Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

Australian Public Assessment Report for Nitisinone

Proprietary Product Name: Orfadin

Sponsor: Orphan Australia Pty Ltd

January 2011

TGA Health Safety Regulation

About the Therapeutic Goods Administration (TGA)

- The TGA is a division of the Australian Government Department of Health and Ageing, and is responsible for regulating medicines and medical devices.
- TGA administers the *Therapeutic Goods Act 1989* (the Act), applying a risk management approach designed to ensure therapeutic goods supplied in Australia meet acceptable standards of quality, safety and efficacy (performance), when necessary.
- The work of the TGA is based on applying scientific and clinical expertise to decision-making, to ensure that the benefits to consumers outweigh any risks associated with the use of medicines and medical devices.
- The TGA relies on the public, healthcare professionals and industry to report problems with medicines or medical devices. TGA investigates reports received by it to determine any necessary regulatory action.
- To report a problem with a medicine or medical device, please see the information on the TGA website.

About AusPARs

- An Australian Public Assessment Record (AusPAR) provides information about the evaluation of a prescription medicine and the considerations that led the TGA to approve or not approve a prescription medicine submission.
- AusPARs are prepared and published by the TGA.
- An AusPAR is prepared for submissions that relate to new chemical entities, generic medicines, major variations, and extensions of indications.
- An AusPAR is a static document, in that it will provide information that relates to a submission at a particular point in time.
- A new AusPAR will be developed to reflect changes to indications and/or major variations to a prescription medicine subject to evaluation by the TGA.

Contents

I. Introduction to Product Submission

Submission Details

Type of Submission	New Chemical Entity
Decision:	Approved
Date of Decision:	15 October 2010
Active ingredient(s):	Nitisinone
Product Name(s):	Orfadin
Sponsor's Name and Address:	Orphan Australia Pty Ltd 300 Frankston-Dandenong Road Dandenong Vic 3175
Dose form(s):	Capsule
Strength(s):	2 mg, 5 mg and 10 mg
Container(s):	High density polyethylene bottle with a tamper proof low density polyethylene cap
Pack size(s):	60
Approved Therapeutic use:	For the treatment of patients with hereditary tyrosinaemia type 1 in combination with dietary restriction of tyrosine and phenylalanine.
Route(s) of administration:	Oral
Dosage:	The dose of nitisinone should be adjusted individually. The recommended initial dose is 1 mg/kg body weight/day divided in 2 doses administered orally. The capsule may be opened and the content suspended in a small amount of water or formula diet immediately before intake.
ARTG Numbers:	164163, 164173, 164174

Product Background

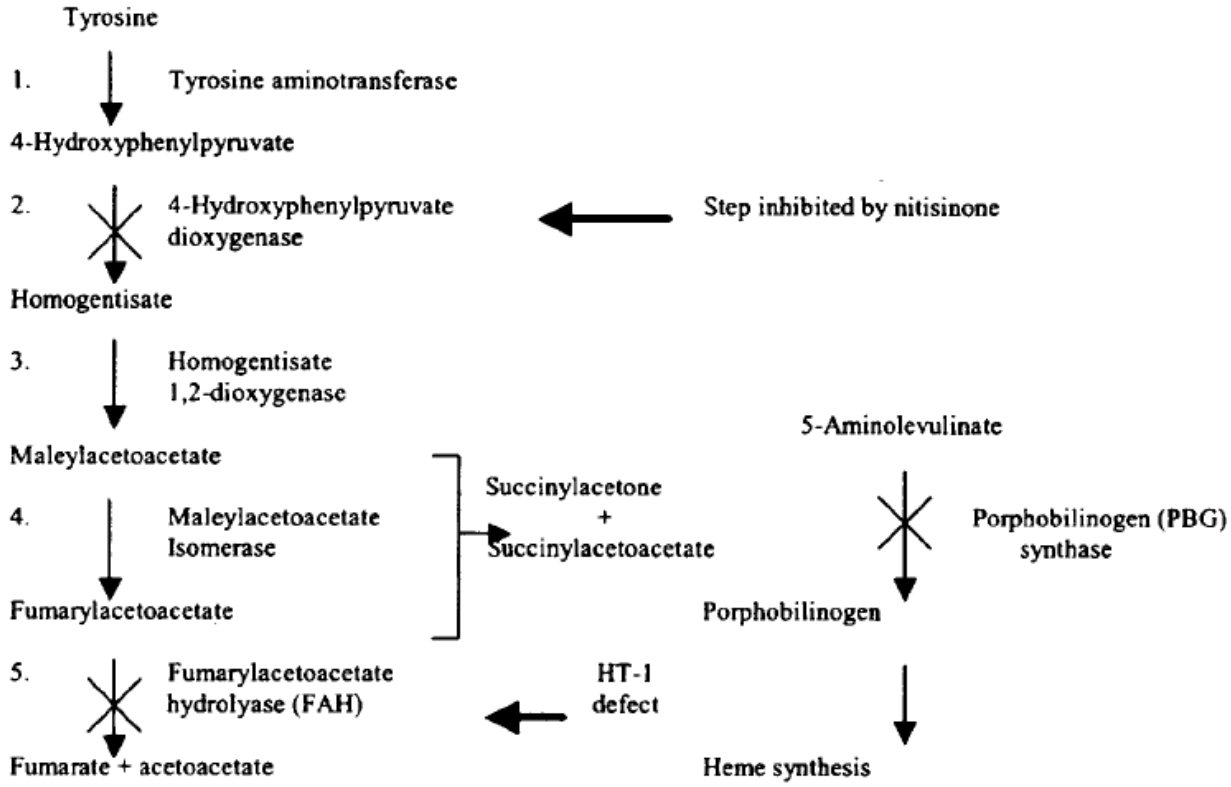
This is an application from Orphan Australia to register nitisinone (Orfadin) for the treatment of hereditary tyrosinaemia type I (HT-1) in capsule strengths 2 mg, 5 mg, and 10 mg.

HT-1 (also known as hepatorenal tyrosinaemia) is an autosomal recessive inherited disease of tyrosine metabolism resulting from a deficiency of the enzyme fumarylacetoacetate (FAH) which is the final enzyme in the tyrosine catabolic pathway (Figure 1). This deficiency leads to accumulation of the toxic intermediates maleylacetoacetate and fumarylacetoacetate and their toxic metabolites succinylacetone and succinylacetoacetate. Succinylacetone causes systemic inhibition of porphobilinogen (PBG) synthase, resulting in increased concentrations of δ-aminolevulinate. Detection of urinary succinylacetone and PBG-synthase inhibition in the blood are important diagnostic tests for HT-1 [Arora et al., 2006] and the detection of urinary succinylacetone is considered to be "pathognomic" [McKeirnan 2006].[1,2] The

[1] Arora N et al. Cardiomyopathy in tyrosinemia type I is common but usually benign. J Inherit Metab Dis 2006; 29: 54-57.

diagnosis is confirmed by measuring FAH activity in fibroblasts or lymphocytes or by demonstrating the presence of pathogenic mutations in FAH [McKeirnan, 2006].[2]

Figure 1: Catabolic pathway of tyrosine and its association with porphyrin synthesis in HT-1.



The clinical features of the condition include severe liver dysfunction, impaired coagulation, painful neurological crises, renal tubular dysfunction, rickets, failure to thrive, increased risk of hepatocellular carcinoma, and "boiled cabbage" odour.[3] The dominant feature of the condition is liver disease (both acute and chronic). The severity of the disease is related to the age of onset. The acute form usually presents during the first 6 months of life with liver failure, ascites, and coagulopathy and the risk of death due to liver failure in the first year of life is high. In the subacute form, onset is in the first 6-12 months of life and the symptoms are similar to the acute form but the clinical course is less rapid. In the chronic form, the onset occurs after 12 months of age and is usually characterised by hypophosphataemic rickets, cirrhosis and renal tubular dysfunction. In children surviving beyond infancy there is a considerable risk of developing hepatocellular carcinoma usually in the first two decades of life [Lindstedt et al., 1992; Arora et al., 2006].[1,4] Prior to nitisinone, treatment consisted of a tyrosine and phenylalanine restricted diet. The diet results in nutritional improvement and

[2] McKieran PJ. Nitisinone in the treatment of hereditary tyrosinaemia type 1. Drugs 2006; 66: 743-750.

[3] Harrison's Principles of Internal Medicine, 16th Edition, 2005.

[4] Lindstedt S, Holme E, Lock EA, Hjalmarson O, Standvik B. Treatment of hereditary tyrosinemia type I by inhibition of 4-hydroxy-phenyl-pyruvate dioxygenase. Lancet 1992: 34A: 8 13-8 I 7.

stabilisation of renal function but does not affect liver function or prevent the risk of hepatocellular carcinoma. Prior to the availability of nitisinone, liver transplantation was considered to be the treatment of choice [Arora et al., 2006].[1] The disease is estimated to have an overall worldwide incidence of 1/100,000 live births, except for a particular region in Quebec, Canada, where 1/20 of the population are carriers due to a founder effect [McKiernan, 2006].[2]

Regulatory Status

Orfadin (nitisinone) was designated as an Orphan Drug for the treatment of hereditary tyrosinaemia type 1 by the TGA on 30 October 2008. The sponsor estimates that there are currently about 10 patients in Australia with the condition. Orfadin is currently being supplied to Australian patients under the provisions of the Special Access Scheme (SAS).

Orfadin was first approved in the US on 18 January 2002, and subsequently in the European Union (EU) via the centralised procedure on 21 February 2005. Orfadin is also available for "named-patient" use in other countries. The drug is not registered in Canada or New Zealand. In the EU, Orfadin is indicated for:

the treatment of patients with confirmed diagnosis of hereditary tyrosinemia type 1 (HT-1) in combination with dietary restriction of tyrosine and phenylalanine.

In the USA, Orfadin is indicated for:

as an adjunct to dietary restriction of tyrosine and phenylalanine in the treatment of hereditary tyrosinemia type I.

Product Information

The approved product information (PI) current at the time this AusPAR was prepared can be found as Attachment 1.

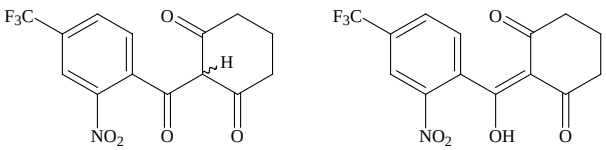
II. Quality Findings

Drug Substance (active ingredient)

This submission is to register Orfadin capsules which contain 2 mg, 5 mg and 10 mg of nitisinone. This drug substance is a new chemical entity that is chemically synthesised. The drug substance is also known as NTBC, an abbreviation of its chemical name.

There are no compendial monographs for the drug substance or for finished products containing this drug substance.

The drug substance is achiral, but can exist in two conformations.



2-[2-nitro-4-trifluoromethylbenzoyl]cyclohexane-1,3-dione

$C_{14}H_{10}F_3NO_5$ Molecular mass = 329.23

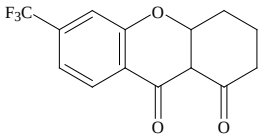
CAS # = [104206-65-7] pK_a = 4.4

Aqueous solubility = 5 mg/mL (0.0005 %w/v, practically insoluble)

The solubility is pH dependent increasing from 1 mg/mL at pH 1 to 9.5 mg/mL at pH 7.2

Biopharmaceutical Classification System (BCS) Class 1 above pH 5.[5]

The drug substance is prone to intramolecular cyclisation at higher temperatures (40°C) to give the degradant 6-trifluoromethyl-1,2,3,4-tetrahydroxanthene-1,9-dione (oxotetrahydro-xanthone). Once manufactured, the drug substance is stored in amber glass bottles.



oxotetrahydroxanthone

No polymorphic forms have been observed. The drug substance is milled during the manufacture of the finished product and the particle size distribution of the drug substance itself is not a critical parameter.

The specification for nitisinone drug substance includes satisfactory limits for assay and related substances. The residual solvents are limited to at or below the limits allowed by International Conference on Harmonisation (ICH) guidance.

Drug Product

The capsules are to be manufactured by Apoteket Products and Laboratory AB in Sweden. The different strengths differ in that the amount of pregelatinised maize starch in the capsule is adjusted so that the fill weight is constant at 216 mg. The process involves simply milling of the drug substance, mixing with the other excipient (pregelatinised maize starch) and filling into size 3 white hard-gelatin capsules. Identification marks in black ink are added and these are different for each strength. The bulk capsules are bottled immediately after manufacture.

The drug substance in the capsules was found to undergo a temperature-dependent cyclisation on storage. In order to minimise this, the bottles of product are to be stored for the most part between 2-8°C with "Refrigerate. Do not freeze." and the rider that 'After first opening, may be removed from the refrigerator and stored below 25°C for a period of 3 months after which the product must be discarded'.

The specifications have acceptable expiry limits and release limits that allow for the change over the shelf life.

Stability data were provided to support an unopened shelf life of 2 years when stored between 2-8°C ("Refrigerate. Do not freeze.") in the bottles. The storage condition 'After first opening, may be removed from the refrigerator and stored below 25°C for a period of 3 months after which the product must be discarded' is also used.

Biopharmaceutics

Clinical Background

[5] The Biopharmaceutics Classification System (BCS) is a guidance for predicting the intestinal drug absorption provided by the U.S. Food and Drug Administration. According to the BCS, drug substances are classified as follows: Class I: high permeability, high solubility; Class II: high permeability, low solubility; Class III: low permeability, high solubility; Class IV: low permeability, low solubility.

The Phase III clinical efficacy studies were performed with capsules containing lactose rather than pregelatinised maize starch as a filler. Dosing during the Phase III clinical efficacy studies was with food.

Studies submitted

The submission included one bioavailability study to compare the bioavailability of the capsule containing lactose with an oral solution.

Study CCT/96/001 was a two-way crossover study in 10 subjects (all completed) performed in 1996. The study was of an appropriate design, and the test method used was mostly appropriate to determine the levels of sapropterin in the subject plasma samples. However, only limited data were provided to demonstrate the stability of nitisinone in plasma, and, if the quality control samples were checked against current acceptance criteria, two sample runs would have had to be rejected.

The results suggest bioequivalence.

- 90% confidence interval for the maximal plasma concentration (C_{max}) was 0.86-0.97 (ratio 0.92).
- 90% confidence interval for the area under the plasma concentration time curve from time zero to infinity ($AUC_{0-\infty}$) was 0.94-1.06 (ratio 1.00).

Justification for Not Providing Bioavailability Data

No bioavailability data were provided comparing the two proposed formulations to the Phase III clinical efficacy formulations, but it was accepted that they would be bioequivalent. The dissolution profiles were similar and nitisinone is a highly permeable drug substance and highly soluble (in relation to the BCS at pH 5 and above) and simple changes to the formulation are unlikely to affect bioavailability or the gastric emptying times as the capsules are given with food.[1] In addition to this the sponsor performed a population pharmacokinetic study comparing the pharmacokinetic results for patients on the lactose and pregelatinised starch formulations (not evaluated by the quality evaluator)

The sponsor did not perform a bioavailability study to determine the absolute bioavailability in humans. As mentioned above nitisinone is a highly permeable drug substance and highly soluble and the company used this as a justification for not performing such a study. This was accepted. Further to this, study CCT/96/001 against an oral solution was provided.

The same justification was provided and accepted in relation to the bioavailability of the different strength capsules.

No data were provided on the effect of food on the bioavailability. However, during the Phase III clinical efficacy studies all patients were co-administered the drug with food. It is recommended that the PI states that all dosing occur with food and this is indeed the case.

PSC Consideration

Details of this submission were presented at the 131st meeting of the Pharmaceutical Subcommittee (PSC) of the Advisory Committee on Prescription Medicines (ACPM) in March 2010. The PSC:

- Concluded that an absolute bioavailability study was not required for this product.
- Concluded that it should be recommended that all dosing occur with food.

Quality Summary and Conclusions

Approval of the application was recommended with respect to chemistry and quality control.

In relation to bioavailability:

1. The formulation of capsules used in the Phase III clinical efficacy studies contained lactose rather than pregelatinised maize starch as a filler. No bioavailability data were provided comparing the two formulations, but it was accepted that they would be bioequivalent. In addition to this the sponsor performed a population pharmacokinetic study comparing the pharmacokinetic results for patients on the lactose and pregelatinised starch formulations.
2. No data were provided on the effect of food on the bioavailability. However, during the Phase III clinical efficacy studies all patients were co-administered with food. It is recommended that the PI states that all dosing occur with food and this is indeed the case
3. No data were provided to investigate the absolute bioavailability of the capsules. Instead the sponsor provided a study (CCT/96/001) comparing the capsules containing lactose to an oral solution. This study indicated bioequivalence. However, as it was performed in 1996, it was not performed to current regulatory standards and the results should be taken as indicative rather than definitive. Having said that, nitisinone drug substance is highly permeable and highly soluble (in relation to the BCS at pH 5 and above) and simple changes to the formulation are unlikely to affect bioavailability.
4. The same (acceptable) justification was provided and accepted in relation to the relative bioavailability of the different strength capsules.

III. Nonclinical Findings

Introduction

Nitisinone was originally developed as a herbicide in the 1980s, but was abandoned when it was found to cause eye lesions in rats. Therefore, the toxicological program for nitisinone was developed according to the standards for herbicide development at that time. Moreover, most of the non-clinical data provided in submissions to the European Medicines Agency (EMEA) and the FDA have been derived from studies conducted with the herbicide technical grade material containing 90-92% nitisinone. However, the currently proposed drug substance is manufactured by the same route and processes as the herbicide except for a modification of the last purification step to yield a substance with purity ≥98%.

The Australian nitisinone submission is based on data reviewed by the EMEA and FDA but also includes further information provided to the EMEA during the review process and post-approval safety updates. This included histological analysis of archived samples in two rodent repeat dose toxicity studies, four new reproductive toxicity studies in mice and rabbits and an additional literature reference regarding ocular toxicity. These new studies are examined in detail in the main body of the report. However, assessment of the core nonclinical studies for nitisinone will be based on the EMEA and FDA reviews of the common dossier.

The toxicological program of nitisinone was deficient with respect to the quality of the majority of the studies submitted, many of which were not compliant with Good Laboratory Practice (GLP), and more importantly, did not include carcinogenicity studies for a substance likely intended for long-term use.

However, it should be noted that the toxicology studies were performed in several animal species chronically dosed and were able to identify the target organs of toxicity, as well as potential for genotoxicity, reproductive and developmental effects. The poor design and inadequate conduct of several studies submitted is reflective of the quality of studies

performed at the time (1980s) and this product's original development program as a herbicide.

Pharmacology

Primary pharmacodynamics

The primary pharmacological effect of nitisinone is the inhibition of the enzyme 4-hydroxy-phenylpyruvate dioxygenase (HPPD), an enzyme which precedes fumarulacetoacetate hydrolase (FAH) in the tyrosine catabolic pathway (Figure 1). Inhibition of HPPD reduces the accumulation of the toxic metabolites, succinylacetone (SA) and succinulacetoacetate (SAA), which are believed to be responsible for liver and renal failure in HT-1 patients, and inhibits porphobilinogen synthase, leading to the accumulation of 5-aminolevulinate, which is thought to be the cause of neurological disorders.

