substance and with each other.

HDPE bottles sealed with tamper proof LDPE caps are used as primary packaging. All materials comply with Eur Ph and are adequate to support the stability and use of the product.

- Manufacture of the Product

The manufacture comprises (1) grinding and sieving of active ingredient, (2) mixing of active ingredient with pregelatinised starch (3) capsule filling, (4) transfer of the capsule to a bulk container, and (5) filling/labelling of the container and packaging.

The manufacturing process has been validated by a number of studies for the major steps of the manufacturing process in 5 production-scale batches (three consecutive batches of 2 mg and one batch of 5 mg and 10 mg capsules)**.** The manufacturing process has adequately been validated and is satisfactory. The in process controls are adequate for this hard capsule preparation.

The batch analysis data show that the hard capsules can be manufactured reproducibly according to the agreed finished product specification, which is suitable for control of this oral preparation.

- Product Specification

The product specifications include tests by validated methods for appearance, identification of nitisinone (HPLC, UV), identification starch (Ph. Eur.), degradation products (HPLC), assay (HPLC, 95-105% of the label), microbial purity (Ph Eur), dissolution, content uniformity and mass uniformity.

Degradation products are controlled and their limits are justified by reference to stability studies and toxicology studies.

The tests and limits of the specifications for the finished products are appropriate to control the quality of the finished product for their intended purpose.

Batch analysis data on three production-scale batches of each capsule strength confirm satisfactory uniformity of the product at release.

- Stability of the Product

Stability data of 5 batches (1 production and 4 pilot batches) of the strengths 2 mg and 10 mg in hard capsules was provided. One production and two pilot scale batches of each of the strengths were stored at 5˚C, 25ºC/60% RH, 30ºC/60% RH and at 40ºC/75% RH for 12, 18 and 28 months, respectively.

The parameters investigated were appearance, disintegration, dissolution, microbial purity, assay and degradation products.

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

Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the drug substance and drug product have been presented in a satisfactory manner. The results of tests carried out indicate satisfactory 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 the clinic.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product. The applicant gave a Letter of Undertaking and committed to resolve these as Follow Up Measures after the opinion, within an agreed timeframe.

3. Non-clinical aspects

Introduction

Nitisinone was originally developed as a herbicide in the 1980s, but abandoned when it was found to cause eye lesions in rats. Subsequently this was shown to be related to high plasma-levels of the amino acid tyrosine. Most of the non-clinical data in the present submission derive from studies conducted at that time with technical grade material containing 90-92% nitisinone. The proposed drug substance is manufactured by the same route and processes as the would-be herbicide except for a modification of the last purification step to yield a substance with purity ≥ 98%. Since technical grade nitisinone tested positive in some early genotoxicity studies, these tests were repeated with the purified drug substance. When development of nitisinone as a herbicide was discontinued, ongoing animal studies were halted and not reported, although the main findings and conclusions were summarised in interoffice memoranda. The Applicant has subsequently prepared more detailed reports on four repeat-dose toxicity studies based on such memoranda and the available raw data.

Pharmacology

The documentation submitted to support the primary pharmacodynamic consists of 3 original non-GLP reports (dated 1989 and 1984) and 3 literature papers published by the same group of researchers on international journals from 1995 and 2000, without any information on product preparation or on batches used, unless otherwise stated. No safety pharmacology and pharmacokinetic (PK) studies, according to current guidelines, have been submitted.

- Primary pharmacodynamics (*in vitro/in vivo*)

Nitisinone is a potent *in-vitro* and *in-vivo* inhibitor of hepatic HPPD in rodents ($IC_{50} \approx 40$ nM ≈ 13 ng/ml). It has been established that nitisinone is not irreversibly bound to HPPD-inhibitor [EI] complex but slowly dissociate with a recovery of 37% of the 7 hour period. In rodents, HPPD inhibition was associated with elevated plasma tyrosine levels and an increase in hepatic tyrosine transaminase activity, presumably due to enzyme induction. There is no information about the effects of nitisinone on renal HPPD or the kinetics of HPPD inhibition in human liver or kidney tissue. Published data from mouse models with mutations resulting in a deficiency of FAH activity suggests that levels of nitisinone resulting in a lack of detectable SA in the urine may not completely inhibit HPPD activity. It has been demonstrated that a homologous mutation in mice resulting in a lack of fumarylacetoacetate hydrolase (FAH) activity result in neonatal death, which can be reversed by a second mutation resulting in a lack of hydroxphenylpyruvate dioxygenase (HPPD) (Sun et al., 2000). Animals in which the second mutation has been introduced develop normally without evidence of the liver or kidney pathology that is associated with HT-1. The administration of nitisinone in the FAH deficient mice has been demonstrated to decrease the levels of urinary SA, in some cases to below the limit of detection (Al-Dhalimy et al., 2002). While nitisinone was able to reverse the lethal nature of the mutation, it was not able to protect against the changes seen in the liver and kidneys (Al-Dhalimy et al., 2002; Dieter et al., 2003). This lack of protection, combined with the data from the double-knock out mice studies, suggests that complete blockade of the HPPD activity is required in order to protect against the hepatic and renal changes seen.
The clinical consequences of any residual activity of HPPD will be influenced by a number of factors including dietary adherence and the level of back mutation resulting in FAH activity that may be occurring in each patient.

- Secondary pharmacodynamics

Secondary pharmacodynamics was not investigated, although other studies showed no effect on rat and guinea pig α_1, α_2 or β_2 adrenoreceptors at 10^{-5} M ≈ 30 μg/ml. As nitisinone has a high specificity for HPPD, no inhibition of other enzymes of the same family is expected. However, the potential for undesired pharmacological effects and pharmacodynamic interactions with other medicines is unknown, as the general receptor binding profile of nitisinone has not been established.

- Safety pharmacology

Safety pharmacology studies comprised screening tests for behavioural effects, muscle relaxation and cardio-respiratory effects in rats. Oral doses of 500 mg/kg but not 200 mg/kg caused CNS depression, prolonged halothane-induced sleeping time and induced muscle weakness. There were no significant changes in heart rate, blood pressure, ECG recordings or respiration rate at 350 mg/kg p.o. However, there is little confidence in these results as none of the safety pharmacology studies were GLP-compliant and they all included small numbers of animals of a single gender. Furthermore, the rat is not a suitable model for assessing the potential risk for QT interval prolongation in humans.

- Pharmacodynamic drug interactions

Analysis of the key hepatic enzymes involved in tyrosine catabolism showed that HPPD activity was markedly inhibited soon after dosing, the activity recovering very slowly, while the activity of tyrosine aminotransferase (TAT) in the liver was induced about two-fold and homogentisic acid oxidase (HGO) was not affected.

In summary, the available pharmacodynamic data and the increase in Tyr plasma levels clearly demonstrate the effect of nitisinone on Tyr catabolism, which is provided by the inhibition of the HPPD enzyme. In addition, the data provide an indication that no major effects on the main body systems are expected due to nitisinone administration. The preclinical data, except for information on QT prolongation, although not extensive, provide sufficient pharmacodynamic evidence to support the proposed therapeutic indication.

Pharmacokinetics

The available pharmacokinetic studies are limited and the lack of comparative animal and human data preclude an appraisal of the relevance of the animal species used in the toxicity testing for human safety assessment.

- Absorption- Bioavailability

In rats, nitisinone had a terminal half-life of about 9 hours and was rapidly and completely absorbed after oral administration. There are no data on basic pharmacokinetic parameters in other animal species.

- Distribution

Tissue distribution has been investigated in the rat and the mouse and the results published (Lock et al, Toxicol. Appl.Pharm: 1996 and Lock et al Toxicology 2000). In both species, administration of a single oral dose of [^{14}C]-nitisinone [either 0.3 or 30µmol/kg (0.1 or 10mg/kg) body weight in rats, and 30µmol/kg (10mg/kg) body weight in mice] led to selective retention of radiolabel in the liver(>90% associated reversibly with the cytosol fraction) and in kidneys and to a lesser extent in the rat Harderian gland. Since the time course of the distribution to the liver is paralleled by the time course of HPPD inhibition and tyrosinaemia, these findings probably result from binding of nitisinone to the enzyme. There are no data on plasma protein binding.
No retention of radiolabel was detected in the eye, where nevertheless ocular lesions occurred with incidence of 35% and 75% at low and high doses used, respectively. Associated with this finding there was a marked increase maximum of Tyr at 24 hours after dosing, both in the plasma (about 2500nmol/ml, rats) and in the ocular fluid (3500-4000nmol/ml, rats).

- Metabolism *(in vitro/in vivo)* and excretion

The route of elimination of nitisinone has been investigated both in animals and man. In an autoradiographic study in rats excretion of nitisinone and two hydroxylated metabolites was found in the urine. In an earlier study in rats, labelled nitisinone was found to be excreted in both the faeces and urine at approximately equal levels. Information about the metabolism and excretion of nitisinone is limited to data from a pilot study in 4 rats sacrificed at 6 hours after oral administration of a single dose of radiolabelled substance. The tentative identification of hydroxylated metabolites and 2-nitro-4-trifluoromethylbenzoic acid in urine points to the involvement of the cytochrome P450 system in the metabolism of nitisinone. A repeat-dose toxicity study in mice also produced findings that are consistent with liver enzyme induction. Investigations on the potential for drug-drug interactions between nitisinone and other medicines that induce or inhibit cytochrome P450 activity have been studied in vitro on human microsomes (see clinical PK, metabolism and interaction studies)

In addition, the likelihood that the metabolism of xenobiotics and endogenous substances other than tyrosine might be affected by the inhibition of HPPD and other enzymes of the same family as well as by the possible induction of tyrosine kinase was discussed. As other potential substrates (phenylpyruvate, dihydroxyphenylpyruvate and α-ketoisocaproate) can still undergo normal degradation via other metabolic pathways, no clinically significant effect is expected. The inhibition of tyrosine kinase complex is also unlikely.

- Summary of pharmacokinetic parameters (in different species)

Only limited pharmacokinetic data are available in animals The tentative identification of hydroxylated metabolites and 2-nitro-4-trifluoromethylbenzoic acid in urine points to the involvement of the cytochrome P450 system in the metabolism of nitisinone. Findings in mice are consistent with liver enzyme induction.
No clinically significant effect is expected for a possible inhibition on metabolism of xenobiotics or endogenous compounds other than tyrosine or for an effect on tyrosine kinase complex.

Toxicology

Toxicological data are mainly derived from comprehensive records based on original toxicological investigation studies of nitisinone as an herbicide, which were terminated earlier than originally scheduled because the developmental plan for nitisinone as herbicide was cancelled. The species used for these studies were rats, mice, rabbits, dogs and monkeys.

- Single dose toxicity

The acute oral toxicity of nitisinone appears to be low, but is poorly documented, with no description of acute clinical signs. The oral LD50 in rat is probably equal or superior to 1000 mg/kg and in mice between 600 to 800 mg/kg. Among others, the available studies are not GLP-compliant, clinical signs are not described and autopsy was not performed. Single-dose toxicity following i.v. administration was not investigated.

- Repeat dose toxicity (with toxicokinetics)

In all animal species tested in repeated toxicity studies, except in monkeys, ocular lesions resulted to be the main findings. Repeat-dose oral toxicity studies were conducted in the mouse, rat, rabbit, dog and rhesus monkey. The rodent studies were terminated early due to the abandon of the product as a herbicide.
The chronic oral toxicity in mice, originally a carcinogenicity at dose levels of 0, 10, 350, 1500 or 3500ppm (approximately 0, 2, 70, 300 or 700mg/kg body weight) was terminated early after 6.5 month of treatment. The target organs in the mouse were the liver, the kidney and the peripheral nervous system. Liver lesions included centrilobular hypertrophy with nuclear enlargement consistent with enzyme induction. Although there was evidence of kidney enlargement and impaired renal function, this organ was not examined microscopically. The incidence and severity of sciatic nerve degeneration seemed dose-related. The applicant draws no conclusions about the NOAEL; however, it seems to be in the order of 10 ppm or approximately 2 mg/kg/day.
The chronic rat oral study, originally a 2 year combined toxicity/carcinogenicity (dose levels 0, 1, 5, 40, 120, 320, 800 ppm in the diet) was terminated early at 12 month with a planned interim termination at 3 month. The target organs were the cornea and, to a lesser extent, the liver. Corneal lesions observed, at all dose levels were inflammatory in nature and included oedema and hyper-vascularisation. They were not reversible, as ghost vessels remained after a 3-month recovery period. The LOAEL for this effect was 1ppm or 0.05 mg/kg/day. A NOAEL was not established.
In rabbits, oral administration of 50 or 250 mg/kg/day for 3 months caused lethality, but no gross eye abnormalities.
In the dog 3 month oral toxicity study (doses levels of 0, 0.1, 0.5, 1.5 or 5mg/kg/day), the target organs were the eyes, gastrointestinal tract and nervous system. Eye lesions comprised degenerative and inflammatory changes of the cornea and were transient and reversible in nature. The LOAEL for this effect was 0.1 mg/kg/day. A NOAEL was not established.
Nitisinone was well tolerated and did not produce eye lesions in rhesus monkeys administered up to 10 mg/kg/day for up to 13 weeks 5days/week. As there are no toxicokinetic data, human safety margins cannot be determined.

- Genotoxicity in vitro and in vivo

Technical grade nitisinone was mutagenic in bacteria and mutagenic and clastogenic in mammalian cells in vitro. The purified drug substance tested negative in bacteria, but was mutagenic in mammalian cells in vitro and was not tested for chromosome damage in vitro. In vivo, technical grade material tested negative in the mouse bone marrow micronucleus test, whereas purified nitisinone produced a weakly positive response with

no clear dose-response relationship. Given the positive tests for genotoxicity *in vitro* and the presence of structural alerts (electrophilic centres and an aromatic nitro group), an *in vivo* mouse liver unscheduled DNA synthesis assay was subsequently performed at the request of CHMP. The results showed no induction of DNA repair in the mouse liver after oral doses of nitisinone up to 250 mg/kg. The overall available data seem to indicate that there no evidence of *in vivo* genotoxicity.

- Carcinogenicity (with toxicokinetics)

Proper tests for carcinogenic potential have not been performed although nitisinone is intended for chronic treatment. Carcinogenicity studies were commenced in rats and mice, but interrupted after 12 and 6½ months (see section repeat dose toxicity). There were no indications of any treatment-related tumours. However, no definitive conclusion can be drawn from these studies, as they were not conducted according to current standards, and because of the relatively short duration of exposure.
The only long-term safety study was performed in the FAH knock-out mouse published by Al-Dhalimy et al. (2002), with many of the characteristics of hereditary tyrosinaemia type 1 also develops hepatocellular carcinomas. The animals received up to 6 mg/kg/day of nitisinone for >2 years. In this study, nitisinone improved the poor condition of the animals. Histology included the liver and the kidney and was apparently only performed on organs with macroscopic changes, but there were no indications of other treatment-related tumours. The high dose of the compound rather decreased the incidence of hepatocellular carcinomas, though without preventing its occurrence.

- Reproductive and developmental studies

Four preliminary non-GLP studies, including 3 embryo foetal studies and one fertility study in rats have been conducted.
Maternotoxicity and embryotoxicity (reduction of pup survival and mean weight) were observed from 20 mg/kg/day and 50m/kg/day and above, respectively. No evidence of teratogenicity was found in the rat.
In the preliminary fertility study, no treatment related effects on pregnancy rate, but significant litter effects were seen at the dose of 100 mg/kg/day (only dose tested).
Four additional GLP studies have been subsequently performed, including an embryo-foetal study in the mouse (doses 5, 50, 250 mg/kg/day) and rabbit (doses 5, 12, 25 mg/kg/day), a fertility and early embryonic study in the mouse (doses 5, 50, 250 mg/kg/day) and a pre and postnatal in the mouse (doses, 5, 50, 250 mg/kg/day).
The results of these new studies indicate that nitisinone show embryo foetal toxicity in the mouse and the rabbit, for both of which the NOELs was not determined (< 5mg/kg/day). In light of the lack of toxicokinetic data for the reproductive and developmental toxicity studies, and of adequate pre-clinical PK data for nitisinone, the comparison for safety factor can be possible only on a mg/kg basis and not on exposure.
Nitisinone induced dose-dependent malformation (umbilical hernia or gastroschisis) in rabbits and changes in ossification at all dose levels in mice and rabbits, although not always in a dose-dependent manner. The alteration on ossification noted in both species is considered treatment related. Moreover, nitisinone reduced pup growth and pup survival in mice at doses which correspond to several multiples of the maximum human recommend dose. The NOAEL for maternal effect can be considered 5mg/kg/day in both species. These results are reflected in the SPC (section 5.3)

- Other toxicity studies

<u>Ocular lesions</u>

A series of studies were performed in order to characterise the ocular lesions. From these studies resulted that histologically the first lesion to be observed was a focal epithelial disorganisation in the cornea. Diet changes (lowering protein content) several weeks after dosing with nitisinone did not influence the corneal lesions. The raised plasma tyrosine due to nitisinone administration probably caused the ocular keratitis more than a direct effect on eyes of the compound itself.

<u>Tyrosinemia induction .</u>

Nitisinone (10mg/kg) produced a marked (about 50-70%) increase of tyrosine levels in plasma and ocular fluid in the dog, rabbit and monkey, reversible upon cessation of dosing. Only dogs developed corneal lesions.

Environmental assessment
Nitisinone belongs to a class of potent herbicides (triketones) with potential risk for the environment. However, the $PEC_{surfacewater}$ and PEC/PNEC ratio are far below the action limit, and the data presented in the expert report, including data from other chemicals of the same class, do not indicate that nitisinone could pose a potential risk for the environment in the conditions of use.

- Summary of toxicological findings

Ocular lesions (cornea) were the main finding in repeat-dose toxicity studies in all species except the monkey. Other identified target organs were the liver, kidney and peripheral nervous system. No effect levels were either not determined or were close to the therapeutic dose. However, the lack of toxicokinetic data precludes the determination of human safety margins.
In the genotoxic battery, some tests were positive or equivocal, however the overall data suggest that there is no evidence of *in vivo* genotoxicity.
The results of the available long-term studies do not suggest a tumorigenic potential. However, as no proper tests for carcinogenic potential have been performed, no definitive conclusion can be made.
Reproductive and developmental toxicity studies have shown malformations in mice and rabbit (mainly ossification abnormalities). As increased plasma levels of tyrosine have been reported to induce developmental toxicity, it is not known whether the findings were due to nitisinone, to increased plasma levels of tyrosine, or to a combination of both.

Discussion on the non-clinical aspects

The available pharmacodynamic data and the increase in Tyr plasma levels clearly demonstrate the effect of nitisinone on Tyr catabolism, which is provided by the inhibition of the HPPD enzyme. The data provide an indication that no major effects on the main body systems are expected due to nitisinone administration. The preclinical data, although not extensive, provide sufficient pharmacodynamic evidence to support the proposed therapeutic indication.
Only limited pharmacokinetic data are available in animals. The cytochrome P450 system is involved in the metabolism of nitisinone and findings in mice are consistent with liver enzyme induction.
No clinically significant effect is expected with regard to inhibition of metabolism of xenobiotics or endogenous compounds other than tyrosine. In addition no effect is expected on the tyrosine kinase complex.
In repeat-dose toxicity studies, the main target organ was the eye, with corneal lesions resulting probably from the accumulation of tyrosine.
The overall genotoxicity data suggest that there is no evidence of *in vivo* genotoxicity.
The results of the available long-term studies do not suggest a tumorigenic potential. However, as no proper tests for carcinogenic potential have been performed, no definitive conclusion can be made.
Reproductive and developmental toxicity studies have shown malformations in mice and rabbit and this is reflected in the SPC. Furthermore, mothers receiving nitisinone should not breast-feed since a transfer of nitisinone in the maternal milk is possible (see SPC sections 4.3 and 5.3).
There is no indication that nitisinone could pose a potential risk for the environment in the conditions of use.
In view of the rare occurrence and seriousness of the disease, the lack of therapeutic alternatives and the obvious clinical efficacy, the CHMP was of the opinion that shortcomings considered in the non-clinical investigations should not preclude a recommendation for a marketing authorisation under exceptional circumstances.