Pharmacology studies provided to support this primary effect were limited to three original non-GLP reports conducted in the 1980s and three literature references published by the same group of researchers in international journals from 1995 and 2000.

Nitisinone was shown to be a potent inhibitor of rodent hepatic HPPD *in vitro* (median inhibitory concentration [IC_{50}] @40 nM @13 ng/mL) and in rats, rabbits, dogs, and monkeys *in vivo*, based on elevated plasma tyrosine levels. While nitisinone is not irreversibly bound to HPPD, it is slow to dissociate from the HPPD-inhibitor complex, with a recovery of 37% over a 7 hour period. This HPPD inhibition was associated with elevated plasma tyrosine levels in all species, and an increase in hepatic tyrosine transaminase activity in rodents, potentially due to enzyme induction.

Published data from mouse mutation models show that in FAH-deficient mice, nitisinone reduces urinary SA levels and reverses the lethal nature of this mutation, although it does not necessarily protect against potential liver and kidney dysfunction. Neonatal death occurring in FAH deficient mice was prevented by induction of a second mutation resulting in a lack of HPPD activity. Animals in which the second mutation has been introduced were reported to develop normally without evidence of the liver or kidney pathology that is associated with HT-1. Overall, the data suggest that inhibition of HPPD alleviates the clinical condition associated with HT-1, but complete blockade of the HPPD activity is required in order to protect against the hepatic and renal damage.

Secondary pharmacodynamics and safety pharmacology

Only a single non-GLP compliant combined secondary and safety pharmacology study of nitisinone was performed. Nitisinone had no effect on rat and guinea pig α_1, α_2 or β_2 adrenoreceptors at 10^{-5}M (@30 µg/mL) *in vitro*. Safety pharmacology assessment *in vivo* was limited to examination of some behavioural, muscle relaxation and cardiorespiratory effects in rats given oral (PO) nitisinone. At a dose of 500 mg/kg (but not 200 or 350 mg/kg) nitisinone caused general signs of toxicity, central nervous system (CNS) depression, prolonged halothane-induced sleeping time and induced muscle weakness. There were no significant changes in heart rate, blood pressure, electrocardiogram (ECG) recordings or respiration rate at 350 mg/kg (only dose examined for cardiovascular effects). However, the results from this study should be interpreted with caution, given the limited study methodology, low animal number of a single gender, and poor choice of animal model for examining ECG effects. Renal or gastrointestinal effects were not examined in any studies.

Pharmacokinetics

Pharmacokinetic studies for nitisinone were limited. With the exception of rats, there are no data on basic pharmacokinetic parameters in animal species employed in nonclinical toxicity

testing (mice, rabbits, dogs or monkeys). No toxicokinetic data were obtained in the toxicity studies.

Available studies in rats (2.6 or 3.0 mg/kg PO) demonstrated rapid and complete oral absorption of nitisinone, with a terminal half-life of 9 hours. In humans, oral absorption after capsule or liquid nitisinone formulations (1 mg/kg) was also rapid, but with a slower elimination rate (terminal half-life = 54 hours).

Tissue distribution studies in rats (0.1 or 10 mg/kg PO) and mice (10 mg/kg PO) showed selective retention of radiolabelled nitisinone in the liver, kidneys and to a lesser extent, Harderian gland. No retention of radiolabel was detected in the eye.

The route of nitisinone elimination was investigated in rodents and humans. Nitisinone and two hydroxylated metabolites were detected in rat urine, with nitisinone also detected in rat faeces (at approximately equal levels to those in urine). Information about the metabolism and excretion of nitisinone was limited to data from a pilot study in rats sacrificed 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 oxidation probably by the cytochrome P450(CYP) system.

In vitro studies conducted in human microsomes examined whether nitisinone was a substrate for a range of CYP isozymes (1A2, 2C9, 2C19, 2D6, 2E1 and 3A4). Incubation with pooled human microsomes identified a single NAPH-dependent metabolite (accounting for about 2% of the sample radioactivity). A single metabolite with a similar retention time was also seen on the chromatogram when nitisinone was incubated with expressed 3A4 isozymes. This was identified this as a hydroxylated metabolite. Thus, *in vitro* data suggests some involvement of CYP3A4 in the metabolism of nitisinone.

Pharmacokinetic Drug Interactions

Investigations on the potential for drug-drug interactions between nitisinone and other medicines that induce or inhibit cytochrome P450 activity were studied in human microsomes *in vitro*. Incubation with nitisinone had no effect on the metabolism of selective substrates for CYP1A2, CYP2C19 or CYP3A4. Nitisinone was a moderate inhibitor of CYP2C9, with an IC_{50} value of 46 μM. It was also found to be a weak inhibitor of CYP2D6 and CYP2E1, with IC_{50} values >100 μM. Based on the absence of an effect on CYP1A2, CYP2C9 and CYP3A4 and the high IC_{50} values of the other CYP isozymes examined, it was considered unlikely that nitisinone would markedly inhibit the clearance of drugs that are metabolised via the CYP450 system.

Toxicology

The toxicological profile of nitisinone was characterised by limited acute toxicity studies in rodents, repeat-dose toxicity studies in mice (≤6.5 months), rats (≤12 months), rabbits, dogs and monkeys (≤3 months; primarily ocular toxicity studies), genotoxicity studies *in vitro* and *in vivo*, and reproductive toxicity studies in mice, rats and rabbits. With exception of the reproductive toxicity studies performed in mice and rabbits, none of the toxicity studies were performed according to GLP and were at least partly deficient in terms of both study design and quality. In the case of repeat dose toxicity studies, histopathological analysis was conducted in a range of tissues for rats, but limited to the eye and liver initially in mice, eye only in rabbits and dogs, and no tissues were examined histologically in monkeys. However, it is important to note that a retrospective histological analysis of all archived samples from the two chronic rodent toxicity studies was conducted and the data were provided for evaluation. It should also be noted that toxicological assessments in the three month toxicity studies did not include any organ weight assessments, necropsy or histopathological

examinations (except the eye/cornea) in rabbits, dogs and monkeys. No urinalysis assessment was performed in monkeys nor were any urinalysis, clinical chemistry or haematology assessments performed in rabbits. Thus, the small number of parameters assessed in these species (rabbits, dogs and monkeys) limits their ability in defining the toxicological profile of nitisinone.

Nonetheless, toxicity studies were performed using the intended clinical administration route (PO), and for the most part, employed appropriate animal numbers and doses (based on range-finding studies). While the adequacy of the animal models for human toxicity testing could not be established, the range of species examined and number of studies performed at least enabled identification of the primary target organs of toxicity. The limited duration and conduct of the longest non-rodent toxicity study (3 months), absence of adequate repeat dose studies in a non-rodent species and absence of completed carcinogenicity studies are considered major deficiencies for a product intended for long-term human use. However, the absence of such studies may be acceptable for a life-threatening disease with currently no other therapeutic treatment options.

Relative exposure

Conventional toxicokinetic data were not provided for any toxicity studies. Therefore, clinical safety margins for nitisinone were based on BSA comparisons between animal doses employed in primary toxicity studies and the maximum recommended human dose (MRHD) (2 mg/kg/day). The relative BSA exposures are shown in Table 1 with No Observable Adverse Effect Levels (NOAELs) indicated in bold.

Table 1: Relative BSA Exposures

Species	Study type	Study Protocol/Duration	PO Dose (mg/kg/day)	BSA (mg/m^2/day)	Relative BSA exposure
Human	MRHD	Daily	2	66[1]	-
Mouse	Suspended Carcinogenicity → Repeat dose	6.5 months (dietary)	**2** 70 300 700	**6** 210 900 2100	**0.1** 3.2 14 32
Rat	Suspended Carcinogenicity → Repeat-dose	12 months (with 3 months interim group) (dietary)	0.05 0.25* 2 6* 16 40	0.3 1.5* 12 36* 96 240	<0.1 <0.1* 0.2 0.5* 1.5 3.6
Rabbit	Repeat-dose (ocular toxicity)	6 weeks 3 months (gavage)	10 50 250	110 550 2750	1.7 8.3 42
Dog	Repeat-dose (ocular toxicity)	3-4 months (gavage)	0.1 0.5 1.5 5.0	2.0 10 30 100	<0.1 0.2 0.5 1.5
Monkey	Repeat-dose (ocular toxicity)	3 months (5 days/week) (gavage)	**10**	**120**	**1.8**
Mouse (GLP compliant)	Fertility	4 weeks M & 2 weeks F (premating) → 2 weeks M/F (mating) → GD7 (F)	**5**$^{\varphi}$ **50**$^{\#}$ 250	**15**$^{\varphi}$ **150**$^{\#}$ 750	**0.2**$^{\varphi}$ **2.3**$^{\#}$ 11
	Embryofetal	GD7-16 (10 days)	5 **50**$^{\#}$ 250	15 **150**$^{\#}$ 750	0.2 **2.3**$^{\#}$ 11
	Pre- & postnatal	GD7-PPD21 (5 weeks)	**5**$^{\#}$ 50 250	**15**$^{\#}$ 150 750	**0.2**$^{\#}$ 2.3 11
Rat	Fertility/ Embryofetal	8-9 weeks M; 2 weeks F (premating) → PPD21 (F)	100	600	9.1
	Embryofetal	GD8-15 (8 days)	200	1200	18
	Embryofetal	GD8-20 (13 days)	**20**$^{¥}$ 50 125	**120**$^{¥}$ 300 750	**1.8**$^{¥}$ 4.5 11
	Cross-fostering Pre- & postnatal	GD8-20 or GD8-PPD20	100	600	9.1
Rabbit (GLP compliant)	Embryofetal	13 days (GD7-19) daily infusion	5 12 25	55 132 275	0.8 2.0 4.2

[1] Based on a worse case scenario of a 50 kg person (this is likely an overestimation of patient weight and therefore BSA conversion factor given the predominantly paediatric patient population); *3 month interim group only; M = male; F = female; $^{\#}$Maternal/Paternal NOAEL; $^{\varphi}$Fertility/Reproduction NOAEL; $^{¥}$Fetal NOAEL; GD = gestation day; PPD = post-partum day; "-" = not applicable.

NOAELs could not be determined in the majority of toxicity studies. Moreover, several of the non-GLP studies only examined a limited number of toxicity parameters, suggesting that any NOAELs determined in these studies may be of limited value. Nonetheless, at the highest doses administered in the repeat-dose toxicity studies for each species, the animal doses, based on BSA, were at least similar to (1.5-3.6-fold; rats, dogs and monkeys) or significantly greater (32-42 fold; mice and rabbits) than the MRHD, and with the exception of monkeys, appeared to be constrained by dose-limiting or target organ toxicity. However, in the absence of a NOAEL in the majority of toxicity studies or the low NOAEL's (0.1-2-

fold MRHD based on BSA) determined in a limited number of studies, it is apparent that adequate clinical safety margins for toxicological effects were not established for any nonclinical species.

Single Dose toxicity

The assessment of acute toxicity was limited to a single non-GLP compliant study in rats and published findings for mice. In rats (10/sex), an oral dose of 100 mg/kg was without mortality, while a dose of 1000 mg/kg resulted in 8 out of 10 female rat deaths (only females were treated at this dose). Therefore, the oral median lethal dose (LD_{50}) in rats is likely between 100-1000 mg/kg. The oral LD_{50} in mice was around 600 mg/kg in male and 800 mg/kg in female mice.

These studies suggest the acute oral toxicity of nitisinone is low. However, it should be noted that these studies were poorly documented, with no parameters outside of lethality assessed and are therefore of limited value. It should also be noted that acute toxicity following intravenous (IV) dosing was not investigated.

Repeat Dose Toxicity

Repeat-dose oral toxicity studies conducted in the mouse, rat, rabbit, dog and monkey identified the eyes (cornea), liver, kidney and nervous system as primary target organs of toxicity.

In mice given nitisinone in the diet for 6.5 months, the liver, kidney and peripheral nervous system were identified as target organs at doses ranging from 70-700 mg/kg/day. Liver lesions included centrilobular hypertrophy with nuclear enlargement, which was consistent with enzyme induction. There was evidence of kidney enlargement and impaired renal function, however this organ was not examined microscopically at the time. Sciatic nerve degeneration was also dose-dependently increased in both incidence and severity, but also not examined microscopically at the time. Recent retrospective histological assessment of all tissue samples collected in this study that were not previously analysed, found an increased incidence of sciatic nerve demyelination (never fibre degeneration) with nitisinone treatment at 300-700 mg/kg/day, but no evidence of pre-neoplastic or neoplastic changes in any other organs/tissues. Based on the liver, kidney and nervous systemic changes reported, a NOAEL of 2 mg/kg/day was determined.

In rats given nitisinone in the diet for 12 months, the eyes and to a lesser extent, the liver, were identified as target organs at doses ranging from 0.05-40 mg/kg/day. The corneal lesions (keratitis) observed were inflammatory in nature, included oedema and hyper-vascularisation and were irreversible. As with mice, the liver lesions related to a slight centrilobular hypertrophy. Recent retrospective histological assessment of all tissue samples collected in this study that were not previously analysed, found no evidence of preneoplastic or neoplastic changes in any other organs/tissues. An NOAEL was not established.

In rabbits, the potential for ocular affects was assessed following administration of oral nitisinone at 50 or 250 mg/kg/day for 3 months. Only clinical signs, body weight and ophthalmology were examined. While no gross eye abnormalities were reported, this result is of limited value, given all four rabbits in the 250 mg/kg/day group died within the first week of treatment and all but one of four rabbits in the 50 mg/kg/day group died during weeks 2 to 7. However, a related study by Lock *et al* (2006) also shows an absence of

corneal effects in male rabbits given oral nitisinone at 10 mg/kg/day for 6 weeks.[6] While one rabbit died on Day 7 and one was euthanised on Day 14, the four remaining animals survived to the end of the study without any clinical signs or body weight differences relative to controls. An NOAEL was not established in either study.

In dogs given oral nitisinone for 4 weeks or 3 months, the eyes, gastrointestinal tract and nervous system were identified as target organs. In the 4-week dose range finding study, gastrointestinal haemorrhage/irritation and corneal lesions were noted at≥5 mg/ kg, and mortalities and clinical signs of neurotoxicity including ataxia, convulsions, reduced activity, stiff or weak hind limb, head tremors, and poor pupillary response to light were observed at ≥10 mg/kg/day. Only eyes were examined in the 3-month study. The eye lesions comprised of degenerative and inflammatory changes of the cornea at all doses (0.1 -5 mg/kg/day) and were transient and reversible in nature. However, the value of 3-month study is limited by the absence of a full toxicological (necropsy, organ weight and histopathological (eyes only)) assessment. In a recent study by Lock *et al* (2006), corneal opacities were also observed in dogs following 18 weeks oral nitisinone dosing at 0.1-5 mg/kg/day.[6] These lesions were mainly focal, confined to the epidermis and also reversible. A NOAEL was not established.

In monkeys, oral nitisinone at 10 mg/kg/day (5 days/week) over 3 months was well-tolerated, with no evidence of systemic or ocular toxicity (via ophthalmology assessment). However, the value of this study is also limited by the absence of a full toxicological (including urinalysis, necropsy, organ weight and histopathology) assessment. Moreover, in the absence of dose-limiting toxicity in this and a previous dose range-finding toxicity study (0.1-10 mg/kg/day over 2 weeks), it is unclear why a higher dose was not employed. A similar finding was published by Lock *et al* (2006) for 6 monkeys given 10 mg/kg/day (5 days/week) oral nitisinone for 12 weeks without evidence of corneal lesions, clinical signs or body weight effects.[6] An NOAEL of 10 mg/kg/day (5 days/week) was determined in both studies.

Nitisinone has been associated with elevated plasma and ocular tyrosine levels through its primary inhibitory action on HPPD in all species. However, while marked and sustained tyrosinaemia leads to ocular toxicity in rats and dogs, mice, rabbits and monkeys appear resistant to the corneal effects. While the basis for the lesions is thought to be due to a marked and sustained build up of tyrosine in the eye, tissue distribution studies in rodents suggest there is no selective ocular retention of radiolabelled nitisinone. Thus, while marked tyrosinaemia may be a prerequisite for the development of corneal lesions, other factors such as species differences in ocular antioxidant defence enzymes or ocular protein binding capacity for tyrosine may also be relevant. Therefore the relevance to humans is unclear. Thus, regular clinical monitoring of plasma tyrosine levels may be warranted in order to minimise the potential for ocular effects. Regular eye examination is also recommended.

Genotoxicity

Nitisinone (technical grade material as initially developed; 90-92%) was tested for potential genotoxic effects in a standard battery of *in vitro* (bacterial reverse mutation, forward gene mutation and chromosomal aberration) and *in vivo* (mouse micronucleus) test systems. All studies were conducted according to GLP utilising appropriate doses/concentrations.

[6] Lock EA, Gaskin P, Ellis M, Provan WM, Smith LL. Tyrosinemia produced by 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione (NTBC) in experimental animals and its relationship to corneal injury. Toxicol App Pharmacol 2006; 215: 9-16.

Technical grade nitisinone was weakly mutagenic in bacterial cells, mutagenic and clastogenic in mammalian cells *in vitro*, but not clastogenic in mouse erythrocytes *in vivo*.

Given the positive results for the technical grade material in several *in vitro* genotoxicity studies, these tests were repeated (according to GLP with appropriate doses/concentrations) with the purified (≥98%) drug substance proposed for therapeutic use. Purified nitisinone was not mutagenic in bacterial cells, but was mutagenic in mammalian cells (mouse lymphoma forward mutation test) *in vitro*, while no test for chromosome damage *in vitro* was performed. Purified nitisinone also produced a weakly positive response in the mouse micronucleus test *in vivo*. At the request of the EMEA, a mouse liver unscheduled DNA synthesis (UDS) assay *in vivo* was subsequently performed in light of the positive genotoxicity results obtained and presence of structural alerts (electrophilic centres and an aromatic nitro group) for nitisinone. This study was adequately performed and showed no evidence of DNA damage *in vivo*.

Nonetheless, there is some, albeit limited evidence of genotoxic potential for therapeutic quality nitisinone *in vitro* and *in vivo*.

Carcinogenicity

Although nitisinone is intended for lifetime treatment, adequate assessments of carcinogenic potential have not been performed. Carcinogenicity studies were commenced in mice and rats, but interrupted after 6½ and 12 months dosing, respectively, following cessation of the initial development program for nitisinone. While no evidence of pre-neoplastic or neoplastic changes were observed in any organs/tissues, the duration of these studies is insufficient for an adequate evaluation of carcinogenicity. In a paper by Al-Dhalimy *et al*. (2002)[7], FAH knock-out mice (showing many of the characteristics of HT-1, including development of hepatocellular carcinomas) given 3-6 mg/kg/day nitisinone in drinking water for >2 years did not show any evidence of treatment-related tumours, but did not prevent the development of liver tumours, which are associated with HT-1. Histological analysis in this study included the liver and the kidney and organs with macroscopic changes. Renal tubular injury (no detailed description of the histological lesions) was observed in high dose animals (3-6 mg/kg/day).

Overall, the absence of completed carcinogenicity studies for nitisinone may be acceptable for a life-threatening disease for which there are currently no other therapeutic treatment options. While a number of positive results from genotoxicity studies and structural alerts suggest a need for carcinogenic evaluation and cause for clinical concern, it should be noted that without effective treatment, lethal hepatocarcinomas can actually develop in patients with HT-1.

Reproductive toxicity

Reproductive toxicity studies with nitisinone included four preliminary non-GLP studies in rats and four more recently conducted GLP-compliant studies in mice and rabbits. The studies in rats (fertility, embyrofetal, pre- and post-natal) were limited in terms of animal numbers (predominantly 10-12/group) employed, doses examined (only one dose in all but one embryofetal study; see *Relative exposure* section) and timing of dosing and parameters assessed. In contrast, studies performed in mice (fertility, embyrofetal, pre- and post-natal) and rabbits (embryofetal) employed appropriate animal numbers, adequate doses (based on range-finding studies) and examined relevant parameters. As with other toxicity studies, no

[7] Al-Dhalimy M., Overturf K., Finefold, M. and Grompe M. Long-term therapy with NTBC and tyrosine-restricted diet in a murine model of hereditary tyrosinemia type 1. Molecular Genetics and Metabolism 2002; 75: 38-45.

toxicokinetic data was obtained in these studies and thus clinical exposure comparisons are based on BSA (see *Relative exposure* section).

In rats given 100 mg/kg/day PO nitisinone prior to mating through weaning, no effects on pregnancy rate but evidence of both maternotoxicity (clinical signs, weight loss and reduced food consumption) and embryotoxicity (reduced live births, pup birth weight and survival to post-partum day 4) was observed (9 times the MRHD, based on BSA). In embryofetal development studies in rats, dosing occurred during Days 8-15 (preliminary study) or 8-20 (dose range-finding study). Given the normal rat organogenesis period occurs during Days 6-15, it is important to consider the implications of a delayed dosing regimen for teratology assessment. In the preliminary study, 200 mg/kg/day given to rats over Gestation Days (GD) 8-15 was associated with maternotoxicity (clinical signs, weight loss, reduced food consumption) and embryotoxicity (increased stillbirths, reduced live births, pup birth weight and survival to post-partum Day 4) (18 times the MRHD, based on BSA). Teratogenic effects were limited to a 2-fold increase in urinary tract findings (kidney pelvis dilated, ureter dilated or convoluted) and an increased incidence of skeletal variants (extra ribs, incomplete ossification of the fifth vertebrae and constriction of the vertebrae centra) compared to control animals. However, it is important to note that only 3 out of 10 litters of treated dams survived to postpartum Day 4 in this study, therefore these results are of limited value. In the range-finding study, 20 mg/kg/day and 50 mg/kg/day given to rats over GD 8-20 was associated with maternotoxicity (clinical signs, weight loss, reduced food consumption, increased kidney weight) and embryotoxicity (increased stillbirths, reduced live births, pup birth weight and survival to post-partum Day 4), respectively (1.8 and 4.5 times the MRHD based on BSA, respectively; NOAEL for embryofetal effects was 1.8 times the MRHD, based on BSA). While no evidence of a teratogenic effect was reported, only a single summary table reporting "findings in incidental pup deaths" was presented. Therefore, the extent of teratology assessment is unclear and combined with the low numbers of litters delivered (7-10 per group), also limits the relative value of this study. In rats given 100 mg/kg/day PO nitisinone during gestation (Days 8-20) or during gestation through weaning (post-partum Day 20), maternotoxicity consisted of clinical signs, weight loss, reduced food consumption, increased corneal opacities, increased liver and kidney weights. Intra-uterine exposure to nitisinone reduced pup survival, pup weight and delayed eye opening. Lactational exposure to nitisinone resulted in reduced pup weight and the development of corneal opacities. Lens lesions developed in pups with prior intra-uterine nitisinone exposure irrespective of dietary exposure during an eight week post-weaning phase of the study. In contrast, corneal lesions developed in pups with exposure to nitisinone in the diet for 8 weeks irrespective of prenatal exposure. No NOAEL could be established in the single dose study.

Maternotoxicity was observed in both mice and rabbits given PO nitisinone. Doses of 250 mg/kg/day in mice (prior to mating, during organogenesis and/or during post-partum period) and from 5 mg/kg/day in rabbits (during organogenesis) were associated with increased clinical signs, weight loss and/or reduced food consumption. Prolonged pre-coital interval and increased post-implantation loss was associated with female mouse treatment at 50 mg/kg/day PO nitisinone prior to mating (2.3 times the MRHD, based on BSA). It is unclear why male reproductive organs only were weighed and examined in this fertility study given the clear attribution of fertility effects to female mice. Gestation length was also increased in pregnant mice given PO nitisinone at doses from 50 mg/kg/day (2.3 times the MRHD, based on BSA) from GD 7. In both species, there was evidence of fetotoxicity (decreased foetal weights, increased early intra-uterine deaths and/or increased implantation loss) and increases in fetal abnormalities (incomplete skeletal ossification in mice; increased umbilical hernia, gastroschisis, reduced or absent lung, abnormal development of first and

second thoracic ribs, lengthening of thoracic costal cartilages, extra centres of ossification in the sternum and thoracolumbar border, and minor changes in ossification at the cranial end of the cervical vertebrae and in the fore and hind paws in rabbits) at maternal PO doses from 5 mg/kg/day given during organogenesis (0.2-0.8 times the MRHD, based on BSA). Reduced pup survival, weight gain and developmental (sensory and learning) effects were observed following maternal treatment of mice at PO doses from 5 mg/kg/day during organogenesis through weaning (0.2 times the MRHD, based on BSA).

Nonclinical Summary and Conclusions

The biochemical defect in HT-1 is a deficiency of fumarylacetoacetate hydrolase (FAH), the final enzyme of the tyrosine catabolic pathway.

A maximum recommended human dose (MRHD) is 2 mg/kg/day PO for all patients.

The Australian nitisinone submission is based on the EU and FDA submissions as well as two repeat dose toxicity study histology sample reanalyses, additional reproductive toxicity studies and an additional literature reference.

The toxicological program of nitisinone was deficient with respect to the quality and conduct of the majority of the studies submitted, many of which were not GLP compliant. The poor design of these studies is reflective of the product's original development program as a herbicide.

Nitisinone was shown to inhibit 4-hydroxy phenylpyruvate dioxygenase (HPPD), the enzyme preceding FAH in the tyrosine catabolism pathway in animals *in vitro* and *in vivo*.

A single safety pharmacology study in rats showed evidence of systemic toxicity and CNS effects at high PO doses (500 mg/kg; 45-fold the MRHD, based on BSA).

Pharmacokinetic studies were limited. Nitisinone was shown to be, at least in part, metabolised by CYP3A4 *in vitro*. Based on *in vitro* studies in human hepatic microsomes, nitisinone is not expected to significantly inhibit the clearance of drugs that are metabolised via the CYP450 system.

The acute oral toxicity of nitisinone was low. Acute IV toxicity was not investigated.

Repeat-dose toxicity studies conducted in the mouse, rat, rabbit, dog and monkey were largely deficient in terms of study design and parameters assessed. However, they employed the intended clinical administration route (PO), generally adequate animal numbers and doses. The adequacy of the animal models for toxicity testing could not be established due to the lack of adequate pharmacokinetic studies, however the animal species were responsive to the pharmacological action of nitisinone, as indicated by elevated plasma tyrosine levels and the studies performed enabled identification of the primary target organs.

The eyes, liver, kidney and nervous system were identified as primary target organs of toxicity. A NOAEL was not established in the majority of toxicity studies, and the NOAEL's that were determined in a limited number of studies were low (0.1-2-fold MRHD based on BSA). Elevated tyrosine levels were observed following HPPD inhibition by nitisinone in all species examined. While tyrosinaemia is likely a prerequisite for some of the observed toxicities, species differences were also apparent. In the absence of adequate pharmacokinetic profiling, the relevance to humans remains unclear. Nonetheless, regular monitoring of patient tyrosine levels and adverse effects on the eyes, liver, kidneys and the nervous system may be considered appropriate to mitigate potential nitisinone toxicities. It is noted that hepatic, renal and neurological disorders are also associated with untreated HT-1.

There was some limited evidence of genotoxic potential for nitisinone *in vitro* and *in vivo*.

No carcinogenicity studies were completed for nitisinone, nor any adequately conducted non-rodent chronic toxicity study. This is considered a major deficiency for a product intended for long-term human use.

Reproductive toxicity studies in mice, rats and rabbits highlighted fertility, embyrofetal, pre- and post-natal effects of nitisinone at maternotoxic doses which often provided little to no apparent safety margin, suggesting its use in pregnancy or during lactation should be avoided.

In conclusion, there were significant deficiencies in the nonclinical submission for nitisinone with little to no clinical safety margin for many observed toxicological effects. No carcinogenicity studies or adequate repeat dose toxicity study in a non-rodent species were conducted. There were no toxicokinetic data or adequate pharmacokinetic studies. The limited nonclinical studies identified the eye, liver, kidney and nervous system as the target organs of toxicity.

Given the rare occurrence, seriousness of the disease and the lack of therapeutic alternatives, a reduced toxicology program and limited safety margin may be considered acceptable. The significant shortcomings of the non-clinical investigations should not preclude a recommendation for registration under these circumstances, if the therapeutic benefit is adequately demonstrated by the clinical data and patients are regularly examined for plasma tyrosine levels and eye, liver, kidney and nervous system disorders.

Significant reproductive effects of nitisinone were observed in animal species exposed prior to mating, *in utero* or through lactation. Its use in pregnancy and during lactation should be avoided.

IV. Clinical Findings

Introduction

The Australian submission was based on the application originally submitted to the EU. The submission included one main clinical efficacy and safety study. The NTBC Study was a multi-national, multi-centred, open-label, uncontrolled study in which nitisinone was provided on a compassionate use basis to patients with HT-1. The NTBC Study results were provided in a main analysis which included 207 patients and a complementary analysis which included 250 patients. It is estimated that the analyses shared about 150 patients enrolled in a common overlapping time period. The submission also included a publication from Lindstedt et al (1992) which reported experience with the first 5 HT-1 patients treated with nitisinone.[4] The submission also included a brief communication from the Quebec NTBC Study Group comparing clinical outcomes in patients treated with nitisinone plus diet (n=34) with diet alone (n=27). The submission included no randomized, controlled studies investigating the efficacy and safety of nitisinone.

The submission included limited pharmacokinetic (PK) data on nitisinone. There was one study in 10 healthy young males which explored the PKs of nitisinone after administration of capsule and liquid formulations and assessed the bioequivalence of the two formulations. There was one study which assessed a limited number of PK parameters in 7 patients with HT-1. There was one subgroup study involving patients (n=239) from the NTBC Study which assessed the changes in nitisinone serum concentration over 6 years of treatment. The bioequivalence of an early nitisinone and lactose formulation and a later nitisinone and pre-gelatinized starch formulation intended for marketing was assessed in one subgroup crossover analysis (n=47), and one parallel group analysis (n=53-55). Both of these retrospective bioequivalence analyses involved patients from the NTBC Study. The submission included a communication from one of the principal investigators of the NTBC

Study explaining that in clinical practice nitisinone was most likely to be administered with food.

In addition to the safety data from the NTBC Study, the submission also included a number of safety reports prepared prior to the EU "birth date" of the drug on 21 February 2005.[8] It was stated in the sponsor's *Clinical Overview* prepared for the EU submission that a total of 401 patients had been treated with nitisinone since the first patient started in February 1991 until April 2001. The submission also included updated safety reports prepared since the EU birth date (Periodic Safety Update Reports [PSURs] 1-6 and a PSUR Summary Bridging Report). The most recent of these was a PSUR Summary Bridging Report (with addendum) for the period 21 February 2005 to the data lock on 10 July 2009. This report estimated that 679 patients (2941 patient years) had been treated with nitisinone over the approximate 4 years and 4 months covered by the report. In addition to the PSUR documents prepared since the EU birth date of nitisinone the submission also included five studies published since 2005 which the sponsor considered provided relevant information on the safety of nitisinone [Arora et al 2006; McKiernan 2006; Koelink et al 2006; Masurel-Paulet 2008; Santra et al 2008].[1,2,9,10,11]

In addition, the submission included a sponsor's *Clinical Overview* (dated 20 February 2002) of the data provided to the EU. No updated *Clinical Overview* was included in the submission. However, the sponsor provided an overview of the PSUR Summary Bridging Report and the five studies referred to above. The NTBC Study did not comply with the Good Clinical Practice (GCP) guideline of the International Conference on Harmonization (ICH).

Pharmacokinetics

Introduction

There were limited pharmacokinetic (PK) data on nitisinone in patients with HT-1. There were data on volume of distribution in three children with HT-1 aged from 2 months to 2.25 years and terminal half-life in six children with HT-1 aged from 2 months to 6 years, and data on these two nitisinone parameters in one adult aged 21 years with HT-1. The C_{max}, AUC and terminal half-life of nitisinone following administration of capsule and liquid formulations (1 mg/kg) were calculated in ten healthy adults. This study also included bioequivalence data on the oral and liquid formulations. There was no formal bioequivalence study comparing the lactose formulation of nitisinone used in many of the patients in the NTBC Study with the pre-gelatinized formulation proposed for registration. However, the submission included retrospective bioequivalence data on the two formulations in patients from the NTBC Study. There were reasonable data from the NTBC Study on the change in serum nitisinone concentration over 6 years of treatment, and limited data on dose proportionality.

[8] The EU birth date is the date it was first was authorised in the EU.

[9] Koelink et al. Tyrosinemia type I treated by NTBC: How does AFP predict liver cancer. Molecular Genetics and Metabolism 2006; 89: 310-315.

[10] Masurel-Paulet A et al. NTBC treatment in tyrosinemia type I: Long-term outcome in French patients. J Inherit Metab Dis 2008; 31: 81-87.

[11] Santra et al. Renal tubular function in children with tyrosinaemia type 1 treated with nitisinone. J Inherit Metab Dis 2008; 31: 399-402.

Absorption

PK data on capsule (1 mg/kg) and liquid (1 mg/kg) nitisinone formulations in young, healthy, male adults (n=10) showed that absorption from the capsule (1 mg/kg) was slower and more variable than from the liquid. The mean (standard deviation [SD]) time to maximal plasma concentration (t_{max}) following administration of the capsule was 4.2 (3.0) hours and ranged from 1.6 to 11.1 hours. Nitisinone was absorbed rapidly from the liquid formulation with the C_{max} in 6 of the 10 subjects being reached prior to the first sample taken 15 minutes after administration. The mean terminal half-life was long for both capsule and liquid formulations (54.5 and 53.6 hours, respectively). Overall, the data showed that absorption of nitisinone from capsule and liquid formulations was rapid and that elimination was slow.

Bioavailability

There are no data on the absolute bioavailability of nitisinone. Assessment of bioequivalence using two non-standard statistical methods showed that the capsule and liquid formulations were bioequivalent as regards the AUC (both methods) and the C_{max} (one of the two methods).

Effect of Food on Bioavailability

No formal study investigating the effects of food on the PKs of nitisinone was submitted. However, the submission included a "To whom it may concern" letter dated 20 February 2001 from one of the two principal investigators of the NTBC Study relating to administration of nitisinone with food. In the letter, it was stated that, "it is reasonable to assume that NTBC has been given together with food in the majority of patients since the message given orally or in the letter accompanying the first shipment of NTBC to a new local investigator was: 'We mix the NTBC with lactose and dispense it in capsules (easy to open and mix the content with e.g. formula diet)'". The letter goes on to state that there are no data on the palatability of NTBC, but there have been no complaints regarding "difficulties in administering the drug and no reports on reactions, which indicate either good or bad taste of the drug".

In view of the instructions given with the drug it appears likely that in clinical practice the capsules are broken open and sprinkled on food or mixed with formula. Furthermore, many of the patients treated with nitisinone are too young to be able to swallow capsules. It is unusual that a liquid formulation for marketing has not been developed. A liquid formulation exists as it was used in the pharmacokinetic study in healthy adults.

Bioequivalence

The submission seeks to register a nitisinone formulation containing pre-gelatinized starch in 2, 5, and 10 mg strengths. In the first years of the NTBC Study a hard gelatine capsule containing nitisinone and lactose was used. Nitisinone in hard gelatine capsules containing lactose in 5, 6, 7, 8 and 10 mg strengths were distributed until 1998. Hard gelatine capsules containing nitisinone 2 mg and pre-gelatinized starch were distributed from 1996, and hard gelatine capsules containing nitisinone 5 mg and 10 mg and pre-gelatinized starch were distributed from 1998. Given the dates of supply of the two formulations it appears likely that the majority of patients in the NTBC Study were treated with the lactose formulation. No formal bioequivalence study has been undertaken comparing the pre-gelatinized starch and lactose formulations. However, the submission included two retrospective "bioequivalence" studies comparing the biochemical and the clinical effects of the two formulations.

In one study, a retrospective crossover analysis was undertaken of nitisinone serum concentrations and relevant biochemical markers in patients who initially received the lactose

containing formulation and subsequently switched to the pre-gelatinized starch formulation. The results showed that the two formulations are likely to have similar clinical activity based on the similarity of the biochemical parameters following administration of the two formulations. The ratio of nitisinone serum concentration (starch/lactose) in 47 patients was 1.016 [95% confidence intervals [CI]: 0.935, 1.103]. In the other study, a retrospective parallel-group analysis was undertaken of nitisinone serum concentration, relevant biochemical markers, and clinical outcomes in patients treated with the lactose or pre-gelatinized starch formulations. The results showed that the two formulations are likely to have similar clinical activity based on the similarity of nitisinone serum concentrations and biochemical markers. The probability of survival without liver transplantation at 1 and 2 years was greater with the lactose than with the pre-gelatinized starch formulation, but the differences were not statistically significant and the number of events was small. The evaluations of these studies are summarised immediately below.

Swedish Orphan AB Study Report 2000 010 04

The objective of this retrospective crossover analysis was to adequately link the clinical data for the nitisinone formulation containing pre-gelatinized starch proposed for US marketing with the nitisinone formulation containing lactose used in the clinical studies. The analysis was undertaken to address FDA concerns relating to the absence of bioavailability and/or clinical data linking the two formulations. The pre-gelatinized formulation is the one proposed for Australian registration. The analysis included patients who satisfied the following three criteria: at least one year on nitisinone treatment in the NTBC Study before the date of the first assessment; assessments during treatment with the lactose and pre-gelatinized starch formulations not to be separated by more than one year; and nitisinone daily dose at the assessment of the lactose formulation to be within ±20% of the daily dose at the assessment of the pre-gelatinized starch formulation. The principal investigators were located at a Swedish hospital and the analysis took place between 19 May 1997 and 22 April 1999.