4. Clinical aspects

Introduction

Efficacy evaluation is based on a large compassionate use program open to all patients with HT.
The promising results of a pioneering study on the first five patients with HT-1 treated with nitisinone were published in 1992 (Lindstedt S et al., Lancet, 1992). A worldwide study was then started (the NTBC Study), co-ordinated by the team from Sahlgrenska University Hospital (SU), Gothenburg, Sweden, to document the effects of nitisinone treatment. On request, nitisinone was distributed from SU to hospitals all over the world

on a compassionate use basis. The physicians/investigators sent blood and urine samples for analysis to SU at regular intervals, according to a protocol designed by the research team at SU, together with results of local laboratory tests and clinical information. The NTBC Study period was from February 1991 to August 1997. From late 1994 the distribution of nitisinone was gradually shifted from SU to Swedish Orphan AB, Stockholm, Sweden. Some patients, who were not included in the NTBC Study, were also treated with nitisinone.

The clinical documentation in the present application consists mainly of the analysis of the results of the NTBC Study. The safety analysis also includes those patients given nitisinone on compassionate basis but not participating in the NTBC Study. In addition, one pharmacokinetic study on 10 healthy adult volunteers has been completed.

Pharmacokinetics

The final formulation to be used for commercialisation is a hard gelatine capsule containing the compound mixed with pregelatinized starch.
Most of the available information derives from a study in healthy adult volunteers comparing a liquid formulation with a hard gelatine capsule containing the compound mixed with lactose, i.e. the first formulation nitisinone.
The pharmacokinetic studies in healthy volunteers used coupled column HPLC liquid chromatography for measurement of nitisinone in plasma. The method has been validated.
In the NTBC study, the nitisinone plasma/serum concentration was determined by an enzyme inhibition assay that was based on the release of 14CO2 from the 4-hydroxy[1-14C]phenylpyruvate. This method had been validated for linearity and repeatability. For repeatability an internal control was prepared and analysed during the period of two years. The overall coefficient of variation for the 306 measurements during that period with a mean nitisinone concentration of 37.7µmol/L was estimated to be 3.6%
In the study in healthy subjects, the paired difference of pharmacokinetic parameters, i.e. after administration of the capsule and liquid formulation, were evaluated by the Pitman randomisation test based on Wilcoxon matched paired sign rank test. Bioequivalence was established by the Weslake's 95% interval for untransformed values and the Hauschke-Steinijans nonparametric test for bioequivalence.
In the bioequivalence analysis of the preliminary and final formulation performed as a intra-patient comparison, the nitisinone serum concentrations were compared using the two one-sided 95% confidence interval bioequivalence procedure (ANOVA, log ratios, 90% two sided confidence interval within 0.8-1.25). A 90% two-sided confidence interval for the difference between the treatment groups after 12 months of nitisinone treatment was also calculated.

- Absorption – Bioavailability

The pharmacokinetics of nitisinone have been studied in healthy volunteers (n = 10) after administration of 1mg/kg as capsule and liquid formulations. Absorption of nitisinone was rapid for the liquid formulation and in six patients the maximum concentration (Cmax) appeared prior to the first sample, i.e 15min after dosing. For the capsules, the first formulation of nitisinone containing the active compound mixed with lactose, the rate of absorption (Tmax) was slower and more variable, ranging from 1.6 to 11.1h (mean 4.2 h). Other pharmacokinetic parameters are show below:

Table 1. Mean pharmacokinetic parameters of nitisinone in healthy volunteers

Parameters[a]	Capsule formulation	Liquid formulation
Tmax (h)	4.2 (3.0)	< 0.2 5
Cmax (µg/mL)	7.7 (1)	7.8 (1.4)
AUC (µg/mL .h)	599 (153)	598 (142)
T1/2 (h)	54.5 (13.0)	53.6 (8.2)

(a) Data from pharmacokinetic modelling. Each value is the mean (SD) of 10 subjects. The dose was 1 mg/kg.

The NTBC study reveals that this drug is rapidly absorbed and slowly eliminated, regardless of the formulation.

- Bioequivalence

No formal bioequivalence study has been performed. However, the study in healthy adult volunteers comparing a liquid formulation with a hard gelatine capsule containing the compound mixed with lactose, i.e. the first formulation nitisinone, shows an equivalence of the 2 formulations for most of PK parameters (except Tmax) as described above.
Furthermore, retrospective data analysis from patients a) receiving the first formulation compared with data from patients receiving the to-be-marketed formulation - a hard gelatine capsule containing the compound mixed with pregelatinized starch - or b) shifting from the first to the final formulation suggest that the formulations used in clinical documentation are essentially bioequivalent.
The possibility of developing a paediatric formulation was investigated by the applicant but the stability results of the liquid formulation were unsatisfactory. Therefore the applicant decided to keep using the capsule which can be opened for young children who cannot swallow. CHMP was of the opinion that a paediatric formulation would be preferable, and requested the applicant to consider to investigate the development of such a formulation in the future.

- Distribution

The pharmacokinetics of nitisinone were studied during the first doses (0.2-0.6mg/kg) in four patients in the NTBC Study, three children (aged 2 months to 27 months) and one adult (aged 21 years) with untreated HT-1. The one-compartment pharmacokinetic analysis suggested a small volume of distribution of 0.3L/kg (0.1 to 0.5L/kg).

- Metabolism and Elimination

The knowledge on metabolism and excretion is limited.
In vitro investigations were conducted to identify whether nitisinone was a substrate for a range of P450 isozymes (1A2, 2C9, 2C19, 2D6, 2E1 and 3A4). Incubation with pooled human microsomes identified a single NAPH-dependent metabolite which accounted for approximately 2% of the sample radioactivity. A single metabolite with a similar retention time on the chromatogram was seen also when nitisinone was incubated with expressed 3A4 isozymes. LC-MS/MS evaluation has identified this as a hydroxylated metabolite with the hydroxylation on the cyclohexanedione moiety. The *in vitro* data thus suggests some involvement of CYP3A4 in the metabolism of nitisinone.
This was further reinforced by the presentation of data from a single patient who was on concurrent treatment with phenobarbital. Upon withdrawal of phenobarbital, an inducer of CYP 3A4, there was a significant increase in the plasma concentration of nitisinone. The metabolism via CYP 3A4 would be in agreement with the excretion of hydroxylated metabolites that was seen in the rat study.

Therefore, it appears that the mode of elimination of nitisinone is via hydroxylation with subsequent excretion in both the urine and faeces.

In order to try and further characterise the pharmacokinetic profile in the patient population, the available data from the NTBC Study has been subjected to a population kinetic analysis. Data on the following parameters was available for 207 patients on nitisinone treatment for up to 54 months: body weight, dose and plasma nitisinone concentration. However, no data is available on the time of dose administration or the time from last dose to sampling. As the patients received the drug twice daily and the absorption rate is considered to be small compared to the elimination rate, it was assumed that the patients rapidly reached an oscillatory steady-state and that this was maintained throughout the treatment period. As the data was assumed to be steady-state, the only parameter that could be estimated was clearance. It was determined that the clearance for the population contained within the NTBC Study was 0.0956L/kg/day. In addition, the $T_{1/2}$ for the population was estimated based on the VD of 0.1 – 0.5L/kg. The average terminal half-life ($T_{1/2}$) was found to vary between 17.3 and 86.9 days with a mean value of 52.1 hours which corresponds well to the

value of 54 hours (median) that was seen in healthy volunteers. Given the heterogeneity of the patient population further characterization of the PK profile is not possible.

- Dose proportionality and time dependencies

A total of 239 patients treated on "named patient" basis between July 93 and March 2000 were included in the second, complementary analysis. There was no evidence of a non-linear dose-concentration relationship.
The dose normalized nitisinone serum concentrations increased from about 30µmol/L per mg/kg/day during the first years to about 42µmol/L per mg/kg/day during the fifth year after start the nitisinone treatment for the patients who started before 24 months of age. The patients who started nitisinone after 24 months of age had higher dose normalized drug concentrations than younger patients. The regression analysis showed that nitisinone serum concentrations increased by about 1.2-2 µmol/L per year of increased start age. Also, a significant increase in the nitisinone serum concentrations was observed during the first three years of treatment, 19% between year 1 and 2 and 14% between year 2 and 3. After that, there was only a moderate increase in nitisinone concentrations, less than 6% per year. This increase per treatment year was in the same order of magnitude as the increase for each year of increased age at start of treatment.

- Special populations

Pharmacokinetic studies in patients with HT-1 are limited to case reports during the first three doses and after treatment discontinuation in seven patients (mainly children) and suggest that the drug elimination $T_{1/2}$ is probably shorter in children (patients) than in adults (healthy). Drug measurements over time suggest that the nitisinone serum concentrations are higher in older children than in younger, and increase with time during the first three years of therapy.

- Interaction studies

A series of *in vitro* studies have been performed to investigate the potential involvement of nitisinone on six P450 isozymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4).
It was found that incubation with nitisinone had no effect on the metabolism of selective substrates for the CYP1A2, CYP2C19 or CYP3A4. nitisinone was determined to be a moderate inhibitor of the CYP2C9 isozyme with an IC50 of 46µM. It was also found to be a weak inhibitor of the CYP2D6 and CYP2E1 isozymes with IC50 values of >100µM. Based on the absence of an effect on CYP1A2, CYP2C9, and CYP3A4 and the high IC50 values of the other CYP isozymes, it is considered that nitisinone will not inhibit the clearance of drugs that are metabolised via the cytochrome P450 system.

The effect of the co-administration of an inhibitor of CYP3A4 and nitisinone is not known but the relatively low degree of metabolism observed in the microsomal preparation expressing a singly isozyme may suggest that there will be no significant effect of a P450 inhibitor on nitisinone plasma concentrations. However, until further interaction data is available one cannot exclude that a dose-adjustment will be necessary when nitisinone is co-administered with inducers and inhibitors of CYP3A4. This is also reflected in the SPC.