The study was designed to undertake assessments with the pre-gelatinized formulation at least 91 days after switching from the lactose to the pre-gelatinized formulation. There were 47, 41, 47, and 44 patients who satisfied inclusion criteria and were analysed for nitisinone serum concentration, urinary succinylacetone, plasma succinylacetone, and erythrocyte PBG-synthase activity, respectively. The mean (SD) age at the start of nitisinone treatment of the 47 patients was 32.2 (44.4) months [range: 0.2, 171], and 22 (46.8%) of the patients were males and 25 (53.2%) were females. The patients came from various countries in Europe, North America, and the Middle East. The mean time on the lactose formulation before assessment was about 45 (range: 18-85) months, and the mean time between the first (lactose) and second (pre-gelatinized starch) assessments was about 6.5 (range: 3.3-12.0) months. The mean daily dose of nitisinone was about 1.0 mg/kg during treatment with both formulations. The majority of patients (67%) had a daily dose of 0.8-1.2 mg/kg, with about 20% having a lower dose and about 13% having a higher dose. There was no significant difference in nitisinone serum concentration between the two formulations (Table 2).

Table 2: Nitisinone serum concentrations following the lactose and starch formulations.

Lactose (n=47)	Starch (n=47)	Ratio starch/lactose [95%CI]
45.7 (22.4) µmol/L	46.3 (23.1) µmol/L	1.016 [95%CI: 0.935 – 1.103]

Of the 41 patients with urinary succinylacetone concentrations, 40 had normal concentrations (< 1.0 mmol/creatinine) with both formulations and only 1 had abnormal concentrations with both formulations. Of the 47 patients with plasma succinylacetone concentrations, 44 had normal concentrations (< 0.1 µmol/L) on the lactose formulation and 42 had normal concentrations on the pre-gelatinized formulation (p=0.414). The mean (SD) erythrocyte PBG-synthase activity (nkat/g Hgb) in 47 patients was 1.0 (0.26) with the starch formulation and 1.01 (0.26) with the pre-gelatinized formulation (mean difference 0.011 [95%CI: -0.035, 0.050]). The mean (SD) urinary 5-ALA concentration (mmol/mol creatinine) in 44 patients was 10.0 (19.3) with the starch formulation and 7.5 (6.0) with the pre-gelatinized formulation (mean difference -2.482 [95%CI: -7.078, 2.114]). The higher mean (SD) value for urinary 5-ALA observed with the starch formulation was due to 1 patient who had highly fluctuating values before and after the shift in formulations. Excluding the results of this patient resulted in mean urinary 5-ALA values for the lactose and pre-gelatinized formulations of 7.2 and 7.4 mmol/mol creatine, respectively.

Swedish Orphan AB Report 2000 010 02

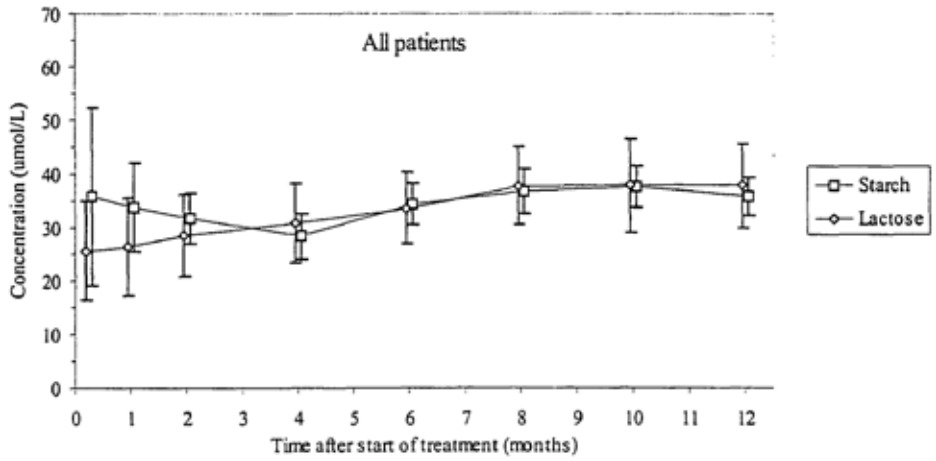
This was a parallel-group analysis comparing patients who started on either the lactose or pre-gelatinized starch formulations. The following assessments were undertaken: nitisinone serum concentration during the first 12, 18 and 24 months of treatment; urine and plasma succinylacetone concentrations, PBG-synthase activity, and urinary 5-ALA concentrations during the first 12 months of treatment; and death, liver transplantation, liver cancer, or liver failure leading to death or liver transplantation (Kaplan-Meier analysis).

The analysis was undertaken in patients who received nitisinone only from Swedish Orphan AB and included patients who started treatment between 1 January 1996 and 31 December 1999. The pre-gelatinized starch group included patients who had only used this formulation from the start of treatment. The lactose group included patients who started treatment with this formulation and had an order date for delivery of the first pre-gelatinized formulation. During the time of the analysis 1 mg/kg body weight daily was generally recommended as the initiation dose.

The analysis included 53 patients in the lactose group and 55 patients in the pre-gelatinized starch group. In the lactose group (n=53), 47% (n=25) of patients were < 6 months of age and 28% of patients were 6-24 months of age. The mean (SD) age of all patients in the lactose group (n=53) was 1.6 (2.4) months [range: 0-13.9], and 49.1% (n=26) were female and 50.9% (n=27) were male. In the pre-gelatinized starch group (n=55), 65% (n=36) of patients were < 6 months of age and 22% (n=12) were 6-24 months of age. The mean (SD) age of all patients in the pre-gelatinized starch group (n=55) was 1.0 (2.0) months [range 0-12.0], and 43.6% (n=31) were female and 56.4% (n=31) were male.

The nitisinone serum concentrations for all patients in both formulation groups were similar (Figure 2). At 12 months, the mean (SD) dose normalized nitisinone serum concentrations for all patients was 35.9 (9.8) and 37.9 (16.1) µmol/L for the pre-gelatinized starch (n=29) and lactose (n=17) formulations, respectively. The difference between the mean concentrations of the two formulations at 12 months was 2.0 µmol/L [95%CI: -4.3, +8.4]. The corresponding figures for the < 6 month age group were 38.3 (9.3) and 36.9 (14.8) µmol/L for the pre-gelatinized starch (n=23) and the lactose (n=7) formulations, respectively. The corresponding figures for the 6-24 month age group were 25.5 (5.3) and 30.7 (16.3) µmol/L for the pre-gelatinized starch (n=5) and the lactose (n=4) formulations, respectively.

Figure 2: Nitisinone serum concentrations (mean+SEM) in all patients followed until the 12 month visit or later (29 patients in the pre-gelatinized starch group and 17 patients in the lactose group).



The results for urinary and plasma succinylacetone concentrations, urinary 5-ALA concentration, and erythrocyte PBG-synthase activity were similar for both formulations. In most patients, urinary succinylacetone concentrations had reached normal values within 0-13 days of initiation of treatment with both formulations. Plasma urinary succinylacetone concentrations did not start to move towards normal values until about 45-59 days after the start of treatment with both formulations and reached normal values in most patients at about 12 months. In most patients, erythrocyte PBG-synthase activity reached normal levels within 14-29 days of initiation of treatment with both formulations. In most patients, urine 5-ALA concentration reached normal values within 0-13 days of initiation of treatment with both formulations.

Deaths (all cause) and survival without liver transplantation were analysed using pre-specified Kaplan-Meier estimates. In the analysis using the pre-specified censoring dates, which were different for both groups, there appear to have been 3 deaths in the pre-gelatinized starch group (2 due to liver failure and 1 due to complications of prematurity) and 4 liver transplantations (2 elective and 2 suspected tumour), and 1 death in the lactose group (liver failure). The study also included a *post hoc* Kaplan-Meier analysis in which the censoring date was the same for both treatment groups (1 January 2000). This *post hoc* censoring date was used in order to provide similar exposure times for patients in the lactose and pre-gelatinized starch treatment groups. In this analysis, there were an extra 5 events in lactose treated patients (all liver transplants) giving a total of 6 events (1 death + 5 transplants) and no additional events in the pre-gelatinized starch group giving a total of 7 events (3 deaths + 4 transplants). The Kaplan-Meier survival estimates using both pre-specified and *post hoc* censoring dates showed that the 1 and 2 year survival probabilities (all cause death) were non-statistically significantly higher in the lactose group than in the pre-gelatinized starch group, as were the 1 and 2 year survival probabilities for survival without liver transplant. The difference in the probabilities between the two groups was greater in the Kaplan-Meier analysis using the pre-specified censoring dates compared with that using the *post hoc* censoring dates.

Evaluator Comment

The results for the nitisinone serum concentrations and the pharmacodynamic biochemical parameters were similar for the pre-gelatinized starch and lactose formulations. The 1 and 2 year survival (all cause death) and survival without liver transplantation probabilities were

both higher in the lactose group than in the pre-gelatinized starch group, but the differences were not statistically significant. Overall, the results suggest that the two formulations are likely to be bioequivalent. The imbalance between the two formulations in "survival" events is likely to have arisen by chance.

Study CCT/96/0001

In this UK Phase I study, nitisinone was administered to 10 healthy adult males with the objective of determining the rate and extent of absorption from oral liquid and capsule formulations

The study was an open-label, randomized, balanced, two-way crossover design. Nitisinone (1.0 mg/kg) was administered as a single oral dose in liquid (2 mg/mL) and capsule formulations (5, 6, 8 and 10 mg) with a washout period of at least 14 days between treatments. The capsules contained nitisinone in combination with lactose.

The pharmacokinetics of nitisinone were evaluated by PK modelling and non-compartmental techniques. The plasma concentration time profiles after the administration of the capsule formulation were best described by a one compartment model with a first order absorption phase. The plasma concentration time profiles after administration of the liquid formulation were best described by a two compartment model with a zero order absorption phase. The results from the pharmacokinetic modelling for both the liquid and capsule formulations for all 10 subjects are summarised in Table 3. The reported results did not specify whether the AUC was $AUC_{0-\infty}$ or the area under the plasma concentration time curve over a dosing interval (AUC_t).

Table 3: Mean (SD) nitisinone pharmacokinetic parameters from pharmacokinetic modelling in 10 healthy male subjects.

Parameter		Nitisinone Liquid (1 mg/kg)	Nitisinone Capsule (1 mg/kg)
AUC	µg•h/mL	598 (142)	599 (153)
Cmax	µg/mL	7.81 (1.40)	7.69 (0.95)
Tmax	hour *	< 0.25 [range: <0.25, 1.95]	2.84 [range: 1.66, 11.10]
T1/2	hour	53.6 (8.2)	54.5 (13.0)

* median value [range]

The bioequivalence results for the liquid and capsule formulations calculated using non-standard methods from pharmacokinetic modelling are summarised in Table 4. The protocol specified that the criteria for bioequivalence were that the 90% CI for the AUC being within 80-125%. The non-parametric method showed that the two formulations were bioequivalent as regards the AUC as did the "Westlake 95% interval" method. The inter-individual variations in bioavailability as assessed by the coefficients of variation for the AUC were 25.6% and 23.7% for the capsule and liquid formulations, respectively.

Table 4: Study CCT/96/0001 – Bioequivalence evaluation.

Parameter	Differences [3]	Westlake's 95% Interval	Non-parametric BE evaluation
AUC [1]	P=0.99	92.4-107.6%	94.5-105.6%
AUC [2]	P=0.99	93.1-106.9%	95.4-105.9%
T1/2	P=0.78	87.5-112.5%	90.8-109.1%
Cmax	P=0.023	77.4-122.6%	102.6-124.3%
Cmax/AUC	P=0.002	81.3-118.7%	105.2-121.5%

[1] Non-compartmental analysis
[2] Pharmacokinetic modelling
[3] Paired differences were evaluated by the Pitman randomization test based on Wilcoxon matched paired sign rank test.

Evaluator Comment

This study appears to be the first PK study of nitisinone in humans. The general quality of reporting and the analysis of bioequivalence do not meet contemporary standards. Nevertheless, it provides useful information on the basic PKs of nitisinone in adults. The AUC results for the capsule and the liquid suggest that the relative bioavailability of the capsules compared with the liquid is high. The non-parametric assessment of bioequivalence showed the two formulations to be bioequivalent as regards the AUC and C_{max} as the 90% CI for the ratios were within the pre-specified equivalence limits of 80-125%. Non-parametric methods to assess bioequivalence are not mentioned in the relevant TGA-adopted EU guideline.[12] The "Westlake's 95% interval" method is no longer used to assess bioequivalence. Westlake's original method involved construction of 95% CIs symmetrical about zero using untransformed data for differences between parameters or about unity for ratios.[13,14] In the current study, the two formulations were bioequivalent as regards the AUC using "Westlake's 95% interval" as the interval is enclosed within the specified bioequivalence interval of 80-125%, but not as regards the C_{max} as the "Westlake's 95% interval" was not enclosed within the 80-125% interval.

Swedish Orphan AB Study Report 2000 010 07

The aim of this Swedish/Canadian Phase I study was to investigate the PKs of nitisinone in 6 children and 1 adult with HT-1 after oral administration of a capsule formulation. The study reported individual PK parameters calculated using a one-compartment model with first order absorption and elimination. In three previously untreated patients, the PK parameters were assessed during the first three dosing intervals following initiation of nitisinone treatment. In three other patients, PK elimination parameters were assessed following discontinuation of nitisinone. In two of these patients, nitisinone treatment had been maintained for about 2 years and then discontinued for 36 hours for experimental reasons. In the other patient,

[12] EMEA. Committee for Proprietary Medicinal Products (CPMP), 26 July 2001. Note for Guidance on the Investigation of Bioavailability and Bioequivalence. CPMP/EWP/QWP/1401/98.

[13] Westlake WJ. Use of confidence intervals in analysis of comparative bioavailability trials. J Pharm Sci 1972; 61: 1340-1.

[14] Westlake WJ. Symmetrical confidence intervals for bioequivalence trials. Biometrics 1976; 32: 74-44.

nitisinone treatment had been maintained for 10 months before discontinuation due to eye problems.

Evaluator Comment

This study presented case reports of individual PKs in 6 children and 1 adult patient. The rate of absorption was similar in the 1 adult patient to the mean value of this parameter in 3 children while the volume of distribution was lower in the 1 adult compared with the mean value for the 3 children. The elimination half-life was similar in the 1 adult to the mean value for all 6 children. However, in the 3 children in whom nitisinone had been initiated the terminal half-life was generally shorter than in the 3 children who were on stable nitisinone doses before the drug had been discontinued. The elimination half-life in patients with HT-1 in this study was nearly half that observed in the healthy adults in study CCT/96/0001. The reason for this difference is unknown but the investigators speculate that it might reflect an age related difference in the metabolism of nitisinone.

Distribution

Data on distribution is limited. The mean (SD) volume of distribution (L/kg) was 0.3 (0.2) [range: 0.11-0.55] in the third dosing interval in three children aged 2 months, 9 months, and 2.25 years.

Elimination

There were no *in vivo* data in humans on metabolism. There were limited data on elimination. The mean (SD) terminal half-life in 10 healthy patients following oral administration of a single capsule (1 mg/kg) was 54.5 (13.0) hours and 53.6 (8.2 hours) following oral administration of a liquid formulation. The mean (SD) terminal half-life in six children aged from 2 months to 6 years was 25.3 (6.9) hours.

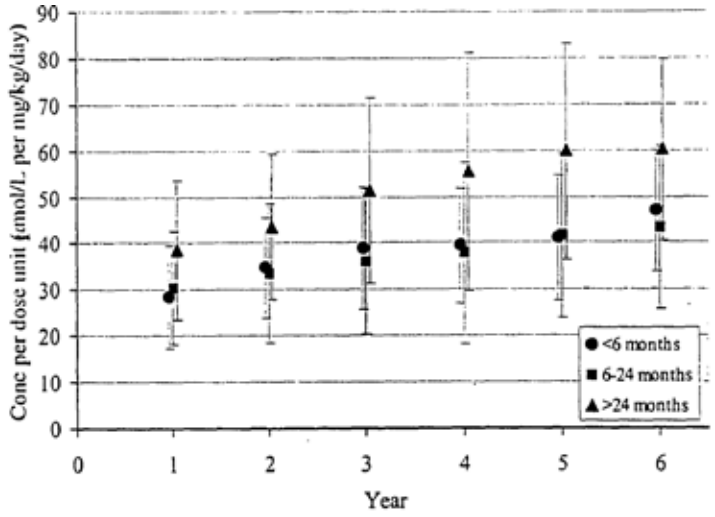
Dose Proportionality and Time Dependency

An analysis was provided of nitisinone serum concentrations in patients enrolled in the NTBC Study between 1 July 1993 and 28 March 2000. The objectives of the analysis were to evaluate serum nitisinone concentration during the first 6 years of nitisinone treatment in patients with start ages of ≤ 6 months, 6 to 24 months, and ≥ 24 months. The analysis included 239 patients: at the start of treatment 116 were ≤ 6 months of age, 67 were aged 6-24 months, and 56 were aged ≥ 24 months. The total nitisinone treatment period ranged from 0.1 to 80.5 months. There were more males than females in the study.

There were three nitisinone dose groups: low (≤ 0.70 mg/kg/day); high (≥ 1.5 mg/kg/day); and recommended (> 0.70 mg/kg/day and < 1.5 mg/day). Most of the patients started on the recommended dose (81.6%, n=195). The high dose group included only 6 (2.5%) patients and the low dose group included 38 (15.9%) patients. The median age for the total population at the start of nitisinone treatment was 6.7 (range 0-260) months.

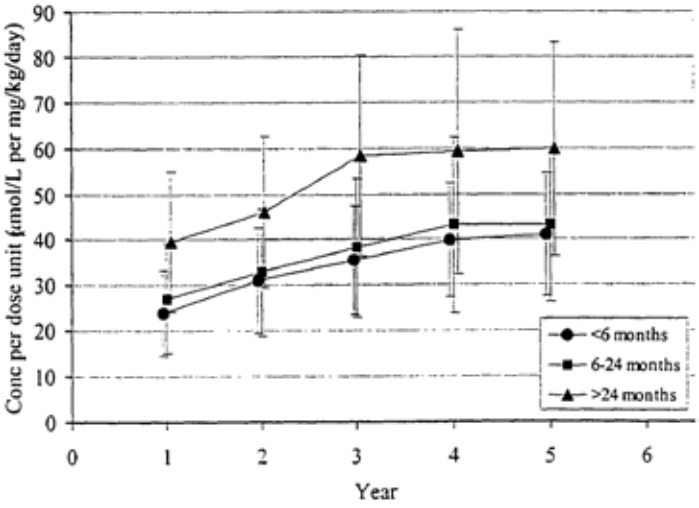
The mean dose normalized nitisinone serum concentrations increased from the first to the fifth year after the start of nitisinone treatment from 29 to 41 µmol/L in patients with a starting age < 6 months, from 30 to 42 µmol/L in patients with a starting age 6-24 months, and from 39 to 60 µmol/L in patients with a starting age≥ 2 4 months. The mean dose normalized nitisinone serum concentration at the fifth year of treatment was similar in the two youngest starting age groups (mean starting age of 3 and 12 months, respectively) and higher in the oldest starting age group (mean starting age about 7 years). The number of patients at the year 1 and year 6 assessments were, respectively, 115 and 11 for the starting age < 6 months group, 66 and 10 for the starting age 6-24 months group, and 55 and 11 for the starting age ≥ 24 months start group. The results are summarised in Figure 3.

Figure 3: NTCB Study – Mean values (SD) of dose normalized nitisinone serum concentration (µmol/L per mg/kg/day) for all evaluable patients.



In the analysis of patients followed for 5 years (that is, patients evaluable for at least 5 years) the mean dose normalized serum concentration increased mainly in the first 4 years of treatment in the two youngest age groups and during the first 3 years in the oldest age group. The results are summarised in Figure 4.

Figure 4: NTCB Study – Mean values (SD) of dose normalized nitisinone serum concentration (µmol/L per mg/kg/day) in patients evaluable at least until year 5 (n=23, 19, and 20 in the low, mid, and high start age group, respectively).



The increase in dose normalized nitisinone serum concentration from one year to the next (ratio with previous year) in all evaluable patients was 1.19 [95%CI: 1.14, 1.25] for year 2/1 (n=173), 1.14 [95%CI: 1.10, 1.18] for year 3/2 (n=137), 1.06 [95%CI: 1.01, 1.12] for year 4/3 (n=104), 1.02 [95%CI: 0.95, 1.09] for year 5/4 (n=63), and 1.04 [95%CI: 1.02, 0.95] for year 6/5 (n=32). The increase from year 1 to 2 was 19% and from year 2 to 3 was 14%, and after 3 years the increases were 6% or less for each of the subsequent years. The changes were similar for the three age groups.

No meaningful conclusions about dose proportionality between the lowest and recommended doses in the two youngest age groups (< 6 months and 6-24 months) could be made because of the small patient numbers in these age groups treated with the low dose (7 and 9 patients, respectively, at year 1 of treatment). In the oldest age group (≥ 24 months), no dose proportionality was observed between the low (n=22) and recommended (n=33) doses in the first year of treatment with the lower dose resulting in higher mean dose normalized nitisinone serum concentrations than the recommended dose.

Special Populations

Nitisinone serum concentrations were similar in patients aged < 6 months and 6-24 months at the start of treatment, and higher in patients aged ≥ 24 months at the start of treatment. In the two youngest age groups, nitisinone serum concentrations increased over the first 3 years of treatment after which time concentrations appeared to stabilize. In the oldest age group, nitisinone serum concentration increased over the first 4 years of treatment after which time concentrations appeared to stabilize.

The limited data suggests that the mean terminal half-life in six children with HT-1 aged between 2 months and 6 years is similar to that observed in one adult with HT-1 aged 21 years (~ 25 hours and 21 hours, respectively). The observed terminal half-lives in the children and the adult with HT-1 were shorter than the terminal-half life observed in healthy young adult males (~ 54 hours for the capsule and liquid formulations). The volume of distribution was smaller in one adult with HT-1 compared with three children aged from 2 months to 2.25 years (0.07 and 0.3 L/kg, respectively).

Evaluator's overall conclusion on pharmacokinetics

The submission included only limited PK data on nitisinone in patients with HT-1. There was no absolute bioavailability study, no formal bioequivalence study, no formal study examining the effect of food on bioavailability, no *in vivo* metabolism study, no mass balance study, no studies defining terminal half-lives, volume of distribution, or renal excretion following IV administration, no PK drug-drug interaction studies, no studies in special populations, and no PK studies in patients with renal or hepatic impairment. Overall, it was considered that the PKs of the drug have not been satisfactorily characterised.

A study in healthy males showed that following administration of a single oral nitisinone capsule (1 mg/kg) the mean (SD) AUC was 599 (153) µg•h/mL, the C_{max} was 7.69 (0.95) µg/mL, and the terminal half-life was 54.5 (13.0) hours, while the median t_{max} [range] was 2.84 hours [1.66-11.0]. This study also showed that capsule and liquid formulations were bioequivalent, but the liquid formulation was absorbed more rapidly than the capsule formulation. In six children with HT-1 the mean terminal half-life was 25 hours compared with 21 hours in 1 adult with HT-1. The mean volume of distribution was 0.3 L/kg in 3 children with HT-1 and 0.07 L/kg in 1 adult with HT-1. The terminal half-life of nitisinone in patients with HT-1 was significantly shorter than that in healthy, young, adult males.

There was no formal study assessing the effect of food on the bioavailability of nitisinone. However, information provided by one of the principal investigators in the NTBC Study indicates that it is likely that nitisinone has been added to food or formula in most patients treated with the drug. There was no formal bioequivalence study comparing the nitisinone lactose formulation used in many of the patients in the NTBC Study with the nitisinone pre-gelatinized starch formulation proposed for registration. However, retrospective data in patients with HT-1 from the NTBC Study showed that the two formulations are likely to be bioequivalent. Data from the NTBC Study showed that serum nitisinone concentrations increased over time reaching a plateau after about 3 years of treatment in patients with a

starting age of ≥ 24 months and after about 4 years in patients with starting ages of < 6 months and 6-24 months.

Pharmacodynamics

There were no formal pharmacodynamic (PD) studies in humans. However, the PD of nitisinone were reflected in efficacy outcomes in the NTBC Study and are reviewed below and in the *Efficacy* section of the clinical evaluation report.

Mechanism of Action

HT-1 is caused by a mutation in the gene encoding the enzyme fumarylacetoacetate hydrolase (FAH), the enzyme which catalyses the final step of tyrosine degradation. This defect results in the accumulation of the highly reactive compounds maleylacetoacetate (MAA) and fumarylacetoacetate (FAA), which account for the characteristic local liver and kidney damage. The pathogenic toxins MAA and FAA undergo further metabolism to the toxic metabolites succinylacetone (SA) and succinylacetoacetate (SAA); SA is particularly toxic and has both local and systemic toxicity. SA causes systemic inhibition of porphobilinogen (PBG) synthase, resulting in increased concentrations of 5-aminolevulinic acid. Detection of urinary SA and PBG synthase inhibition in blood are important diagnostic tests.[1] Nitisinone inhibits the activity of the enzyme 4-hydroxyphenylpyruvate dioxygenase, the enzyme which catalyses the second step in the degradation of tyrosine pathway. Inhibition of this enzyme by nitisinone prevents accumulation of MAA, FAA, SA, and SAA, but does not prevent accumulation of tyrosine. The tyrosine catabolic pathway is summarised in Figure 1.

Primary pharmacology

The data from the NTBC study showed that nitisinone at the dose proposed for registration rapidly reduced urinary excretion of succinylacetone and 5-ALA, rapidly normalized erythrocyte PBG-synthase activity, and slowly reduced plasma succinylacetone concentration. The results from the complementary NTBC analysis for the primary pharmacodynamic (biochemical) parameters indicating inhibition of 4-hydroxyphenylpyruvate dioxygenase following nitisinone treatment are reviewed below. The results are also discussed in the *Efficacy* section.

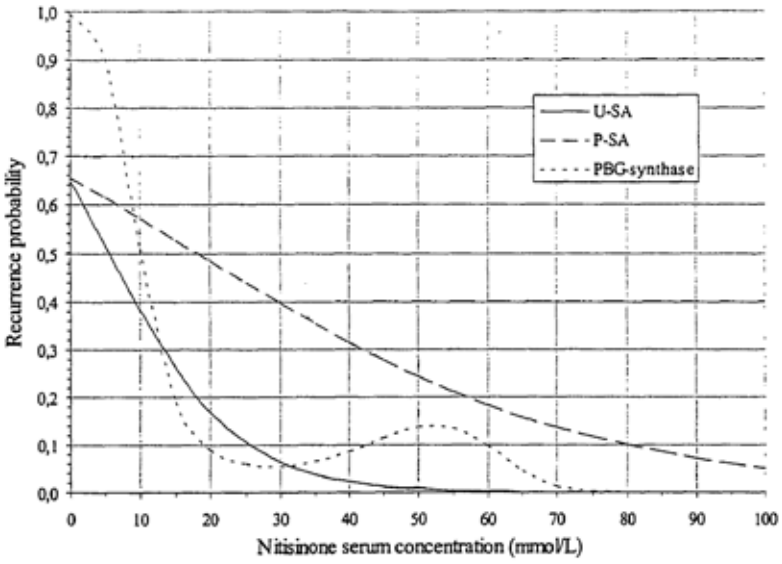
More than 90% of treated patients achieved urine succinylacetone concentrations of < 1 mmol/mol creatinine (reference range) within the first week of treatment and virtually all patients achieved this concentration within the first six months of treatment. In the first month of treatment, no patients achieved plasma succinylacetone concentrations of < 1 µmol/L (reference range) but by 6 and 12 months of treatment 90% and nearly 100% of patients, respectively, were reported as having normalized concentrations. The slower time to normalization of plasma succinylacetone concentration compared with urinary succinylacetone excretion is due to plasma succinylacetone being bound to plasma proteins which slows its disappearance time.[2]

Urinary excretion of 5-ALA rapidly fell after initiation of nitisinone with more than 80% of patients achieving levels below the reference level during the first week of treatment and nearly all patients achieving this level during the first month of treatment. Erythrocyte PBG-synthase activity rapidly increased after initiation of nitisinone with about 90% of patients having values above the lower reference limit (0.58 nkat/g Hgb) after the first month of treatment and nearly 100% after the second month of treatment.

Relationship between plasma concentration and effect

The NTBC Study included an assessment of the relationship between nitisinone serum concentration and the probability of recurrence of values outside the normal range for the primary PD parameters. In the complementary NTBC analysis, there was a statistically significant inverse relationship between nitisinone serum concentration and the probability of recurrence of values outside the normal ranges for urine and plasma succinylacetone and erythrocyte PBG-synthase. At a nitisinone serum concentration of 30 µmol/L, the estimated probability of recurrence of values outside the normal range was about 6% for both urine succinylacetone and erythrocyte PBG-synthase, and about 40% for plasma succinylacetone. The results of the logistic regression analyses of the relationship between nitisinone serum concentration at the time of recurrence and the probability of recurrence of values outside the normal ranges during the second and third year of treatment after being normalized during the first year of treatment for urine and plasma succinylacetone and erythrocyte PBG-synthase are summarised in Figure 5. For urine succinylacetone there were 9 events in 103 patients, for plasma succinylacetone there were 36 events in 102 patients, and for PBG-synthase there were 16 events in 98 patients. The number of observations for urine 5-ALA was too small to perform a meaningful analysis on this variable.

Figure 5: Complementary NTBC Analysis - Probability of recurrence of laboratory values outside normal ranges during the second and third year after start of treatment after being normalized during the first year versus NTBC serum concentration in patients who were followed for at least 2 years.



Note: The normal ranges were defined as < 1 mmol/mol creatinine for U-succinylacetone, <0.1 µmol/L for P-succinylacetone and > 0.58 nkat/g Hgb for PBG-synthase.

Evaluator's overall conclusions on pharmacodynamics

The data from the NTBC Study showed that nitisinone at the dose proposed for registration rapidly reduced urinary excretion of succinylacetone and 5-ALA, rapidly normalized erythrocyte PBG-synthase activity, and slowly reduced plasma succinylacetone concentration. These results indicate that nitisinone can effectively inhibit 4-hydroxyphenylpyruvate dioxygenase at the dose proposed for registration. The estimated probability of recurrence of values outside the normal range was about 6% for both urine

succinylacetone and erythrocyte PBG-synthase at a serum nitisinone concentration of 30 µmol/L, and about 40% for plasma succinylacetone at this concentration.

Efficacy

The submission included one, multi-national, multi-centred, open-label, uncontrolled, compassionate use Phase III study in patients with HT-1 treated with nitisinone (the NTBC Study). The NTBC Study clinical data were presented in two separate parts, the main analysis and the complementary analysis. The NTBC Study was the only clinical efficacy and safety study submitted in support of approval of nitisinone for the treatment of HT-1. The submission included no randomized, controlled, double-blinded studies assessing the efficacy and safety of nitisinone (NTBC).

Dose Response Studies

The submission included no formal dose response studies. The first clinical study of NTBC (as noted in Section II, nitisinone is also referred to as NTBC) in HT-1 (n=5) assessed daily doses ranging from 0.1 to 0.6 mg/kg body weight with dose adjustment being based on biochemical response. On the basis of this pilot study, the initial daily dose in the NTBC Study was 0.6 mg/kg body weight. However, with increasing clinical experience the initial recommended dose was increased to 1.0 mg/kg body weight per day during the course of the NTBC Study. Dose adjustments in individual patients were undertaken based on increasing body weight with age and on NTBC serum concentrations and biochemical response to treatment.

Main Clinical Study – NTBC Treatment of Tyrosinaemia Type 1

The NTBC Phase III Study was the main clinical study. NTBC was distributed on a compassionate use basis to any hospital in the world presenting a patient for inclusion in the study

The NTBC Study was performed in accordance with the principles adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, and later revisions. Individual investigators were responsible for obtaining informed consent from patients or their parents. The study was not undertaken according to the Good Clinical Practice Guidelines (GCP) of the International Conference on Harmonization (ICH). Local investigational sites and investigators were not monitored. In view of the improvement observed in the Phase II pilot study the principal investigators concluded that it would be unethical to refuse any treatment requests for NTBC. Consequently, the NTBC Study does not include a placebo control or diet alone control. However, the NTBC Study included a comparison of selected relevant clinical outcomes with those from an international survey of 108 patients treated only with a tyrosine and phenylalanine restricted diet.[15] The clinical outcome data from this international survey can be considered to come from "historical controls".

The *main NTBC analysis* was conducted from 23 February 1991 to 21 August 1997 and included 207 patients. The *complementary NTBC analysis* was conducted from 1 July 1993 to 28 March 2000 and included 250 patients. The complementary analysis included data on patients entered in the coordinating university (CU) NTBC database supplemented by information held by the European sponsor, Swedish Orphan AB (SOAB). The total number of patients in the NTBC Study enrolled between 23 February 1991 and 28 March 2000 did

[15] Van Spronsen FJ et al. Hereditary tyrosinemia type 1: A new clinical classification with difference in prognosis on dietary treatment. Hepatology 1994; 20: 1187-1191.

not appear to be explicitly stated in the submission. However, from the data provided in the two NTBC analyses it is estimated that about 290 patients were enrolled from the beginning of the main analysis (23 February 1991) to the conclusion of the complementary analysis (28 March 2000). There was an overlapping time period for the main and complementary NTBC analyses running from the start of the complementary analysis on 1 July 1993 to the end of the main analysis on 21 August 1997. Consequently, the two analyses shared a substantial number of patients. The number of shared patients in the two analyses did not appear to be explicitly stated in the submission. However, from the data provided in the two NTBC analyses it is estimated that the analyses shared about 150 patients. As a result of the substantial number of shared patients, the description of the results of the two analyses tended to be similar and often described the same patients. For completeness the results from both analyses are discussed in this report.

Evaluator Comment

The reason for presenting the results of the NTBC Study in two separate parts was unclear. It would have been preferable to have presented the NTBC Study as one report including a single analysis of all patients enrolled from 23 February 1991 (start of the main NTBC analysis) to 28 March 2000 (conclusion of the complementary NTBC analysis). This would have simplified the interpretation and avoided repeating the analysis in different but overlapping patient groups. The decision not to use a dietary control is reasonable. Nevertheless, it is considered that an ethical case could have been made for including such a control despite the encouraging results in the pilot Phase II study. However, in view of the rarity of HT-1 it is likely that recruitment to a randomized controlled trial would have presented a number of difficulties. Overall, it is considered that the pragmatic approach adopted by the principal investigators was a reasonable compromise.

Objectives

The principle objectives of the NTBC Study as outlined in the main and/or complementary analyses were to investigate the occurrences of death, liver transplantation, death due to liver failure and liver transplantation due to liver failure, hepatocellular carcinoma (HCC), and porphyric crisis in patients with HT-1 treated with NTBC in combination with a tyrosine and phenylalanine restriction diet. The study also investigated the effects of NTBC on those laboratory parameters specifically associated with HT-1 including urine and plasma succinylacetone concentrations, erythrocyte PBG-synthase activity and urine 5-aminolevulinate (5-ALA) concentration. In addition, the study also investigated the effect of NTBC on the hepatic manifestations of HT-1 by measuring serum α-fetoprotein (AFP) concentration, prothrombin complex, and serum concentrations of alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), bilirubin and albumin. The effects of NTBC on the renal manifestations of HT-1 were also investigated by measuring urine excretion of α_1-microglobulin and amino acids, and serum concentrations of ALP, phosphate and creatinine. The effects of NTBC on the haematological manifestations of HT-1 were also investigated by measuring haemoglobin parameters, erythrocyte count, thrombocyte count and neutrophil count. Also investigated were the effects of NTBC on plasma concentrations of tyrosine and other amino acids, and on the urinary excretion of phenolic acids. The effects of NTBC on somatic development and clinical condition were also investigated, as was the safety of NTBC treatment.

Patients

The *main NTBC analysis* included 207 patients treated by 96 local investigators at 87 hospitals in 25 countries including 1 patient from Australia. The *complementary NTBC*

analysis included 250 patients from 30 countries including 2 patients from Australia. As mentioned above it is estimated that the two analyses share about 150 patients. The NTBC study was open to all patients with HT-1, without restrictions relating to clinical type, age, sex, ethnic origin, or nationality. Patients were included only after succinylacetone had been demonstrated in the urine or plasma. Patients who had undergone liver transplantation were not included in the study, but there were no other exclusion criteria. The reasons for withdrawal from the study were death, liver transplantation and parents' or patients' wish to withdraw. The protocol stated that after the start of NTBC treatment local investigators should examine patients at regular intervals (8 times in the first year and then 4 times each subsequent year). At each visit, blood and urine samples were collected and sent to SU for analysis of serum nitisinone, urine succinylacetone, plasma succinylacetone, erythrocyte PBG-synthase, urine 5-ALA, serum AFP, tyrosine and other amino acids and α_1-microglobulinaemia. Additional standard laboratory analyses and clinical investigations were performed locally and the results sent to the principal investigators for inclusion in the CU database.

Treatment

NTBC was administered orally twice daily (bd) either in a hard gelatine capsule or as a powder. In the main NTBC analysis, the initial recommended dose was 0.6 mg/kg per day based on the Phase II pilot study. However, in 1994 the initial dose was increased to 1 mg/kg per day as experience had shown that in most patients NTBC serum concentrations of 20-30 µmol/L were required to reach the desired biochemical effect. In the majority of patients this target serum concentration was not achieved with an initial dose of 0.6 mg/kg per day. Individual dose adjustments subsequent to the initial dose were made based on weight gain, NTBC serum concentration (from 1994) and the biochemical profile, which included erythrocyte PBG-synthase activity, plasma succinylacetone concentration, and urine 5-ALA and succinylacetone concentrations. No daily dose exceeded 3.0 mg/kg per day. In the *main NTBC analysis* the median duration of treatment was 22.2 months [range: 0.1-77.9]. In the *supplementary NTBC analysis*, the treatment period ranged from 0.1 to 80.5 months (no median duration could be identified in the submitted data).