No formal food interactions studies have been performed. However, nitisinone has been co-administered with food during the generation of efficacy and safety data. Therefore, it is recommended that if nitisinone treatment is initiated with food, this should be maintained on a routine basis.

Pharmacodynamics

The pharmacological effect of nitisinone is to prevent the development of clinical phenotype of HT-1. HT-1 is a life–threatening disease and the only other effective treatment available is liver transplantation.

- Mechanism of action

Nitisinone inhibits the enzyme 4-hydroxy phenylpyruvate dioxygenase (HPPD), i.e. the second step in tyrosine degradation, both in vitro and in animals. This was only indirectly verified in man. This inhibition prevents the accumulation of maleylacetoacetate and fumarylacetoacetate that occurs in HT-1 patients as a consequence of their inherited defect of fumarylacetoacetate hydroxylase activity, the last step in the tyrosine degradation.

Through this mechanism of action, nitisinone enhances the effects of a diet restricted in tyrosine and phenylalanine, which is used to alleviate the HT-1 progression. This diet is concomitantly required, since HPPD inhibition causes tyrosinemia, which in man can produce ocular toxicity. The efficacy of this approach was only indirectly verified in man, but it has recently been confirmed in double mutant Fah-/-Hpd-/-mice (Endo and Sun, J Inherit Metab Dis 25: 227-34, 2002). These mice grew normally without evidence of liver and renal disease.

- Primary and Secondary pharmacology

Nitisinone prevents accumulation of maleylacetoacetate and fumarylacetoacetate. These compounds are toxic and reactive metabolites. Their accumulation may be related to the development of hepatocellular carcinoma seen in HT-1 patients (Endo et al., JBiolChem, 272, 24426-32, 1997). They are also partially converted to succinylacetone and succinylacetoacetate, which inhibit the PBG-synthase activity in the heme synthesis pathway. This leads to accumulation and increased excretion in the urine of 5-aminolevulinate (5-ALA). This compound is likely responsible of the development of neuropathic porphyria –like symptoms in HT-1 patients.
Nitisinone treatment leads to normalised porphyrin metabolism with normal erythrocyte porphobilinogen synthase activity and urine 5-ALA and decreased urinary excretion of succinylacetone.
However, as in the animal experiments, plasma tyrosine and phenolic acid excretion increased in all patients treated with nitisinone.

- Discussion on Pharmacokinetics and Pharmacodynamics

The pharmacokinetic studies supporting the present application are rather scarce. There is limited or no information on the drug distribution, including binding to blood components, main route(s) of elimination and physiological and pathological factors that may alter the absorption, distribution and elimination of this drug in humans. This is considered to be acceptable taking into account the extreme rarity of the disease and the heterogeneity of the patient population.
Most of the available information derives from a study in healthy adult volunteers comparing a liquid formulation and a hard gelatine capsule containing the compound mixed with lactose, i.e the first formulation of nitisinone. This study tells us that this drug is rapidly absorbed and slowly eliminated, regardless of the formulation.
Pharmacokinetic studies in patients with HT-1 are limited to case reports and suggest that the drug elimination is probably faster in children (patients) than in adults (healthy). Drug measurements over time suggest that the nitisinone serum concentrations increase with treatment time during the first three years of therapy.
The relationship between the nitisinone serum concentration and the inhibition of the target enzyme HPPD has not been directly measured in man. The optimisation of the dose has been derived from the relationship between nitisinone serum concentration and the probability to obtain a decline in plasma and urine succinylacetone and urine 5-ALA, to reverse inhibition of PBG-deaminase and to normalize serum alpha-fetoprotein (see section “Clinical efficacy”). This approach is considered acceptable since the mechanism of action is clear and the biochemical parameters monitored are markers of the clinical phenotype of HT-1.
Infants reportedly required a higher dose to obtain the effects.
The lack of studies regarding genetic differences in pharmacodynamic response is acceptable considering the limited number of patients.

Clinical efficacy

The clinical development programme consists of one uncontrolled, compassionate use, multicentre trial (NTBC Study), which was co-ordinated by the Sahlgrenska University Hospital (SU), Gothenburg, Sweden. The study was not performed according to current GCP rules.

- Dose response study(ies)

No formal dose-finding study was done. An initial experience based on five patients (Lindstedt S et al., 1992) tested a daily dose of 0.1 to 0.6mg/kg bodyweight. Treatment duration was 7-9 months and evaluation criteria were Urinary-succinylacetone, Plasma-SA, PBG-synthase, Urinary-5-ALA, α-fetoprotein, liver

function, tyrosine. Based on results of this study, the 0.6mg/kg dose was recommended until it became clear to the investigators that it was generally too low. From mid 1993, an initial daily dose of 1mg/kg has been recommended. Adjustments to the dose are recommended if the response is inadequate, as judged by the effect on the HT-1 specific parameters and/or low nitisinone serum concentrations.

- Main studies

METHODS

Evidence of clinical efficacy is mainly based on the compassionate use in 207 patients enrolled in the NTBC Study, a large uncontrolled study co-ordinated by the team from Sahlgrenska University Hospital (SU), Gothenburg, Sweden, and involving 96 local investigators at 87 different hospitals in 25 countries.
Nitisinone was administered orally twice daily, initially at a daily dose of 0.6mg/kg bodyweight until it was clear that this dose was generally too low. From 1994, 1mg/kg was recommended as total daily initiation dose. The individual response to treatment was evaluated and the dose was adjusted if considered necessary. No daily dose exceeded 3.0mg/kg. Nitisinone treatment was always combined with a diet restricted in tyrosine and phenylalanine.
Besides the main analysis regarding the 207 patients who were included between 23 February 1991 and 21 August 1997, a complementary analysis refers to the 250 patients who were included between 1 July 1993 and 28 March 2000, after all investigators had received the recommendations of an initial daily dose of 1mg/kg bodyweight.
HT-1 specific biochemical variables (urine and plasma succinylacetone, erythrocyte PBG-synthase and urine 5-ALA), α-fetoprotein, death, liver transplants, hepatocellular carcinoma and porphyria-like crises were evaluated.

RESULTS ON EFFICACY

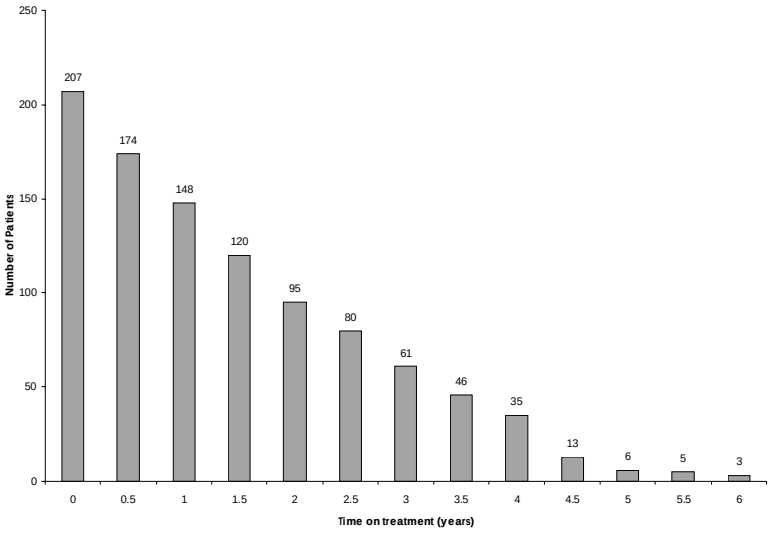
Survival

The primary efficacy variables for the study consisted of the following: survival, survival without transplantation, death due to liver failure, transplantation due to liver failure and hepatocellular carcinoma.
The total patient population of 207 patients in the database is the result of the cumulative enrolment into the study over the period from February 23, 1991 to August 21, 1997, which has been used as the pivotal study period. At the time of data cut off, out of the 207 patients enrolled there were 169 patients still on treatment and a total of 38 withdrawals.
The reasons for withdrawal are shown in the following table:

Reason for withdrawal	**Number of patients**		
	Survived	**Died**	**All**
Death during nitisinone treatment			
liver failure	-	7	7
HCC	-	2	2
multiorgan failure	-	1	1
All	-	10	10
Liver transplantation			
Elective	4	3	7
liver failure	7	0	7
suspected HCC, verified	5	2	7
suspected HCC, not verified	6	0	6
All	**22**	**5**	**27**
Parents' wish to discontinue	-	1	1
All withdrawals	**22**	**16**	**38**

Since patients have been enrolled on an ongoing basis and all patients entered up to the point of data cut-off (August 21, 1997) have been included in the analyses, each patient has been on treatment for a different

period of time. As a result, patients have been on treatment up to a period of 75 months with the number of patients diminishing with the length of treatment. This can be seen in the following figure.



As a result, the number of patients who were available for inclusion in analysis after one, two and four years of treatment was 148, 95 and 35 respectively.

The two-year and four-year survival was 96% (N=95) and 93% (N=35), respectively; two-year and four-year survival also including deaths after stopping nitisinone treatment was 93% (N=109) and 90% (N=43), respectively; two-year and four-year survival without liver transplantation was 84% (N=95) and 78% (N=35), respectively (see Table below).

	n	No. of patients at			Probability (95% confidence interval)		
		1 yr	2 yrs	4 yrs	1 yr	2 yrs	4 yrs
Survival							
Total population	207	148	95	35	96% (93;98)	96% (93;99)	93% (88;98)
Start age 0-2 months	16	12	7	3	88% (71;100)	88% (65;100)	88% (52;100)
Start age 0-6 months	80	55	30	11	94% (88;100)	94% (85;100)	94% (80;100)
Start age 2-6 months	64	43	23	8	95% (90;100)	95% (90;100)	95% (90;100)
Start age >6 months	127	93	65	24	97% (94;100)	97% (94;100)	93% (85;100)
Survival without transplantation							
Total population	207	148	95	35	88% (83;93)	84% (78;90)	78% (69;86)
Start age 0-2 months	16	12	7	3	88% (70;100)	88% (65;100)	88% (52;100)
Start age 0-6 months	80	55	30	11	89% (81;97)	85% (75;95)	82% (66;97)
Start age 2-6 months	64	43	23	8	89% (81;97)	84% (75;94)	80% (68;92)
Start age >6 months	127	93	65	24	88% (82;94)	83% (76;91)	76% (65;87)
Survival incl death after nitisinone stop							
Total population	207	162	109	43	96% (93;99)	93% (89;97)	90% (83;96)
Start age 0-2 months	16	12	7	3	88% (70;100)	88% (65;100)	88% (52;100)
Start age 0-6 months	80	58	33	13	94% (88;100)	92% (84;100)	92% (80;100)
Start age 2-6 months	64	46	26	10	95% (90;100)	93% (86;100)	93% (86;100)
Start age >6 months	127	104	76	30	97% (93;100)	94% (89;99)	89% (81;97)

Liver failure

The improved survival of the younger patients, i.e. patients with the acute form, is reflected in the decreased occurrence of liver failure and transplantation due to liver failure. In the nitisinone study 7/80 (9%) patients with treatment start < 6 months died of liver failure or were transplanted due to liver failure whereas in the

historical control (van Spronsen) 35/83 (42%) patients with symptom onset < 6 months died of liver failure or recurrent bleeding with or without liver failure. Transplantation due to liver failure was performed in the historical control in 6/108 patients (6.4%), in the nitisinone study in 7/207 (3.4%).