Initially, NTBC as a powder was distributed from CU to local investigators. The participating local hospitals were advised to mix NTBC with lactose fill and dispense in a hard gelatine capsule containing one dose. The individual hospitals were responsible for labelling the capsule containers. From November 1994, Swedish Orphan gradually assumed the responsibility for the manufacture and distribution of NTBC. The only instruction given to investigators regarding administration of NTBC was to administer the daily dose on two separate occasions. However, in order to achieve the daily dose with the available dose strengths the required dose could be administered more than twice daily. No other recommendations relating to dose interval or specific timing of the dose were specified.

Efficacy and Safety Variables

Survival analyses

The primary clinical efficacy variables of the NTBC Study were death, survival without liver transplantation, death due to liver failure or transplantation due to liver failure, hepatocellular carcinoma, and porphyric crisis. Other primary efficacy evaluations were the effect of NTBC on urine and plasma succinylacetone concentrations, erythrocyte PBG-synthase activity and urine excretion of 5-ALA, and the relationship between serum NTBC concentration and these biochemical effects.

Laboratory Analyses

At each visit, samples of blood, serum and urine were collected from patients and sent to CU for specified analyses including erythrocyte PBG-synthase, plasma succinylacetone concentration, plasma amino acids concentrations, serum AFP concentration, serum NTBC concentration, and urine α_1-microglobulins, succinylacetone, 5-ALA, amino acids, and phenolic acids concentrations. The normalized values after start of treatment were defined as having a value within the reference range. In addition to the specified analyses undertaken at CU relating directly to HT-1, blood, plasma, and serum concentrations were collected at each visit and sent to the local hospitals for standard analyses.

Adverse Events

The local investigators undertook to report adverse events (AEs) immediately to CU or SOAB. The possible causal relationship to NTBC treatment was evaluated by the medical director of SOAB. In addition, the medical director of SOAB decided on the seriousness of the AE using ICH criteria.

Eye Symptoms

In the *complementary NTBC analysis*, correlation between the occurrence of eye symptoms and plasma tyrosine concentration was evaluated.

Statistical Methods

The occurrences of death, liver transplantation, liver failure leading to death or liver transplantation, liver cancer and porphyric crisis were presented as Kaplan-Meier Survivor Function estimates. In the *main NTBC analysis*, all patients who started treatment from 23 February 1991 until 27 August 1997 were included in the survival analyses and patients were censored after 27 August 1997. In the *complementary NTBC analysis*, all patients who started treatment from 1 July 1993 to 28 March 2000 were included in the survival analyses and patients were censored at 28 March 2000.

No adjustments were made in the analyses for variation due to country and/or centre. Age at the start of treatment was included in a logistic regression model used to test the relationship between NTBC serum concentration and the biochemical response to treatment. Starting age was observed to have no influence on biochemical response and was not included in the final model. All statistical tests were two sided and no formal adjustments for multiple testing were made. Data from patients withdrawing from the study were used up to the point of withdrawal. Missing data were not replaced except in the analyses of NTBC dose and in the description of the extent of exposure where the last reported dose was carried forward. No calculation of sample size was undertaken and all patients willing to participate were included in the study.

In the *main NTBC analysis*, the Wilcoxon signed rank test was used to compare differences in numerous outcomes pre-treatment and post-treatment (that is, 1 year after initiation of NTBC). In both the *main and complementary NTBC analyses* assessments of outcomes were undertaken in the total population and in subgroups based on age at the start of NTBC treatment. In the *complementary NTBC analysis* subgroup analyses based on dose were also undertaken (that is, low, recommended, and high doses).

Demographic and Baseline Characteristics

In the *main NTBC analysis*, the median age at start of treatment was 0.8 years. Of the total number of patients in the study, 39% (80/207) started treatment before 6 months of age, 30% (62/207) between 6 months and 2 years of age, and 31% (65/207) after 2 years of age. The age distribution at inclusion changed during the course of the study with increasing numbers of younger patients being included as the study progressed. Of the 54 patients included during the first 3 years, 30% were younger than 6 months at the start of treatment and 54% were older than 2 years. Of the 71 patients included during the last 2 years of the study, 49% were younger than 6 months at the start of treatment and 25% were older than 2 years. The median treatment time for the total population was 22.2 months [range: 0.1-78]. There were more boys (n=114) than girls (n=93) included in the study.

In the *main NTBC analysis* there were data on the age of onset of HT-1 in 69% (143/207) of patients. In these 143 patients, 98 (69%) presented with symptoms before 6 months of age (which is consistent with an acute form of HT-1), and 45 (31%) patients presented after 6 months of age (which is consistent with a sub-acute or chronic form of HT-1). The majority of patients presented with liver disease (69%), followed by failure to thrive (37%), renal disease (27%) and neurological disease (5%) (Table 5).

Table 5: Main NTBC analysis – Presenting symptoms and age at presentation *.

	Start age 0-24 months (n=99)	Start age > 24 months (n=44)	Total (n=143)
Age at presentation 0-2 m	38 (38%)	4 (9%)	42 (39%)
2-6 m	45 (46%)	11 (25%)	56 (40%)
> 6 m	16 (16%)	29 (66%)	45 (31%)
Failure to thrive	34 (34%)	19 (43%)	53 (37%)
Liver Disease	70 (71%)	28 (64%)	98 (69%)
Renal Disease	25 (25%)	14 (32%)	39 (27%)
Neurological Disease	4 (4%)	3 (7%)	7 (5%)

* One patient could present with more than one symptom.

In the *complementary NTBC analysis*, 49% (123/250) of patients started on NTBC before 6 months of age, 28% (69/250) from 6-24 months, and 23% (58/250) at ≥ 24 months. Most patients (79%) started on the recommended dose and only 6 (2%) patients started on a higher dose.

Results – Survival Analyses

Probability of Survival (Death Due to All Causes)

Death due to all causes was reported in the main and complementary NTBC analyses and the probability of survival estimated. The results for the *main NTBC analysis* for the probability of survival at 1, 2, and 4 years of NTBC treatment are summarised in Table 6. The total number of patients in the analysis (n=207) represented cumulative enrolment from 23 February 1991 to 21 August 1997. The probability of survival was lower in patients in whom treatment was started before 2 months of age than in patients in whom treatment was started after the age of 2 months. There were 10 deaths in patients treated with NTBC during the period covered by the analysis: 7 due to liver failure, 2 due to hepatocellular carcinoma (HCC), and 1 due to multi-organ failure. Patients were censored at 21 August 1997 or at stop date if they stopped for reasons other than death. There were 28 patients censored at stop date (withdrawals) for reasons other than death.

Table 6: Main NTBC Analysis – Probability of survival after 1, 2, and 4 years of NTBC treatment.

	Number of Patients				Probability of survival [95% CI]		
Patients	Start	1year	2 years	4 years	1 year	2 years	4 years
All	207	149	95	35	96% [93, 98]	96% [92, 100]	93% [87, 99]
Start 0-2 m	16	12	7	3	88% [70, 100]	88% [65, 100]	88% [52, 100]
Start 0-6 m	80	55	30	11	94% [88, 100]	94% [85, 100]	94% [80,100]
Start > 6 m	127	94	65	24	97% [94, 100]	97% [94, 100]	93% [85, 100]

The results for the *complementary NTBC analysis* for the probability of survival at 2, 4, and 6 years of NTBC treatment are summarised in Table 7. The total number of patients in the

analysis (n=250) represented cumulative number enrollment from 1 July 1993 to 28 March 2000. There were 15 deaths in patients treated with NTBC during the period covered by the analysis: 8 due to liver failure, 2 due to HCC, and 5 due to other reasons (2 from multi-organ failure, 1 from GI bleeding, 1 from complications of prematurity and 1 from death not specified). The probability of surviving at each of the three time points was higher in children aged > 6 months at the start of treatment compared with children age ≤ 6 months at the start of treatment. For children starting NTBC treatment aged ≤ 2 months all deaths occurred during the first month of treatment. Patients were censored at 28 March 2003 or at the stop date if they stopped for reasons other than death. There were 35 patients censored at the stop date (withdrawals) due to reasons other than death.

Table 7: Complementary NTBC Analysis – Probability of survival after 2, 4, and 6 years of NTBC treatment irrespective of dose.

	Number of Patients				Probability of survival [95% CI]		
Patients	Start	2 years	4 years	6 years	2 years	4 years	6 years
All	250	158	88	16	94% [91, 98]	94% [89, 98]	94% [84, 100]
Start ≤ 2m	60	32	16	2	93% [85, 100]	93% [82, 100]	93% [60, 100]
Start ≤ 6 m	128	75	38	6	93% [87, 98]	93% [85, 100]	93% [74, 100]
Start > 6 m	122	83	50	10	96% [86, 100]	95% [89, 100]	95% [82, 100]

In the international survey of children with HT-1 treated only with a tyrosine and phenylalanine restricted diet, the risk of death within the first 6 months of treatment varied with the age of onset of symptoms.[15] The results for risk of death from the survey are summarised in Table 8.

Table 8: van Spronsen et al., 1994 – Risk of death within a specified time after onset of symptoms.

Age of onset of symptoms	Within 1 month	Within 2 months	Within 6 months
0-2 months	18%	31%	44%
2-6 months	4%	9%	23%
> 6 months	0%	0%	4%

The probability of death within the first 6 months after onset of symptoms in *van Spronsen et al (1994)* in children with HT-1 treated only with dietary restriction was much greater than in children treated with dietary restriction plus NTBC. It is not possible to undertake a strict statistical comparison between the international survey and NTBC patient populations due to differences in the study designs. However, the comparison strongly suggests that NTBC in combination with dietary restriction has a significant benefit on survival compared with dietary restriction alone. The complementary analysis of the NTBC Study included Kaplan-Meier estimates of survival in similar patient groups from the NTBC study and from *van Spronsen et al (1994)*. The analysis of survival probability in NTBC was estimated from the start of NTBC treatment compared with time from first symptoms in *van Spronsen et al*

(1994). However, this difference is unlikely to be significant as regards comparison of the results for the two studies, particular in patients aged less than 6 months.

Probability of Survival without Liver Transplant

The results for the *main NTBC analysis* for the probability of survival without liver transplantation at 1, 2 and 4 years are summarised below in Table 9. In the period covered by the study a total of 27 patients underwent liver transplantation due to liver failure (7 patients), suspicion of HCC (13 patients, confirmed in 7), or as elective transplantation (7 patients).

Table 9: Main NTBC Analysis – Probability of survival without liver transplantation after 1, 2, and 4 years of NTBC treatment.

	Number of Patients				Probability of survival [95% CI]		
Patients	Start	1year	2 years	4 years	1 year	2 years	4 years
All	207	149	95	35	88% [83, 93]	84% [78, 90]	78% [69, 86]
Start 0-2 m	16	12	7	3	88% [70, 100]	88% [65, 100]	88% [52, 100]
Start 0-6 m	80	55	30	11	89% [81, 97]	85% [75, 95]	82% [66, 97]
Start > 6 m	127	94	65	24	88% [82, 94]	83% [76, 91]	76% [65, 87]

The results for the *complementary NTBC analysis* for the probability of survival without liver transplantation at 2, 4, and 6 years are summarised below in Table 10. In the period covered by the complementary analysis a total of 34 patients underwent transplantation due to liver failure (8 patients), suspicion of HCC (16 patients, confirmed in 8), or as elective transplantation (10 patients). The patients were censored at 28 March 2000 or at the stop date if they stopped for reasons other than death or liver transplantation. One patient, who discontinued NTBC treatment due to the parent's request, was censored from the stop date in this analysis.

Table 10: Complementary NTBC analysis – Cumulative probability of survival without liver transplantation after 2, 4, and 6 years of NTBC treatment.

	Number of Patients				Probability of survival [95% CI]		
Patients	Start	2 years	4 years	6 years	2 years	4 years	6 years
All	250	158	88	16	84% [79, 89]	79% [73, 85]	75% [64, 85]
Start ≤ 2m	60	32	16	2	84% [73, 94]	84% [69, 99]	84% [41, 100]
Start ≤ 6 m	128	75	38	6	85% [78, 92]	84% [74, 93]	84% [61, 100]
Start > 6 m	122	83	50	10	83% [76, 90]	76% [67, 85]	69% [54, 84]

Probability of Death due to Liver Failure or Liver Transplantation due to Liver Failure

The results for the *main NTBC analysis* for the probability of death due to liver failure or transplantation due to liver failure at 1, 2, and 4 years are summarised in Table 11. Death due to liver failure or liver transplantation due to liver failure occurred only in patients with a starting age of 0-24 months. There were 7 patients who died due to liver failure and 7 patients

who were transplanted due to liver failure. Of the 80 patients who started NTBC treatment before 6 months of age, 7 (8.8%) died of liver failure or were transplanted due to liver failure. Of the 62 patients who started NTBC treatment at 6-24 months of age, 7 (11.3%) died of liver failure or were transplanted due to liver failure.

Table 11: Main NTBC Analysis – 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.

	Number of Patients				Probability [95% CI]		
Patients	Start	1year	2 years	4 years	1 year	2 years	4 years
Start 0-6 m	80	54	29	11	9% [2, 16]	9% [0, 19]	9% [0, 25]
Start 6-24 m	62	45	30	9	8% [1, 16]	11% [2, 20]	17% [0, 34]
Start 0-24 m	142	99	59	20	9% [3, 14]	10% [4, 16]	13% [3, 22]

The results for the *complementary NTBC analysis* for the probability of survival without death due to liver failure or transplantation due to liver failure are summarised below in Table 12. In the period covered by the complementary NTBC analysis there were 8 deaths due to liver failure and 8 patients were transplanted due to liver failure. All but 1 of the 16 events occurred in patients aged ≤ 24 months at the start of treatment. Of the 128 patients who started NTBC treatment before 6 months of age (6.3%) died of liver failure or were transplanted due to liver failure within the first year of treatment. There were 34 patients censored at the stop date for withdrawal for reasons other than death due to liver failure or transplantation due to liver failure.

Table 12: Complementary NTBC Analysis – Cumulative probability of survival without liver failure or transplantation due to liver failure after 2, 4, and 6 years of NTBC treatment.

	Number of Patients				Probability [95% CI]		
Patients	Start	2 years	4 years	6 years	2 years	4 years	6 years
All	250	156	88	16	94% [90, 97]	94% [89, 98]	92% [84, 100]
Start ≤ 6 m	60	75	38	6	94% [88, 99]	94% [86, 100]	94% [75, 100]
Start 6-24 m	128	40	25	2	90% [82, 98]	90% [80, 100]	86% [51, 100]
Start > 24 m	122	41	25	8	98% [93, 100]	98% [92, 100]	98% [87, 100]

In the international survey, there were 83 patients with the acute form of the disease (that is, presenting with severe liver disease before 6 months of age) and in these patients 35 (42.2%) died of liver failure (n=20) or recurrent bleeding (n=8) of a combination of liver failure and bleeding (n=7).[15] In the 108 patients in the international survey, transplantation due to liver failure was reported in 6/108 (6.4%) patients, compared with 7/207 (3.4%) patients in the main NTBC analysis (3.4%) and 8/250 (3.2%) patients in the complementary NTBC analysis. The figures for death due to liver failure were 27/108 (25%) in the international survey (liver failure plus liver failure/bleeding), 7/207 (3.4%) in the main NTBC analysis and 8/250 (3.2%) in the complementary NTBC analysis.

Hepatocellular Carcinoma (HCC)

The results for the *main NTBC analysis* for the probability of death due to HCC, transplantation due to HCC, or diagnosis of HCC during NTBC treatment at 1, 2, and 4 years are summarised below in Table 13. HCC was observed in 9 patients during NTBC treatment

and 2 of these patients died. Of these 9 patients, 8 were older and one was younger than 24 months at the start of NTBC treatment. There were 6 patients who underwent liver transplant due to HCC. One patient with HCC and suspected lung metastasis was treated with anti-cancer therapy and underwent a partial liver resection. In addition to the 9 patients with HCC verified by histopathology there were an additional 2 patients with suspected HCC based on serum AFP concentrations. Withdrawals (n=29) for reasons other than death due to HCC or liver transplantation due to HCC were censored from the stop date in the analysis. Of the 141 patients who started treatment at 0-24 months of age there was 1 (0.7%) event. Of the 65 patients who started treatment aged > 24 months there were 10 (15.4%) events. In the analysis of the total population (n=206) the risk of an event was 16.5% [95%CI: 7.9, 25.7] higher in patients aged > 24 months at start of treatment compared with patients aged ≤ 24 months.

Table 13: Main NTBC Analysis – Cumulative probability of death due to HCC, transplantation due to HCC or diagnosis of HCC after 1, 2, and 4 years of NTBC treatment *.

	Number of Patients				Probability of no occurrence of HCC [95% CI]		
Patients	Start	1 year	2 years	4 years	1 year	2 years	4 years
All	206	147	94	35	3% [0, 6]	5% [1, 8]	11% [4, 19]
Start 0-24 m	141	99	59	20	1% [0, 3]	1% [0, 3]	1% [0, 5]
Start > 24 m	65	48	35	15	8% [0, 15]	12% [2, 21]	27% [11, 42]

* Two patients with exponential increase in AFP level were included as HCC.

The results for the *complementary NTBC analysis* for the probability of no occurrence of hepatic carcinoma (HCC) at 2, 4, and 6 years are summarised in Table 14. In the period of the complementary NTBC analysis, 10 patients developed HCC. Only 1 of these patients was younger than 24 months at the start of treatment. There were 2 deaths due to verified HCC. Of the 10 patients with HCC, 8 underwent liver transplantation (1 died during the operation). One patient with HCC carcinoma with suspected lung metastasis was treated with chemotherapy followed by partial hepatic resection. This patient was still in the study at 28 March 2000. Withdrawals (n=41) for reasons other than death or transplantation due to HCC were censored from the stop date in the survival analysis. One patient had HCC diagnosed at the start of treatment and was transplanted after two weeks of treatment. This patient was not included in any of the analyses of the probability of no occurrence of HCC.

Table 14: Complementary NTBC Analysis – Cumulative probability of no occurrence of hepatocellular carcinoma (HCC) after 2, 4, and 6 years of NTBC treatment.

	Number of Patients				Probability of no occurrence of HCC [95% CI]		
Patients	Start	2 years	4 years	6 years	2 years	4 years	6 years
All	250	155	86	15	98% [95, 100]	94% [90, 98]	91% [81, 100]
Start ≤ 24 m	193	114	61	8	99% [98, 100]	99% [97, 100]	99% [94, 100]
Start > 24 m	57	41	25	8	92% [84, 100]	82% [70, 95]	75% [56, 95]

Porphyric Crisis

In the *main NTBC analysis*, there was only one patient with a reported porphyric crisis. In the *complementary NTBC analysis* there were only two (0.8%) patients with reported porphyric crises during the time period covered by the analysis: one was aged≤ 24 months at the start of treatment and one was > 24 months of age at the start of treatment. Both patients recovered. Withdrawals (n=50) were censored from the stop data in the survival analysis. The 2, 4, and 6 year probability of no occurrence of porphyric crisis for patients aged≤ 24 months and > 24 months were, respectively, 100% and 100% at 2 years, 99% and 100% at 4 years, and 99% and 91% at 6 years.

Results – Biochemical

Urine Succinylacetone

In the *main NTBC analysis*, excretion of urine succinylacetone decreased very quickly after the start of NTBC treatment and most patients had values below the limit of detection after the first week of treatment. The median time to normalization was 0.3 (range: 0.2, 20.8) months. All but one of the 104 NTBC treated patients had concentrations below the detection limit of 1 mmol/mol creatinine at the 1 year visit. In the *complementary NTBC analysis*, more than 90% of patients achieved urine succinylacetone concentrations in the reference range (< 1 mmol/mol creatinine) during the first week of treatment, and after the first month of treatment almost all patients were in the reference range. After the first 6 months of treatment, only one patient had a urine succinylacetone concentration which was outside the reference range and this was associated with a low nitisinone serum concentration. Later in the study, this patient had urinary succinylacetone concentrations within the reference range and nitisinone serum concentrations were higher. There were an additional three patients who had urine succinylacetone concentrations outside the reference range in the first year of treatment, but these patients had short times from treatment start (0-7 days) to the last observation during the first year.

Plasma Succinylacetone

In the *main NTBC analysis*, plasma succinylacetone concentration decreased at a slower rate than urine excretion of succinylacetone. At the 2 month visit more than 50% of patients still had plasma succinylacetone concentrations above the detection limit. The median time to normalization was 3.9 (range: 0.2, 27.0) months. Most patients had concentrations below the limit of detection (< 0.1 µmol/L) at the one year visit. In the *complementary NTBC analysis*, no patients had plasma succinylacetone concentrations within the reference range (< 0.1 µmol/L) during the first month of treatment. During the sixth month of treatment, about 90% of the patients were reported to be normalized, and at the end of the first treatment year, almost all patients were normalized. During the last six months of the first treatment year, 11 not previously normalized patients reported plasma succinylacetone concentrations above the reference range. All of these patients had low nitisinone serum concentrations. Only one of these patients had nitisinone serum concentrations consistently above 20 µmol/L during the first year of treatment, and one patient had very low nitisinone serum concentrations (0-7 µmol/L). In addition to these 11 patients, there were 23 patients who did not have any normalized plasma succinylacetone concentrations in the first year and these patients had treatment times ranging from 6-105 days from start of treatment. The slower normalization of plasma succinylacetone concentration compared with urine succinylacetone concentration is considered to be due to succinylacetone binding to plasma proteins.

Erythrocyte PBG-synthase

In the *main NTBC analysis,* erythrocyte PBG-synthase activity increased rapidly after the start of NTBC treatment and after one week's treatment about 75% of patients had values above the lower reference limit of 0.58 nkat/g Hgb. The median time to normalization was 0.3 (range: 0.2, 7.5) months. Out of 100 patients, 98 had values below the lower reference limit at pre-treatment, and 97 had values above this limit at one year. In the *complementary NTBC analysis,* more than 90% of the patients had PBG-synthase values in the reference range (> 0.58 nkat/g Hgb) after the first month of treatment, and after the second month of treatment all patients but one were normalized.

Urine 5-aminolevulinate (5-ALA)

In the *main NTBC analysis,* urinary excretion of 5-ALA decreased very rapidly after the start of treatment. The upper limit of urine 5-ALA excretion was defined by the 95th percentile of all values in the study after at least 6 months of treatment (that is, 23 mmol/mol creatinine). Most patients had values below this limit after the first week of treatment. The median time to normalization was 0.2 (range: 0.2, 20.7) months. In the *complementary NTBC analysis,* more than 80% of the patients had urine 5-ALA concentrations in the reference range during the first week of treatment, and after the first month of treatment all patients were normalized except one patient.

Serum α-fetoprotein

In the *main NTBC analysis,* serum α-fetoprotein (AFP) concentrations were markedly elevated before the start of NTBC treatment and concentrations decreased slowly during NTBC treatment. In the *complementary NTBC analysis,* the percentage of normalized serum AFP patients increased gradually over the entire two-year period following initiation of NTBC treatment. After one year of NTBC treatment, about 50% of the patients were normalized, and at the end of the second year of treatment about 90% of patients were normalized (that is, had values below the 90th percentile for a normal population). During the last six months of the first two-year treatment period, 17 not previously normalized patients had serum AFP concentrations above the reference range. Of these 17 patients, 6 had nitisinone serum concentrations generally below 20 µmol/L, and 2 of these patients underwent liver transplantation for suspicion of HCC (verified in one).

Liver Function

In the *main NTBC analysis,* most patients had abnormal *prothrombin complex* values before treatment. The prothrombin complex was assessed differently at different centres (that is, international normalised ration [INR], PT%, or PT ratio). In patients with a start age of 0-24 months (n=54) the median pre-treatment prothrombin complex values were INR 3.37, PT% 45%, and PT ratio 1.81, and about 10% of patients had values with the reference ranges. In patients with a starting age of > 24 years (n=15) the median pre-treatment prothrombin complex values were INR 1.23, PT% 75%, and PT ratio 1.10, and about 50% of patients had values with the reference ranges. In the total population normalized during the study period (n=69), the median time to normalization was 1.1 (range: 0.2, 16.0) months and median times were similar in the younger and older start groups (1.2 [range: 0.2, 16.0] and 1.1 [range: 0.2, 6.0], respectively). Of the total population (n=105), 69 (65.7%) were normalized during treatment, 9 (8.6%) were not normalized during treatment and 27 (24.8%) were normal at pre-treatment. In the early starting group (0-24 months) most patients had elevated serum AST, GGT and bilirubin concentrations pre-treatment and concentrations were normalized during treatment. In most patients in the total population serum ALT concentrations were within the reference range pre-treatment but increased during treatment to concentrations

above the reference range. In most patients in the total population median serum albumin concentrations were within the reference range pre-treatment and increased during treatment but remained within the reference range.

Kidney Function

The *main NTBC analysis* included an assessment of renal function pre- and post-treatment. There were only a small number of patients in the analysis with signs of kidney damage (defined as urine excretion of amino acids above the reference limit) before start of NTBC treatment. The median urine excretion of α_1-microglobulin was higher than the reference range in patients aged > 24 months (n=11). In this age group, the median values were normalized at the 1 year visit, with the difference between pre-treatment and post-treatment values being statistically significant ($p<0.001$). The median serum concentration in patients with signs of renal damage was within the normal range pre-treatment and was still within the normal range at the 1 year visit. Abnormally elevated pre-treatment urine amino acid concentrations in the 0-24 month old start group (n=3) were at normal levels at 1 year after treatment.

Haematology

The *main NTBC analysis* included an assessment of haematological tests pre- and post-treatment. The percentage of patients with median haemoglobin (Hb) concentrations and red blood cell (RBC) counts within the reference ranges in patients aged 0-24 months at start of treatment were 59% and 71%, respectively. In the 0-24 months age group, the median Hb concentration increased from a pre-treatment value of 104 g/L (reference range 100-160 g/L) to 118 g/L after 1 year of NTBC treatment ($p<0.001$), and the median RBC count increased from a pre-treatment value of $3.67x10^{12}$/L (reference range $3.4\text{-}5.2x10^{12}$/L) to $4.26x10^{12}$/L after 1 year of NTBC treatment ($p<0.001$). In patients aged > 24 months at the start of treatment, about 70% had pre-treatment median Hb concentrations and RBC counts within the reference range for both variables and these values did not significantly change after 1 year of NTBC treatment. Pre-treatment thrombocyte counts were low in most patients aged 0-24 months at start of treatment with 66% having values outside the reference range. About 33% of patients in this age group had pre-treatment values < $100x10^9$/L. During the first 3 months of NTBC treatment thrombocyte counts rapidly increased. The median count increased from $105x10^9$/L pre-treatment to $266x10^9$/L after 1 year of NTBC treatment ($p<0.001$). In patients aged > 24 months at the start of treatment, the median pre-treatment thrombocyte count increased from $162x10^9$/L to $182x10^9$/L after 1 year of treatment ($p=0.821$). The neutrophil count was normal in most patients pre- and post-treatment.

Urinary Phenolic Acid Excretion

The *main NTBC analysis* included an assessment of urinary phenolic acid excretion pre- and post-treatment. In the total population (n=96), the median urinary excretion of phenolic acids increased gradually from a pre-treatment concentration of 458 mmol/mol creatinine to 1730 mmol/mol creatinine after 1 year of NTBC treatment ($p<0.001$). This pattern was seen in patients aged 0-24 months and in patients aged >24 months at the start of treatment.

Plasma Amino Acids

The *main NTBC analysis* included an assessment of plasma amino acid concentrations pre- and post-treatment. In the total population (n=110), median pre-treatment plasma tyrosine concentration was 146 µmol/L (which was above normal levels) and increased to 387 µmol/L after 1 year of NTBC treatment. The pattern of increase was similar in both age groups. At the 1 year visit, 16% of patients had plasma tyrosine concentrations less than 250 µmol/L and 10% had concentrations higher than 600 µmol/L. There was little change in plasma

phenylalanine concentration after 1 year of NTBC treatment with pre- and post-treatment concentrations being within the reference range. The 1 year post NTBC treatment concentrations were statistically significantly lower compared with pre-treatment concentrations for methionine, glycine and alanine in patients aged 0-24 months at start of treatment, but not in patients aged > 24 months at start of treatment. The plasma concentration of valine increased significantly in both age groups following 1 year of NTBC treatment.

Clinical Condition and Somatic Development

The *main NTBC analysis* included assessment of a number of clinical parameters pre- and post-treatment. The general clinical condition improved in the 50 patients with data from about 60% reporting good general condition pre-treatment to about 90% after 1 year of NTBC treatment ($p<0.001$). The clinical signs of liver disease improved after 1 year of NTBC treatment. The pre- and post-treatment percentage of patients reporting the following hepatic problems were, respectively, hepatomegaly (80% and 51%, $p=0.002$), hepatic failure (42% and 6%, $p=0.001$), and ascites (33% and 0%, $p<0.001$). The proportion of patients reporting rickets fell from 26% pre-treatment to 5% post-treatment, $p=0.008$. There were no statistically significant differences between pre- and post-treatment reports of signs of bleeding, splenomegaly, muscle tone change, tendon reflex change, and delayed mental or motor development. There were no reports of cardiomyopathy, or central and peripheral neuropathy. Most patients had normal weight and height increases during NTBC treatment.

Supportive Studies

Lindstedt et al., 1992[4]

In 1992, *Lindstedt et al* from the Sahlgrenska Hospital, Sweden, published the first report of nitisinone for the treatment of children with HT-1. The study included five children of approximate ages 1 day, 2 months, 4 months, 5 months, and 2 years 5 months. Of the five children, two were newly diagnosed with HT-1 with one presenting with signs of liver failure and one presenting with rachitic symptoms. In the other three children, one had been a candidate for liver transplantation, one had a six month history of severe renal tubular dysfunction with rachitic symptoms despite dietary treatment, and no history could be identified for the third child. All five children had enlarged livers and all except one also had hepatic nodules and parenchymal changes on CT scan. All children had been treated with dietary tyrosine and phenylalanine restriction since diagnosis and these dietary restrictions were continued during nitisinone treatment. In the first treated child, treatment was initiated with a daily dose of 0.1 mg/kg which was then increased to 0.2 mg/kg and 0.4 mg/kg during the first two months. The next four children had treatment initiated with 0.2 mg/kg, and 3 of these had their dose increased to 0.6 mg/kg because of persistently elevated 5-ALA urinary excretion. Treatment was given for 7 to 9 months with the total daily dose being administered over two (bd) or three (tds) doses.

The biochemical indicators specific for HT-1 responded favourably to nitisinone treatment (that is, plasma and urine succinylacetone, erythrocyte PBG-synthase activity, and urine 5-ALA). All children had laboratory evidence of improved liver function, but one underwent liver transplantation due to increased serum AFP concentration. One of the children aged 2 months had the acute form of the disease (serious symptoms before the age of 2 months) which has a particularly poor prognosis (only 25% survive the first year). The investigator's commented that this child "had an unusually favourable course" which was considered to be due to nitisinone treatment. The other four children had the "sub-acute-chronic form of the disease" in which 65% of patients survive for 10 years but rarely reach the age of 20. These four children all had signs of severe liver disease with portal hypertension due to cirrhosis

and each of these children showed improvement in various parameters of liver function after 7-9 months treatment. The investigators concluded that nitisinone treatment "may offer an alternative to liver transplantation in this otherwise fatal disease". The favorable results from this pilot study encouraged the investigators to initiate the NTBC Study.

Personal Communication

The submission included a personal communication on behalf of the Quebec NTBC Study Group dated 3 September 2001. The communication stated that it could be "shared within your company [SOIAB] and within licencing agencies". The communication included information showing that in a small number of HT-1 patients from the Quebec Region clinical outcomes were statistically significantly better in patients treated with nitisinone than with diet alone. The outcomes were hospitalizations for HT1-complications, neurological crises, hepatic transplantations, and deaths.

Evaluator's Overall Conclusions on Clinical Efficacy

The NTBC Study provides the only adequate efficacy data supporting approval of nitisinone for the treatment of HT-1. In this open-label study, nitisinone was administered with a tyrosine and phenylalanine restricted diet on a compassionate use basis to all patients with HT-1 nominated for treatment irrespective of country of origin, age, severity and characteristics of the disease, and concomitant illness. The only exclusion criterion was patients with HT-1 who had undergone liver transplantation. The study did not comply with GCP (ICH) principles nor were the treatment sites audited. There were no randomized, controlled, double-blind data submitted supporting the efficacy of nitisinone for the treatment of HT-1. However, there were data from "historical controls" from a published study which assisted interpretation of the open-label efficacy data from the NTBC Study. The submission included supportive data in the form of a brief "communication" from the Quebec NTBC Study Group which showed that nitisinone and diet (n=34) was significantly superior to diet alone (n=27) on a number of clinically relevant outcome (hospitalizations for HT-1 complications, neurological crisis, hepatic transplantation, and deaths).

The NTBC Study efficacy and safety data were submitted in two separate reports: a main analysis of 207 patients and a complementary analysis of 250 patients. It is estimated that the two analyses shared a substantial number of patients due to overlapping time periods. Consequently, the reported results for the two analyses were similar whenever the same outcome was assessed. Therefore, when summarizing the efficacy data from the NTBC Study the focus will be on the complementary analysis as this analysis included more patients that the main analysis and patients were followed-up for 6 years rather than 4 years.

In the complementary NTBC analysis, the probability of survival in all patients at 2, 4, and 6 years of nitisinone treatment was 94% at each of the three time points. Survival reflected lower occurrence of death due to all causes. The patient numbers at the various time points were 250 at start, 158 at 2 years, 88 at 4 years, and 16 at 6 years. The probability of survival at each time point was greater in children starting treatment at > 6 months of age compared with children starting treatment at ≤ 6 months of age. This might reflect more serious acute disease in the younger patients. There were 15 deaths in patients treated with NTBC during the period covered by the analysis: 8 due to liver failure, 2 due to HCC, and 5 due to other reasons (2 from multi-organ failure, 1 from GI bleeding, 1 from complications of prematurity and 1 from death not specified). For children starting NTBC treatment aged ≤ 2 months of age, all deaths occurred during the first month of treatment. In the "historical controls", the risk of death within 6 months of the onset of symptoms in children treated with dietary

restriction alone were 44% (0-2 months of age), 23% (2-6 months of age) and 4% (> 6 months of age). It is not possible to undertake a formal statistical comparison between the NTBC and "historical control" data due to differences in study design. However, comparison of the results strongly suggest that nitisinone treatment has a marked survival benefit in children with HT-1, particularly in those in whom treated is started at ≤ 6 months of age.

In the complementary NTBC analysis, the probability of survival without death due to liver failure or transplantation due to liver failure in all patients at 2, 4, and 6 years was 94%, 94%, and 92%, respectively. The probability of experiencing these events was greater at each time point in patients aged 6-24 months at the start of treatment compared with patients aged ≤ 6 months. In the period covered by the complementary analysis there were 8 deaths due to liver failure and 8 patients were transplanted due to liver failure. All but one of the 16 events occurred in patients aged ≤ 24 months at the start of treatment. In the complementary NTBC analysis, the probability of survival without liver transplantation in all patients at 2, 4, and 6 years was 84%, 79%, and 75%, respectively. The probability of surviving without a liver transplantation at each time point was greater in patients aged ≤ 6 months at the start of treatment compared with patients aged > 6 months. In the period covered by the complementary analysis a total of 34 patients underwent transplantation due to liver failure (8 patients), suspicion of HCC (16 patients, confirmed in 8), or as elective transplantation (10 patients). Overall, the results suggest that increased benefits as regards liver failure are related to initiating treatment before 6 months of age.

In the "historical controls", there were 83 patients with the acute form of the disease (that is, presenting with severe liver disease before 6 months of age). Of these 83 patients, 35 (42.2%) died of liver failure (n=20) or recurrent bleeding (n=8) or a combination of liver failure and bleeding (n=7). In the complementary NTBC analysis, there were 8/250 (3.2%) deaths due to liver failure in the total population. In the "historical controls" transplantation due to liver failure occurred in 6.4% (6/108) of patients compared with 3.2% (8/250) of patients in the complementary NTBC analysis. The corresponding figures for death due to liver failure were 27/108 (25%) in the "historical controls" (liver failure plus liver failure/bleeding), 7/207 (3.4%) in the main NTBC analysis and 8/250 (3.2%) in the complementary NTBC analysis. The results suggest that nitisinone treatment significantly reduces the risk of death due to liver failure and transplant due to liver failure compared with diet alone.

In the complementary NTBC analysis, the probability of no occurrence of HCC in all patients at 2, 4, and 6 years was 98%, 94%, and 91%, respectively. The probability of no occurrence of HCC was higher at each of the three time points in children aged ≤ 24 months at t he start of treatment compared with children aged > 24 months. In children who started treatment aged ≤ 24 months, the 6-year probability of developing a HCC was 1% compared with 25% in children aged > 24 months at start of treatment. In the period covered by the complementary analysis, 10 patients developed HCC and only 1 of these patients was younger than 24 months at the start of treatment. There were 2 deaths due to verified HCC. Of the 10 patients with HCC, 8 underwent liver transplantation and there were 2 deaths due to verified HCC (1 occurring in a patient while undergoing liver transplantation). In the complementary NTBC analysis, HCC occurred in 10/250 (4%) of all patients while in the "historical controls" HCC occurred in 18% of patients who had lived for 2 years. In the main NTBC analysis, HCC was reported in 6% of all patients who reached at least 2 years of age during the study period. Overall, the results suggest that nitisinone treatment reduces the risk of developing HCC compared with diet alone. In the complementary NTBC analysis, starting nitisinone treatment at ≤ 24 months of age markedly reduced the risk of developing HCC compared with starting treatment at > 24 months of age.