Table 5.6. Cumulative probability of death due to liver failure or transplantation due to liver failure at 1, 2 and 4 years after start of NTBC treatment.

	n	No. of patients at			Probability (95% confidence interval) at		
		1 yr	2 yrs	4 yrs	1 yr	2 yrs	4 yrs
start age 0-6 months	80	54	29	11	9% (2;16)	9% (0;19)	9% (0;25)
start age 6-24 months	62	45	30	9	8% (1;16)	11% (2;20)	17% (0;34)
start age 0-24 months	142	99	59	20	9% (3;14)	10% (4;16)	13% (3;22)

Heptocellular carcinoma (HCC)

In two retrospective surveys of HT-1 patients on dietary treatment, the occurrence of HCC was 37% (Weinberg et al. 1976) and 18% (van Spronsen et al. 1994), respectively, in all patients older than 2 years. In the NTBC study, the cumulative probability (and 95% CI) of death due to HCC, transplantation due HCC or diagnose of HCC for all patients who started nitisinone treatment after 24 months of age, was 8%, 12% and 27% at 1-, 2- and 4-years respectively. (see Table below).

Table 5.8. Cumulative probability of death due to HCC, transplantation due to HCC or diagnose of HCC during NTBC treatment at 1, 2 and 4 years after start of NTBC treatment.

	n	No. of patients at			Probability (95% confidence interval) at		
		1 yr	2 yrs	4 yrs	1 yr	2 yrs	4 yrs
total population	206	147	94	35	3% (0;5)	4% (0;8)	8% (2;15)
start age 0-24 months	141	99	59	20	1% (0;3)	1% (0;3)	1% (0;5)
start age >24 months	65	48	35	15	6% (0;12)	10% (1;19)	20% (5;35)
total population [1)]	206	147	94	35	3% (0;6)	5% (1;8)	11% (4;19)
start age 0-24 months[1)]	141	99	59	20	1% (0;3)	1% (0;3)	1% (0;5)
start age >24 months[1)]	65	48	35	15	8% (0;15)	12% (2;21)	27% (11;42)

[1)] Two patients with exponential increase in the AFP level were included as cancer

The plasma concentration of nitisinone was not correlated to the risk of development of HCC as there was no difference in the mean plasma nitisinone concentration between those patients who developed HCC and those that did not develop HCC during the NTBC Study period.

The analysis of a possible effect of age at the start of treatment on the risk for developing HCC, examined in a total of 554 patients who had been exposed to nitisinone (cut-off date 31 December, 2003), is as follows:

	Age < 12 months at start of treatment	**Age > 12 months at start of treatment**
Patients without HCC	368	163
Patients with HCC	3	20

There is a significant effect on the time at initiation of treatment in the development of HCC. Patients who initiate treatment after the age of 12 months have a significantly higher risk of developing HCC (Fisher exact test, $p<0.000001$). The relative risk for developing HCC is 13.52 (95% CI: 4.07 – 44.89) if treatment is initiated after the age of 12 months.

Serum α-fetoprotein (AFP)

Serum AFP concentration decreased significantly in most patients during nitisinone treatment, from levels highly above the reference limit at the pre-treatment evaluation to levels slightly above the reference limit at one year after treatment start. The decrease in serum AFP concentration may indicate a change from rapid proliferation to a more normal proliferation rate of hepatic cells.

Liver function

ALT and albumin increased during the first year of treatment, while AST, γGT, and bilirubin decreased (these findings are from about one fourth of the study population). After 6 months of nitisinone treatment, almost 80% of the patients whose values have been reported had normal prothrombin complex levels, while 90% of patients with HT-1 had abnormal prothrombin complex levels before start of nitisinone treatment. Serum α-Fetoprotein concentration significantly decreased (from median 471 to 3.1µg/L, N=104) at one year after treatment start.

Neurological symptoms

Porphyria-like symptoms, which reportedly occur in >40% children with HT-1 on dietary treatment, during nitisinone treatment only occurred in one patient with sub-optimal treatment at the time preceding the event. No information on the occurrence of neurologic crises is provided by van Spronsen except that respiratory failure/porphyria like syndrome was the cause of death in 5/108 patients. Mitchell (1990) reported on neurologic crises in 20/48 patients hospitalised for HT-1.

Biochemical variables

Among the biochemical parameters, it was observed in the main and the complementary analysis that succinylacetone in urine was reduced to below the reference limit (i.e. 1.0mmol/mol creatinine) in two months (N=186) after start of the nitisinone treatment. Plasma succinylacetone also decreased to below the reference limit (0.1µmol/l for plasma succinylacetone) (N=172) but more slowly than in urine (median time to normalisation 3.9 vs. 0.3 months). Urine 5-ALA rapidly decreased within reference limits (median 0.2 month, N=163). PBG-synthase activity increased rapidly up to reference limits within one month in half of patients (median 0.3 month, max 7.5 months) (N=180).

Table 5.11. Time from start of NTBC treatment to the first value within reference range for urine excretion of succinylacetone, plasma concentration of succinylacetone, erythrocyte activity of PBG-synthase and urine excretion of 5-ALA in patients with abnormal pre-treatment values, who were normalized during study.

	Reference values[3]	n[4]	Time to normal (months) median	min	max	Not normalized[5]	Normal at pre-treatment
U-Succinylacetone (mmol/mol creatinine)	<1.0[1]						
total population		186	0.3	0.2	20.8	0	3
start age 0-24 months		128	0.3	0.2	20.8		
start age >24 months		58	0.2	0.2	6.4		
P-Succinylacetone (µmol/L)	<0.10[1]						
total population		172	3.9	0.2	27.0	22	0
start age 0-24 months		115	4.0	0.9	27.0		
start age >24 months		57	3.5	0.2	21.6		
Erc-PBG-synthase activity (nkat/g Hgb)	>0.58[2]						
total population		180	0.3	0.2	7.5	0	2
start age 0-24 months		124	0.4	0.2	7.5		
start age >24 months		56	0.2	0.2	6.4		
U-5-ALA (mmol/mol creatinine)	<23[3]						
total population		163	0.2	0.2	20.7	2	27
start age 0-24 months		121	0.3	0.2	20.7		
start age >24 months		42	0.2	0.2	6.4		

[1] Detection limit. [2] Lower reference limit. [3] For 5-ALA a limit was defined as the 95th percentile of all values in the study after at least 6 months of treatment. [4] Number of patients normalized during study period. [5] Only patients with NTBC treatment lasting to at least the two-month visit were included as not normalized.

The complementary analysis of the 250 patients enrolled between 1 July 1993 and 28 March 2000 (after all investigators had received the recommendations of an initial daily dose of 1mg/kg bodyweight) gives the following information on the normalization of laboratory variables:

U-succinylacetone: more than 90% of the patients had urine succinylacetone values in the reference range already during the first week of treatment, and after the first month almost all patients were normalized.

P-succinylacetone: no patient had plasma succinylacetone values within the reference range during the first month of treatment. During the sixth month, about 90% of the patients were reported to be normalized, and at the end of the first treatment year, almost all patients were normalized.

Erc-PBG-synthase: more than 90% of the patients had urine succinylacetone values in the reference range during the first months of treatment, and during the second month all patients but one were normalized.

U-5-aminolevulinate: more than 80% of the patients had urine 5-aminolevulinate values in the reference range already during the first week of treatment, and after the first month all patients but one were normalized.

S- α -fetoprotein: the percentage of normalized patients increased gradually over the first two-year period of nitisinone treatment. After the first treatment year, a little more than 50% of the patients were normalized, and at the end of the second treatment year, about 90% of the patients were normalized, i.e. had values below the 90th percentile of a normal population.

Haematology
Platelet (N=46) and erythrocyte count (N=40), and blood haemoglobin (N=51) were significantly increased at one year of nitisinone treatment.

Renal function
One year after start of nitisinone treatment, both the urine excretion of amino acids (N=8) and the serum concentration of phosphate (N=12) were within the reference ranges in the subgroup of patients who had signs of renal tubular dysfunction before treatment (the Fanconi syndrome) (N=34).

Effects in relation to nitisinone concentrations
There was a significant inverse relationship between nitisinone concentration and the probability of recurrence of values outside normal ranges for U-succinylacetone, P-succinylacetone and Erc-PBG-synthase (see Table below). For U-5-aminolevulinate, there were reference values only for the group of patients older than 24 months at the start of nitisinone treatment. Therefore, the number of observations was too small to perform a meaningful analysis on this variable. At a nitisinone serum concentration of 30µmol/L, the estimated probability of recurrence was about 6% for both U-succinylacetone and Erc-PBG-synthase

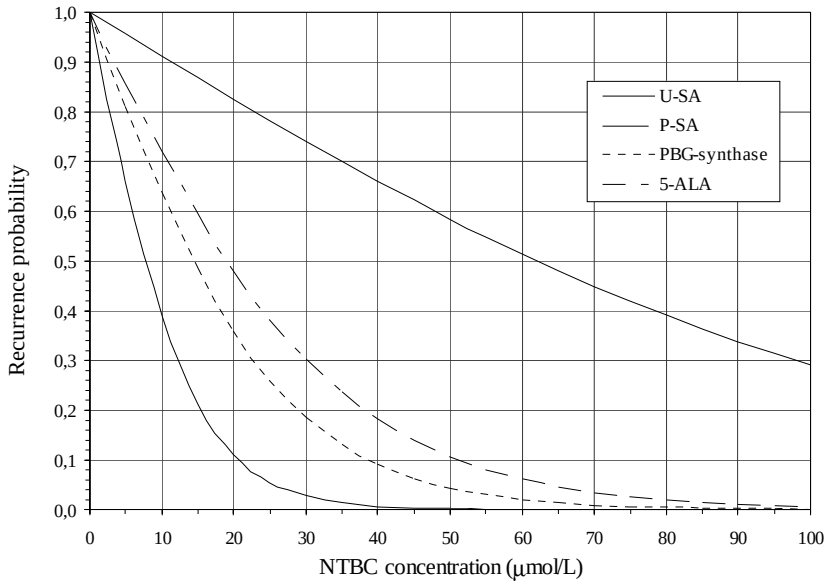


Figure: The probability for recurrence of pathological values after once being normalized during the first two years of NTBC treatment versus NTBC serum concentration in patients who were followed for at least two years. Pathological values were defined as >1 mmol/mol creatinine for U-succinylacetone, >0.1 µmol/l for P-succinylacetone, ≤ 0.58 nkat/g Hgb for PBG-synthase and >23 mmol/mol creatinine for U-5-ALA.

Nitisinone in comparison to diet treatment
The probability of survival for HT-1 patients on nitisinone treatment and diet in the NTBC Study was compared with the survival estimates in the earlier international survey of 108 patients with HT-1 treated with a diet restricted in tyrosine and phenylalanine alone (van Spronsen et al. 1994).
The two- and four-year survival probabilities for patients who started nitisinone treatment at < 2 months were 88% and 88%, respectively, the 2-and 4-year survival probabilities for the patients of the historical control (van Sponsen et al, 1994, dietary treatment) being 0-2 months at onset of symptoms, 29% and 29%, respectively.
The survival probabilities for the patient group on nitisinone with a start of treatment-age between 2-6 months were calculated to be 95%. In comparison, the corresponding values for two and four years in the historical values determined by Van Spronsen et al. (1994) were 74% and 65% respectively .

However, two- and four-year survival probabilities for patients with start of treatment beyond 6 months (nitisinone) and start of symptoms beyond 6 months of age (van Spronsen), respectively, appear to differ only marginally for dietary therapy + nitisinone and dietary therapy only.

The Table below shows the experiences from the Quebec NTBC Study Group (Mitchel GA, personal communication) on a subset of patients from the NTBC Study. These data show the clear impact resulting from the introduction of nitisinone treatment compared with diet alone. The hospitalisations and neurological crises were eliminated, the number of liver transplants was markedly reduced and no deaths occurred except after transplantation.

	Non-nitisinone treated	**During nitisinone treatment**	**p-value***
Patients			
Number	27	34	
Follow-up, pat months	1562**	1529	
Hospitalisations for HT-1 complications			
Number	190	0***	<0.001
Duration, pat months (% of total)	69 (4.4%)	0 (0%)	<0.001
Neurological crises			
Number	92	0	<0.001
Duration, pat months	33.5	0	<0.001
Hepatic transplantation			
Number	19	5	<0.001
Transplants (per patient year)	0.146	0.039	
Age at transplant (months)	24.8 ± 4.1 (4.9 – 78.6)	41.3 ± 12.8 (34.8 – 90.6)	
Deaths			
Total	10	2	0.008
Pre-transplantation	8	0	0.003
Number per patient year	0.061	0	
Age at death (months)	12.6 ± 3.3	-	
Post-transplantation	2	2	0.40
AGE AT DEATH (MONTHS)	5 ; 25	89 ; 104	

- Discussion on clinical efficacy

The NTBC Study has not been designed according to GCP rules. In addition, since HT-1 is a life-threatening disease and nitisinone was considered as the only effective treatment besides liver transplantation, no control group was included. Considering that HT-1 is a very rare disease, the number of patients included in the clinical programme, reached through a high number of centres over many years is considered to be sufficient for a marketing authorisation approval under exceptional circumstances.

Nitisinone treatment in the doses used in the NTBC Study almost completely prevents the formation of the toxic metabolites. The risk for death, or the need for liver transplantation due to liver failure, appear to be small in patients in whom nitisinone treatment is started before six months of age. The risk of hepatocellular carcinoma is also reduced if nitisinone treatment is started early, before the age of 12 months. The risk for life-threatening porphyria-like crises is almost eliminated.

Some areas have not been investigated in great detail in the NTBC Study. For example, there were signs of improved kidney function in a small subgroup of patients with renal tubular dysfunction. It was also suggested that a low thrombocyte count could be related to liver disease, and that these effects were eliminated by nitisinone treatment. The general well-being has also been reported to have improved significantly, but quality of life assessment scores were not administered. Since the international survey by van Spronsen et al. used as a control in the evaluation of the NTBC Study mainly covers an earlier time period and was designed differently, statistical comparisons are not valid. However, the difference between the outcome in the survey, in which the patients had been treated with diet only, and the outcome in the

NTBC Study, in which the patients had been treated with nitisinone and diet, is suggestive: the efficacy of the nitisinone is also confirmed by the Quebec NTBC Study group, which reported a dramatic change in treatment outcome after nitisinone treatment was introduced.
It is concluded that nitisinone seems to be an effective treatment for HT-1, particularly if started early before there is irreversible liver damage.

Clinical safety

- Patient exposure

The total exposure in the NTBC study includes more than 1300 patient years, as summarised in the Table below. The safety evaluation is mainly based on a total of 441 patient years from the NTBC Study (median treatment time 675 days for the total population). During the time period covered by the main analysis of the NTBC Study, 24 patients received nitisinone without being included in the study. After the main analysis of the NTBC Study, 21 August 1997, until 30 April 2001, three periodic safety update reports were prepared, based on a total of 927 patient years of nitisinone treatment in 365 patients.

Patient exposure

	NTBC Study Rep (N=207)	**Safety Add (N=24)**	**PSUR 97-98 (N=266)**	**PSUR 99 (N=282)**	**PSUR 00-01 (N=318)**
Median treatment time	675 days	156 days	497 days	365 days	486 days
Min treatment time		1 day	2 days	3 days	6 days
Max treatment time		707 days	497 days	365 days	486 days
Mean treatment time		194 days	397 days	331 days	440 days
Total exposure	441 patient yrs	13 patient yrs	289 patient yrs	255 patient yrs	383 patient yrs

- Adverse events

All recorded adverse events in the safety evaluations are listed in the Tables below.

Number and percentage of patients with adverse drug reactions,
i.e. adverse events that may be causally related to treatment with nitisinone.

WHO body class	**WHO preferred term**	**N. of patients (% of all)**				
		NTBC Study Rep (N=207)	**Safety Addendum (N=24)**	**PSUR 97-98 (N=266)**	**PSUR 99 (N=282)**	**PSUR 00-01 (N=318)**
Body as a whole, general disorder	Anaemia	-	-	-	1 (0.4%)	-
Platelet, bleeding and clotting disorders	Thrombocytopenia	3+3* (3%)	-	2 (0.8%)	1 (0.4%)	-
Skin and appendages Disorders	Dermatitis exfoliative	2 (1.0%)	-	-	1 (0.4%)	-
	Pruritus	3 (1.4%)	-	-	-	-
	Rash erythematous	-	-	-	1 (0.4%)	-
White cell and RES Disorders	Granulocytopenia	2 (1.0%)	-	2 (0.8%)	-	-
	Leucocytosis	-	-	-	-	1 (0.3%)
	Leucopenia	4 (2%)	-	-	1 (0.4%)	-
Vision disorders	Blepharitis	2 (1.0%)	-	-	-	-
	Conjunctivitis	4 (2%)	-	1 (0.4%)	1 (0.4%)	1 (0.3%)
	Corneal opacity	4 (2%)	-	4 (1.5%)	3 (1.1%)	-
	Eye pain	3 (1.2%)	-	2 (0.8%)	2 (0.7%)	-
	Keratitis	5 (2%)	-	3 (1.1%)	2 (0.7%)	1 (0.3%)
	Photophobia	4 (2%)	-	1 (0.4%)	1 (0.4%)	-

*Serious AE

Number and percentage of patients with adverse events unlikely to be causally related to treatment with nitisinone.

WHO body class	**WHO preferred term**	**N. of patients (% of all)**

		NTBC Study Rep (N=207)	Safety Addendum (N=24)	PSUR 97-98 (N=266)	PSUR 99 (N=282)	PSUR 00-01 (N=318)
Body as a whole, general	Anaemia	-	-	-	-	1* (0.3%)
Disorders	Death	1* (0.5%)	1* (4%)	2* (0.8%)	1* (0.4%)	-
	Pyrexia	-	-	-	1 (0.4%)	-

WHO body class	WHO preferred term	N. of patients (% of all)				
		NTBC Study Rep (N=207)	Safety Addendum (N=24)	PSUR 97-98 (N=266)	PSUR 99 (N=282)	PSUR 00-01 (N=318)
Cardiovascular disorders, General	Cyanosis	1* (0.5%)	-	-	-	-
Central and peripheral nervous system disorders	Convulsions	3* (1.4%)	-	-	-	-
	Encephalopathy	-	1* (4%)	-	-	-
	Headache	1 (0.5%)	-	1 (0.4%)	-	-
	Hyperkinesia	1 (0.5%)	-	-	1 (0.4%)	1 (0.3%)
Gastro-intestinal system disorders	Abdominal pain	-	-	1 (0.4%)	-	-
	Constipation	-	-	-	1 (0.4%)	-
	Diarrhoea	1 (0.5%)	-	-	-	-
	Enanthema	1 (0.5%)	-	-	-	-
	Gastritis	1 (0.5%)	-	-	-	-
	Gastroenteritis	1 (0.5%)	-	-	-	-
	GI haemorrhage	1* (0.5%)	-	1* (0.4%)	-	-
	Melaena	-	-	1 (0.4%)	-	-
	Tooth discoloration	1 (0.5%)	-	-	-	-
Liver and biliary system disorders	Alfa-fetoprotein incr	-	-	-	-	2 (0.6%)
	Hepatic enzymes incr	-	-	2 (0.8%)	-	-
	Hepatic function dis	-	-	1 (0.4%)	-	-
	Liver cirrhosis	-	-	-	2* (0.7%)	-
	Liver enlargement	-	-	1 (0.4%)	-	-
	Liver failure	14* (7%)	-	10* (4%)	6* (3%)	6* (1.9%)
	Porphyria	1* (0.5%)	-	-	2* (0.7%)	-
Metabolic and nutritional disorders	Dehydration	1 (0.5%)	-	-	-	-
	Hypoglycaemia	1 (0.5%)	-	-	-	1 (0.3%)
	Thirst	1 (0.5%)	-	-	-	-
Musculo-skeletal system disorders	Fracture pathological	1 (0.5%)	-	-	-	-
Neoplasm	Brain neoplasm benign	1* (0.5%)	-	-	-	-
	Hepatic neoplasm**	6* (3%)	1* (4%)	1* (0.4%)	2* (0.7%)	1* (0.3%)
	Hepatic neoplasm mali	10* (5%)	-	2* (0.8%)	2* (0.7%)	4* (1.3%)
	Lymphoma malignant	-	-	1* (0.4%)	-	1* (0.3%)
Platelet, bleeding & clotting disorders	Epistaxis	2 (1.4%)	-	-	-	-
	Haematuria	-	-	-	1 (0.4%)	-
Psychiatric disorders	Nervousness	1 (0.5%)	-	-	1	-
	Somnolence	1 (0.5%)	-	-	-	-
Reproductive disorders, female	Amenorrhoea	1 (0.5%)	-	-	-	-
Resistance mechanism disorders	Infection	1 (0.5%)	-	2* (0.8%)	1 (0.4%)	1 (0.3%)
	Otitis	1 (0.5%)	-	-	-	-
	Septicaemia	-	-	1* (0.4%)	-	-
Respiratory system disorders	Bronchitis	1 (0.5%)	-	-	-	-
	Respiratory insuffi	-	1* (4%)	-	-	-
Skin and appendages disorders	Alopecia	1 (0.5%)	-	-	1 (0.4%)	-
	Rash maculo-papular	1 (0.5%)	-	-	-	-
	Skin dry	1 (0.5%)	-	-	-	-
Urinary system disorders	Hyperkalemia	-	-	-	-	1 (0.3%)
	Renal tubular disorder	-	-	-	-	1 (0.3%)
Vision disorders	Cataract	1 (0.5%)	-	2 (0.8%)	-	-
	Retinal disorder	-	-	-	1* (0.4%)	-
Other	Other					
	- elective liver transpl	7* (3%)	1* (4%)	5* (2%)	3* (1.1%)	1* (0.3%)
	- liver transpl for unknown reason			1* (0.4%)	1* (0.4%)	1* (0.3%)

* Serious AE

The most frequently reported adverse event was visual disorder (21 cases), including eye pain, keratitis, conjunctivitis, corneal opacity and photophobia. It is well recognised that a high plasma tyrosine concentration may cause corneal lesions. Corneal lesions, due to the formation of tyrosine crystals, develop in some species treated with nitisinone, e.g. rat and dog, but not in the monkey and mouse. In the main analysis of the NTBC study too, the most common adverse events reported was eye disorder, including conjunctivitis, photophobia, eye pain, keratitis, and corneal lesion, which were reported in a total of 14 patients. The eye symptoms were all transient, but in some patients they reappeared. For the majority of these cases, the final outcome of the eye symptoms was not reported. These events were regarded to be non-serious. They were considered to have a possible causal relationship to the nitisinone treatment: patients with plasma tyrosine concentration above 800µmol/l on one or more occasions, had a significantly increased risk of developing eye symptoms compared with those who never exceeded that plasma tyrosine level. Eye problems during nitisinone treatment disappeared either spontaneously or after improved compliance with the diet.

- Serious adverse event/deaths/other significant events

The 49 serious adverse events reported in the pivotal study included liver failure (14), HCC (10 verified, 6 not verified), multiorgan failure (1), elective liver transplantation (7), and thrombocytopenia (3). The three cases of thrombocytopenia, which were all transient, were the only serious adverse events considered to have a possible causal relationship to nitisinone, whereas the other events mentioned above are well known manifestations of HT-1 and not considered to be causally related to nitisinone treatment.

During the time period covered by the NTBC Study, a total of 5 serious adverse events were reported in a *Safety Addendum* regarding 24 patients who received nitisinone without being included in the NTBC Study. All these events were well-known manifestations of HT-1: encephalopathy (1), hepatic neoplasm (1), respiratory insufficiency (1), and elective liver transplantation (1). In addition, there was one report of a patient who died without further details known, and one report of treatment being discontinued because of the lack of efficacy on renal complications after liver transplantation.
In the three Periodic Safety Update Reports, the serious adverse events (61 events) were liver failure (22), hepatic neoplasm (8 verified carcinoma, 4 suspected but not verified), elective liver transplantation (9), liver transplantation for unknown reason (3), porphyria (2), liver cirrhosis (2), multiorgan failure (1), gastrointestinal haemorrhage (1). All these events are well-recognised manifestations or outcomes of HT-1. The serious adverse event list also includes 3 cases of infection, 1 case of retinal degeneration, 1 case of anaemia related to thalassemia, 2 cases of death without further details known, and one patient who first reported treatment for Hodgkin's lymphoma but died after relapse two years later.

A total of 34 patients died during treatment with nitisinone, mostly due to liver failure (N=19), or gastrointestinal bleeding, encephalopathy or liver cancer (N.=5), 3 due to fever or infection, 2 due to multiorgan failure, 2 due to porphyric crisis or respiratory insufficiency, 1 due to lymphoma and 2 due to unknown reasons.
A total of 61 patients were liver transplanted, 17 due to liver failure, 13 due to liver cancer, in 17 cases an elective transplantation was performed, 11 patients were transplanted because of suspected (not verified) liver cancer, and in 3 cases the reasons are unknown.

- Laboratory findings

In the main analysis of the NTBC Study there were 3 cases of severe thrombocytopenia considered to have a possible causal relationship to treatment. For 7 patients there were reports of leucopenia, thrombocytopenia or a combination of both, which were regarded as not serious adverse events. These events were considered possibly to have a causal relationship to the nitisinone treatment. The tests returned to normal in all cases, some of them spontaneously and some after temporary dose reduction. Some of these cases were reported to occur following infection.
In the three PSUR, 2 cases of granulocytopenia, 2 cases of thrombocytopenia, 1 case of acute pancytopenia, and 1 case of mild leukocytosis on three separate occasions may possibly be causally related to nitisinone treatment. The cases are regarded as non-serious adverse events.

- Safety in special populations

Not addressed

- Immunological events

None reported

- Safety related to drug-drug interactions and other interactions

No AE related to drug-drug interaction have apparently been reported.

- Discontinuation due to adverse events

In the NTBC Study, no patients were withdrawn from the study because of adverse events considered to have a causal or possible causal relationship to the treatment. In the pivotal study 37 out of 38 patients with serious AEs discontinued nitisinone treatment and were withdrawn from the study. However, all these 38 serious adverse events were considered to be complications of HT-1, and to have no causal relationship to nitisinone treatment. There were 3 cases of severe thrombocytopenia considered to have a possible causal relationship to treatment, and another 8 different serious adverse events which were not considered likely to have a causal relationship to treatment. All these 10 patients (one patient had 2 events) remained in the study taking nitisinone, the serious adverse event being resolved.

- Post marketing experience

Nitisinone was authorized in USA in January 2002.
Also based on this experience, a safety update with respect to the data contained in the dossier has been provided. It includes information up to February 28th, 2004.

A total of 566 HT-1 patients have been treated with nitisinone for varying periods of time since February 1991. A total of 439 of the patients remained on nitisinone treatment as of December 31st, 2003. The outcome for the remaining patients is as follows: death (44); transplantation (70); withdrawal (5) and lost to follow-up (6).

The mean exposure is 1555 days (or 4.3 years) with a range of 1-4695 days. This corresponds to a total nitisinone exposure of 2331 patient years. The mean age at start of treatment for the exposed population is 1.7 years (range 0- 21.7 years, n=554, age at start of treatment missing for 12 patients). Fifty percent of the population started treatment below the age of 6 months, 28% started between the age of 6 and 24 months with the remaining (22%) starting at an age greater than 24 months.
Until mid 1993, the recommended dose was 0.6mg/kgbw; since then it is 1mg/kgbw. Due to the individual variation and the multiple parameters influencing the clinical outcome, dosing is based on the composite biochemical response.

A total of 44 deaths have been reported. In the majority of the cases (29 of 44 cases, 66%), the patient died due to liver associated diseases such as hepatocellular carcinoma and or hepatic failure. However, in the remaining cases the underlying liver disease was likely a contributing factor for death, even if the direct cause of death was attributed to another cause. The remaining deaths were as follows: in seven cases it was not possible to obtain sufficient information to classify the cause of death; two cases were due to infection or septicaemia; one case to respiratory insufficiency; one case to encephalopathy; one case to GI haemorrhage; one case to malignant lymphoma and one case to porphyria. Time on nitisinone treatment for patients who died is available for 33 patients. The mean time on treatment is 9.6 months (range: 1 day to 76.6 months).

A total of 70 patients underwent a liver transplantation. Seventeen (24.2%) due to hepatocellular carcinoma (HCC), 17 (24.2%) to liver failure or cirrhosis, 12 were due to suspected but not verified HCC, and 24 (34.3%) were prophylactic (elective) transplantations due to risk for liver cancer or liver failure. The time on nitisinone treatment is available for 68 patients. The mean time on treatment was 24.4 months (median 14.4 months, range 2 days to 90.7 months, see Table 9.). The mean age at start of treatment was 38.5 months, (range 0.65 months to 15.9 years, n=69).

A total of 24 cases of HCC have been identified in the population of 566 patients. One patient (Patient #52) had been diagnosed with HCC prior to treatment with nitisinone and underwent transplantation two weeks after the initiation of therapy. The majority of the patients who have developed HCC had nitisinone treatment initiated after the age of 12 months.
The mean age at start of treatment was 73.4 months ((6.1 years) range 1 month to 18.0 years). The mean time on nitisinone treatment was 27.7 months (range 0.5 month to 114.9 months (9.6 years)). The data indicate that the patients developing hepatocellular cancer started treatment at a rather late age in comparison to the whole study population. While 69% of patients without cancer started treatment below the age of 12 months, only 11% of the patients with hepatocellular cancer started treatment before the age of 12 months. Patients who initiate treatment after the age of 12 months have a significantly higher risk of developing HCC (Fisher exact test, $p<0.000001$). The relative risk for developing HCC is 13.52 (95% CI: 4.07 – 44.89) if treatment is initiated after the age of 12 months. Dose and time on nitisinone treatment and dose in patients who developed HCC did not differ statistically from the population not developing cancer (data not shown).

In addition to hepatic failure, malignancies and liver transplants, a total of 29 patients reported other serious adverse events: The remaining cases of serious adverse events that occurred in more than one patient were: convulsions (6); anaemia (2); unknown cause of death (4); GI haemorrhage (2); septicaemia (2); infection (2); thrombocytopenia (3) and porphyria (3).

Of the non serious adverse events, 83 were visual disorders (most common were blepharitis, corneal deposits, corneal opacity, corneal ulceration, keratopathy or keratitis, conjunctivitis, eye pain or photophobia) and 17 events were of the haematological system (granulocytopenia, leucopenia, thrombocytopenia and leucocytosis).
Symptoms of visual disorders were found to be transient but were seen to re-occur in some patients. However, it was found that these symptoms resolved with increased adherence to the dietary restriction of tyrosine and phenylalanine. It was determined that in patients who had plasma tyrosine concentrations above 800µmol/L, at one or more occasion, had an increased risk of developing eye symptoms. The haematological adverse events may be related to nitisinone treatment. However, no patient has terminated nitisinone treatment as a result of either the visual or haematological adverse events.
A total of six cases of convulsions have been reported and all were rated as serious. The causes of these were as follows: febrile (2); idiopathic (2) hypoglycaemia (1); and not specified (1). The two cases of idiopathic convulsions were placed on carbamazepine and phenobarbital respectively and no further seizures have been reported. There are too few cases to make any meaningful causality assessment.
In comparison with the earlier reported adverse events, the major finding during the latest reporting period is that there is a continuing decrease in the number of patients with liver malignancies or liver transplants. During the latest period, no patients have undergone liver transplantation and no patient has reported liver failure.

- Discussion on clinical safety

Based on the data provided, nitisinone treatment does not appear to raise significant safety concerns. All serious adverse events appear to be well recognised manifestations or outcomes of HT-1 such as life-threatening liver failure or cancer, respiratory failure, porphyria-like neurological crisis and renal tubular dysfunction (Fanconi syndrome).
The most frequently reported adverse events (AE) were visual disorders, which were regarded to be non-serious and to disappeared either spontaneously or after improved compliance with the diet.
Besides eye symptoms, the adverse events, which may be attributed to treatment with nitisinone include thrombocytopenia and leucopenia and skin symptoms.
The AEs possibly related to nitisinone treatment are reported in the SPC, 4.8. Furthermore, in section 4.4 of the SPC, warnings are given addressing the risk of visual disorders, liver function monitoring and platelets/white blood cell monitoring.
Convulsions might represent a new signal which has not been reported during the safety evaluation of the pivotal evaluation period. This would have to be monitored in the future, in association with data on neurological development.
Furthermore, due to the limited number of patients with sufficient follow-up, the long-term safety profile of nitisinone is not well-known, especially the risk of chronic renal or liver disease in patients with the chronic

form of the disease. A post-marketing surveillance programme is aimed at providing such additional data. (see letter of undertaking).

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

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

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product. The applicant gave a Letter of Undertaking and committed to resolve these as Follow Up Measures after the opinion, within an agreed timeframe.

Non-clinical pharmacology and toxicology

The available pharmacodynamic data and the increase in Tyr plasma levels clearly demonstrate the effect of nitisinone on Tyr catabolism, which is provided by the inhibition of the HPPD enzyme.
Only limited pharmacokinetic data are available in animals. The cytochrome P450 system is involved in the metabolism of nitisinone and findings in mice are consistent with liver enzyme induction.
No clinically significant effect is expected with regard to inhibition of metabolism of xenobiotics or endogenous compounds other than tyrosine. In addition no effect is expected on the tyrosine kinase complex.
In repeat-dose toxicity studies, the main target organ was the eye, with corneal lesions resulting probably from the accumulation of tyrosine. However, as not all tissues from the 12- and the six-month studies in rodents have been examined histologically, further investigations on the availability of these additional samples for histology have been required as a specific obligation.
The overall genotoxicity data suggest that there is no evidence of *in vivo* genotoxicity.
The results of the available long-term studies do not suggest a tumorigenic potential. However, as no proper tests for carcinogenic potential have been performed, no definitive conclusion can be made.
Reproductive and developmental toxicity studies have shown malformations in mice and rabbit and this is reflected in the SPC. Nitisinone should not be used during pregnancy. Mothers receiving nitisinone should not breast-feed since a transfer of nitisinone in the maternal milk is possible (see SPC sections 4.3 and 5.3).

Efficacy

Nitisinone treatment in the doses used in the NTBC Study almost completely prevents the formation of the toxic metabolites. The risk for death, or the need for liver transplantation due to liver failure, appear to be small in patients in whom nitisinone treatment is started before six months of age. The risk of hepatocellular carcinoma is also reduced if nitisinone treatment is started early, before the age of 12 months. The risk for life-threatening porphyria-like crises is almost eliminated.
Some areas have not been investigated in great detail in the NTBC Study such as improvement of patients with renal tubular dysfunction, relation between thrombocytopenia and liver disease and their possible recovery under nitisinone treatment, the assessment of the improved quality of life. Since the international survey by van Spronsen et al. used as a control in the evaluation of the NTBC Study mainly covers an earlier time period and was designed differently, statistical comparisons are not valid. However, the difference between the outcome in the survey, in which the patients had been treated with diet only, and the outcome in the NTBC Study, in which the patients had been treated with nitisinone and diet, is suggestive: the efficacy of the nitisinone is also confirmed by the Quebec NTBC Study group, which reported a remarkable change in treatment outcome after nitisinone treatment was introduced.
Overall, nitisinone seems to be an effective treatment for HT-1, particularly if started early before there is irreversible liver damage.

Safety

Based on the data provided, nitisinone treatment does not appear to raise significant safety concerns. All serious adverse events appear to be well recognised manifestations or outcomes of HT-1.
The most frequently reported adverse events (AE) were visual disorders, which were regarded to be non-serious and to disappeared either spontaneously or after improved compliance with the diet.
Besides eye symptoms, the adverse events, which may be attributed to treatment with nitisinone include thrombocytopenia and leucopenia and skin symptoms.
The AEs possibly related to nitisinone treatment are reported in the SPC, 4.8. Furthermore, in section 4.4 of the SPC, warnings are given addressing the risk of visual disorders, liver function monitoring and platelets/white blood cell monitoring.
However, due to the limited number of patients with sufficient follow-up, the long-term safety profile of nitisinone is not well-known, especially the risk of chronic renal or liver disease in patients with the chronic form of the disease.

Benefit/risk assessment

The submitted non-clinical data are in general of poor quality compared with the current standard (not always compliant with GLP). However, taken together, the non-clinical data seem to be sufficient to make a risk assessment. Therefore, in view of the severity of the disease, its rare occurrence, the lack of therapeutic alternatives and the obvious clinical efficacy, the deficiencies of the non-clinical part of the dossier are still considered acceptable for recommending a marketing authorisation under exceptional circumstances. Further investigations on the availability of additional samples for histology in long-term rodent toxicology studies have been required. A letter of undertaking was provided by the applicant to resolve this issue as a specific obligation.

The benefit-risk profile of nitisinone in the treatment of HT-1 seems to be favourable. Nitisinone appears to provide a better outcome for patients than that reported in literature with diet alone. This is reflected by both biochemical marker normalisation and clinical improvement: the risk for death, liver transplantation due to liver failure or porphyria crises seems to be reduced, particularly in patients starting nitisinone before six months of age. There are no apparent major safety concerns: as addressed in the Special warnings section (4.4) of the SPC, the risk of leukopenia and thrombocytopenia must be carefully monitored and the risk of visual disorders due to the formation of tyrosine crystals must be avoided through strict compliance to a diet deficient in phenylalanine and tyrosine and the monitoring of plasma tyrosine levels. Convulsions might represent a new signal which has not been reported during the safety evaluation of the pivotal evaluation period. This would have to be monitored in the future, in association with data on neurological development. The risk of HCC indeed appears to be lower if treatment is initiated as early as possible. However these promising findings have some limitations, as they come from one single uncontrolled study conducted in several centres (87 in 25 countries).

Clinical specific obligations that should be covered on an ongoing basis by a post-marketing surveillance programme for the use of nitisinone in the treatment of Hypertyrosinaemia type 1, include monitoring liver, renal. haematological, neurological and ophthalamic status. In a letter of undertaking, the applicant committed to perform these analyses as specific obligations and provide the details of these commitments in due time.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk ratio of Orfadin in the treatment of "Treatment of patients with confirmed diagnosis of hereditary tyrosinemia type 1 (HT-1) in combination with dietary restriction of tyrosine and phenylalanine" was favourable and therefore recommended the granting of the marketing authorisation **under exceptional circumstances,** since the indication for which the product is intended is encountered so rarely that the Applicant cannot be reasonably expected to provide comprehensive evidence (Directive 2001/83/EC as amended). In addition the medicinal product would be under restricted medicinal prescription (see section 4.2 of the SPC). In a letter of undertaking the applicant committed to perform a number of specific obligations and follow-up measures with a time schedule for fulfilment.