In the complementary NTBC analysis, nitisinone treatment rapidly reduced urinary nitisinone excretion with excretion being normalized in more than 90% of patients within the first week of treatment and nearly all patients being normalized after 6 months of treatment. In the complementary NTBC analysis, plasma succinylacetone concentration was normalized in 90% of patients after 6 months of treatment and in all patients after 1 year, erythrocyte PBG-synthase activity was normalized in more than 90% of patients after 1 month of treatment and in nearly all patients after 2 months, urinary 5-ALA excretion was normalized in more than 80% of patients after 1 week of treatment and in nearly all patients after 1 month. In patients with a nitisinone serum concentration of 30 µmol/L, the probability of recurrence of detectable concentrations of succinylacetone in the urine and erythrocyte PBG-synthase activity below the reference range was estimated to be 6% for both variables.

Serum AFP was normalized in about 50% of patients after 1 year of nitisinone treatment and in about 90% of patients after 2 years. The prothrombin complex normalized after a median of 1.1 months of treatment [range: 0.2, 16.0], serum AST, GGT and bilirubin concentrations normalized during treatment while serum ALT concentrations increased. In a small number of patients with impaired renal function (defined by increased urinary amino acid excretion), nitisinone treatment normalized α_1-micgroglobulin and amino acid urinary concentrations after 1 year. The Hb concentration and RBC counts significantly improved after 1 year of nitisinone treatment in patients aged 0-24 months at start of treatment, but no improvements in these two variables were observed in patients aged > 24 months at start of treatment. The platelet count rapidly increased in patients aged 0-24 months at the start of treatment, but in patients aged > 24 months at the start of treatment the increases were small and not significant after 1 year of treatment. Plasma tyrosine and valine concentrations increased with nitisinone treatment in all patients after 1 year of treatment, but there was little change in plasma phenylalanine concentration. In patients aged 0-24 months at the start of treatment plasma concentrations of methionine, glycine, and alanine were significantly lower after 1 year of nitisinone treatment, but not in patients aged > 24 months at the start of treatment.

In the main NTBC analysis, the general condition of patients (n=50) treated with nitisinone significantly improved after 1 year of treatment. Similarly, after treatment with nitisinone for 1 year significant improvements were also seen in liver function (that is, hepatomegaly, hepatic failure, and ascites) and in the proportion of patients with rickets. There were no significant differences between pre and post-treatment reports of signs of bleeding, splenomegaly, muscle tone changes, tendon reflex changes, and delayed mental or motor development. There were no reports of cardiomyopathy, or central or peripheral neuropathy. Most patients had normal weight and height increases during nitisinone treatment.

Safety

Introduction

The sponsor's *Clinical Overview* included safety information on 406 patients treated with nitisinone to April 2001. The sources were patient databases held by the CU and adverse event reports held by SOAB. The safety data in the *Clinical Overview* was primarily derived from the PSUR 00-01 which included information on 318 patients, and summarised the data from the main NTBC analysis, previous PSURs, and patients not included in the NTBC study who had received nitisinone.

The safety data from the *Clinical Overview* (n=406) and/or PSUR 00-01 (n=318), together with the safety data from the main NTBC analysis (n=207) and the supplementary NTBC analysis (n=250) have been evaluated. These data represents the safety data collected before the EU "birth date" of nitisinone on 21 February 2005. There is obvious overlapping of patients among the submitted safety data. The *Clinical Overview* and PSUR 00-01 focused

predominantly on adverse drug reactions, while the main and supplementary NTBC analyses included assessment of adverse events.

The submission also included additional safety data collected after the date of EU approval. These data included six PSURs summarizing data at six month intervals from 21 February 2005 to 20 February 2009, a PSUR Summary Bridging Report (with Addendum) for the period 21 February 2005 to 20 June 2009, and five publications identified by the sponsor for the period 21 February 2005 to 20 February 2009 containing safety information relating to nitisinone.[1,2,8,9,10]

Patient Exposure

A total of 406 patients were treated with nitisinone from February 1991 until April 2001. The usual daily dose before mid 1993 was about 0.6 mg/kg and after that time it was usually about 1 mg/kg. In the main NTBC analysis, most patients were treated with a daily nitisinone dose of 0.8-1.2 mg/kg. The highest daily dose used in the NTBC Study was 3 mg/kg. The total exposure times in the safety evaluations are summarised below in Table 15. The databases share some of the patients exposed to nitisinone.

Table 15: Patient exposure in the pre-EU birth date safety documents.

	NTBC [Ref 8]	Addendum [Ref 11]	PSUR 97-98 [Ref 12]	PSUR 99 [Ref 13]	PSUR 00-01 [Ref 14]
Patient No.	207	24	266	282	318
Median	675 days	156 days	497 days	365	486
Range		1-707 days	2-497 days	3-365	6-486
Mean		184 days	397 days	331	440
Total Exposure	441 patient-years	13 patient-years	289 patient-years	255 patient-years	383 patient-years

In the complementary NTBC analysis, adverse event data were available on 250 patients who were included in the NTBC Study between 1 July 1993 and 28 March 2000. The total nitisinone treatment varied from 0.1 to 80.5 months.

Adverse Events

Overall

In the NTBC study, an event (symptom or sign) was included as an adverse event only if it was reported on the adverse event form, explicitly mentioned as an adverse event in other communications from the local investigator, or classified as an adverse event by the local investigators. Withdrawals from the study due to death and transplantations were also included as adverse events. The main and complementary NTBC analyses included a description of all adverse events (AEs) reported to CU or SOAB during the period of the analyses. There are shared reports in the two analyses due to the overlapping time periods. The adverse event data from both the main and complementary NTBC analyses have been reviewed and the patterns are similar. Consequently, only the AE data from the complementary NTBC analysis have been described in detail in this report as this analysis included more patients exposed to nitisinone for a longer duration compared with the main NTBC analysis. The number and percentages summarizing the AEs in complementary NTBC analysis were calculated from the provided data if these values were not presented in the study report. The AE data in the NTBC Study were presented in the study report using WHO rather than MedDRA terminology.

In the *complementary NTB analysis*, 128/250 (51.2%) patients experienced a total of 169 adverse events (WHO Preferred Term). Visual disorders accounted for the most commonly reported adverse events (25 patients [10.0%], 60 events) and the most commonly reported individual visual disorders were keratitis (3.2%), corneal opacity (2.4%), photophobia (2.4%) and conjunctivitis (2.0%). The second most commonly reported group of disorders was liver and biliary system (24 patients [9.6%], 26 events). The most commonly reported liver and biliary system AE was hepatic failure (6.4%). The third most commonly reported group of disorders was “neoplasm” (20 patients [8.0%], 20 events). The most commonly reported neoplasm AE was malignant hepatic neoplasm (4%) followed by hepatic neoplasm (3.2%).

In the *complementary NTBC analysis*, the following adverse events were judged by the medical director of SOAB to be possibly related to nitisinone treatment: keratitis (reported by 3% of the patients); corneal opacity (2%); photophobia (2%); conjunctivitis (2%); eye pain (2%); blepharitis (<1%); dermatitis exfoliative (1%); pruritus (l%); rash erythematous (<1%); dry skin (<l%); thrombocytopenia (2%); leucopoenia (1%) and granulocytopenia (1%).

In other studies, the most commonly occurring adverse reactions were liver failure (1.9% to 7%), hepatic neoplasm (0.3% to 4%), elective liver transplantations (0.3% to 4%), thrombocytopenia (0.8% to 3 %), corneal opacity (1.1% to 2%), keratitis (0.3% to 2%), conjunctivitis (0.4% to 2%), and photophobia (0.4% to 2%).

Eye Symptoms in Relation to Plasma Tyrosine

There was a statistically significant relationship between the age at start of nitisinone treatment and the maximum plasma tyrosine concentration during the nitisinone treatment period on the probability of eye symptoms. At a maximum plasma tyrosine concentration of 850 µmol/L, the estimated probabilities of occurrence of eye symptoms were about 11%, 12%, 13% and 15% for patients at start age 0, 1, 2, and 3 years, respectively. At a maximum plasma tyrosine concentration of 500 µmol/L, the corresponding estimated probabilities were about, 5%, 5%, 6% and 6%, respectively.

Haematology Findings

In the *complementary NTBC analysis,* there were 5 cases of thrombocytopenia, 3 of leucopenia, and 2 of granulocytopenia. Of these 10 AEs, there were 2 serious adverse events (SAEs) (both thrombocytopenia). All ten haematological AEs were considered to be possibly related to nitisinone treatment.

Serious Adverse Events and Deaths

Deaths

There were 34 (8.4%) deaths in 406 patients during treatment with nitisinone from February 1991 to April 2001. The most common cause of death was liver failure (n=19) followed by liver cancer (n=3). Other causes of death with 2 patients each were infection/septicaemia, multi-organ failure, and "details unknown". Other causes of death with 1 patient each were encephalopathy, fever (no further details known), GI haemorrhage, malignant lymphoma, porphyria-like crisis, and respiratory insufficiency.

Deaths occurring in the NTBC study have been summarised under *Efficacy*. In the *main NTBC analysis,* there were 10 (4.8%) deaths in 207 patients during the period covered by the analysis: 7 due to liver failure, 2 due to HCC carcinoma, and 1 due to multi-organ failure. In the *complementary NTBC analysis,* there 15 (6.0%) deaths in 250 patients treated with NTBC during the period of the analysis: 8 due to liver failure, 2 due to HCC, and 5 due to other reasons (2 from multi-organ failure, 1 from GI bleeding, 1 from complications of prematurity and 1 from death not specified).

Serious Adverse Events

In the *main NTBC analysis,* there were 49 SAEs reported in 44/207 (21.3%) patients treated between 23 February 1991 and 21 August 1997. The 49 SAEs included hepatic failure (14), hepatocellular carcinoma (16 [10 verified, 6 not verified]), elective liver transplantation (7), multi-organ failure (1), thrombocytopenia (3), and 8 other cases not considered to be causally related to nitisinone. The 3 SAEs of thrombocytopenia were transient. The other specified SAEs were all considered to be well known manifestations of HT-1 and not causally related to nitisinone.

The *complementary NTBC analysis* included 68 SAE reports (excluding withdrawals due to death or transplantation) in 64/250 (25.6%) treated between 1 July 1993 and 28 March 2000. The 68 SAEs included: hepatic failure (16), hepatic neoplasm malignant (11), elective transplantation (10), hepatic neoplasm (8), hepatic cirrhosis (4), convulsion (3), GI haemorrhage (2), porphyria (2), thrombocytopenia (2), multi-organ failure (2), lymphoma

malignant (1), hypoglycaemia (1), brain neoplasm benign (1), cyanosis (1), infection (1), retinal disorder (1), complications of prematurity (1) and fever (1).

During the time period covered by the main analysis of the NTBC Study, 24 patients received nitisinone without being included in the NTBC Study and were included in a safety addendum. A total of 5 SAEs were reported in this addendum and all were considered to be manifestations of HT-l: 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 known details, and one report of treatment being discontinued because of lack of efficacy in a patient with renal complications after liver transplantation.

From the end-point of the main analysis of the NTBC Study, 21 August 1997, until 30 April 2001, three PSURs including 365 patients and 927 patient years of nitisinone treatment were prepared. The PSURs listed 61 SAEs which included liver failure (22), hepatic neoplasm (8 verified carcinoma, 4 suspected and not verified), elective liver transplantation (9), liver transplantation for unknown reason (3), porphyria (2), liver cirrhosis (2), multi-organ failure (1), and GI haemorrhage (1). All these events were considered to be well recognised manifestations or outcomes of HT-l. The SAEs also listed 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 1 patient who first reported treatment for Hodgkin's lymphoma but died after relapse two years later. The investigators considered that nitisinone treatment was not causally related to any of the 61 SAEs

Laboratory Findings, Vital Signs, ECG

There was no systematic assessment of safety data relating to laboratory abnormalities, vital signs (pulse rate, blood pressure) or ECG changes in the NTBC Study. The NTBC Study included measurement of specified laboratory parameters as part of the efficacy assessment for nitisinone and these have been discussed above. In the *main NTBC analysis*, results over time for serum concentrations of bile acids (small number of patients), sodium, potassium, calcium, urea and total protein were provided. Perusal of these results did not give rise to concern relating to the measured laboratory parameters. The number of patients with measurements declined significantly over time with the highest number of patients with measurements being observed within the first 6 months of treatment.

Discontinuations due to Adverse Events

In the *complementary NTBC analysis*, there were 50 (20%) withdrawals out of 250 patients. Of the 50 withdrawals, 15 were due to death, 34 due to liver transplantation and 1 due to the parents' wishes (died 12 months after discontinuation). No patient was withdrawn from treatment due to an AE considered to have a causal or possibly causal relationship with nitisinone.

Post Marketing Experience

PSUR Summary Bridging Report

The submission included a *PSUR Bridging Report* for Orfadin which summarised all the safety information collected from the time the drug was first approved in the EU on 21 February 2005 to the data lock point 4 years and 4 months later on 10 July 2009.

As of 20 June 2009, the sponsor's database contained information on 628 patients (356 male, 268 female, and 4 unknown). The ages and number of the patients in the database were: < 1 month of age (n=0); 1 month to 2 years of age (n=32); 2-12 years of age (n=427); 12-18 years of age (n=140); ≥ 18 years of age (n=27); unknown (n=2). The estimated average daily dose at the end of the period covered by the PSUR was 27 mg based on 425 patients.

Swedish Orphan International aims to capture all safety data available for Orfadin. Data from the following sources irrespective of causality were included in the Summary Bridging Report: spontaneous reports from healthcare professionals; solicited reports from the Orfadin Active Surveillance program (ORFADIN-OAS); solicited reports from the Named Patient Use program (ORFADIN-NPU); reports on adverse events in the framework of contractual arrangements; reports from literature; reports received from regulatory authorities worldwide; and spontaneous reports from patients and other consumers (not medically confirmed).

In total, 97 medically confirmed individual case safety reports (ICSRs) were identified during the period under review, and 70 of these were considered to be serious. Of the 97 ICSRs, 49 were from the EU/European Economic Area (EEA) and 48 were from outside the EU/EEA. In addition to the 97 medically confirmed ICSRs there were 4 non-medically confirmed ICSRs. Of the 97 medically confirmed ICSRs, 38 were from the ORFADIN-OAS, 29 from the ORFADIN-NPU, 20 were spontaneous, 9 were from non-company sponsored clinical trials, and 1 was from the literature.

The highest number of adverse events fell within the MedDRA System Organ Class (SOC) *Surgical and Medical Procedures* (n=27), followed by *Nervous System Disorders* (n=21), *Hepatobiliary Disorders* and *Neoplasms Benign, Malignant and Unspecified (incl. cysts and polyps)* (n=19 each) and *Eye Disorders* and *General Disorders and Administration Site Conditions* (n=18 each). Adverse events under *Hepatobiliary Disorders* and *Surgical and Medical Procedures* included patients whose hepatic function deteriorated and/or patients who underwent liver transplantation. Serious adverse events arising from the SOC *Nervous System Disorders* involved acute events such as hepatic coma, brain oedema and seizures, while non-serious adverse events in this SOC were mainly related to cognitive problems. The most common Preferred Terms under SOC *General Disorders and Administration Site Conditions* were “no therapeutic response” (n=4), “drug ineffective” (n=3) and “death” (n=3), with most events being considered to be related to HT-1. There were 22 fatal outcomes identified during the reporting period and the majority of these were assessed as not related to Orfadin by the reporters.

The most frequently reported serious adverse event was liver transplant (n=37) followed by hepatic neoplasm malignant (n=20), hepatic failure (n=13) and hepatic cirrhosis/gastrointestinal haemorrhage/drug ineffective/no therapeutic response (n=4 each). The SOC *Infections and Infestations* included adverse events such as sepsis and other infections. Serious adverse events in the SOCs of *Nervous System Disorders*, *Gastrointestinal Disorders* and *Blood and Lymphatic System Disorders* included complications related to hepatic impairment (for example, hepatic coma, varices, and bleeding disorders).

The most frequently reported non-serious adverse events were eye disorders (n=27) such as corneal opacity/erosion/deposits/keratitis and various symptoms associated with these conditions. Non-serious adverse events involving the SOC *Nervous System Disorders* included various cognitive problems. Other relatively common non-serious adverse events were abnormal laboratory parameters relating to liver function and bleeding.

Hepatic adverse events (including liver transplant). There were 27 reports of “liver transplant” during the reporting period, and in 5 of these reports “liver transplant” was the only reported term and no additional information was available. The most commonly reported reason for liver transplant was verified or suspected hepatocellular carcinoma. There were 17 reports of “hepatic neoplasm” including 1 report of hepatoblastoma. Hepatic failure was reported in 8 patients.

Renal adverse events: In the period covered by the bridging summary there were reports of 4 patients with a renal disorder. Acute renal failure with a fatal outcome was reported in one severely ill neonate, reported as not related to Orfadin. Azotaemia was reported in one patient with a porphyria like crisis, who recovered after the Orfadin dose was increased; no causality assessment was provided. The remaining two events involved decreased renal function.

Haematological adverse events: There were four patients who experienced serious adverse events: thrombocytopenia in two patients and anaemia in two patients. Lymphadenopathy, assessed as unlikely to be related to Orfadin was reported in one patient participating in a non-company sponsored alkaptonuria study.

Neurological adverse events: Neurologic crises with porphyria, polyneuritis and dystonia can occur in the acute form of HT-1 and in rare cases may be the presenting features of the disease. Porphyria-like crisis was reported in one patient and the symptoms disappeared when the Orfadin dose was increased. Neurological complications were reported in two patients during episodes of gastroenteritis; acute polyneuropathy in one patient, and seizures in one patient. Single reports of coma/hepatic coma (2 patients), cerebral oedema (1 patient), cerebral vascular haemorrhage (1 patient) and epileptic seizure (1 patient) have also been reported. A fatal outcome was reported in some of these cases.

Cognitive dysfunction adverse events: The sponsor has been closely monitoring reports of learning difficulties and/or disturbances of cognitive function in patients treated with nitisinone. There have been nine reports of adverse events related to disturbances in cognitive function, and in none of these have progressive decreases in cognitive/developmental function been observed despite continued Orfadin treatment. The addendum to the *Bridging Summary PSUR* refers to an abstract *"Does treatment with NTBC affect cognitive functioning in Tyrosinemia type 1?"*, presented by van Hasselt *et al*. at the Netherland's national metabolic society symposium in Nijmegen 18-19 June 2009. The authors suggest that HT-1 patients treated with nitisinone exhibit mild mental retardation compared with healthy siblings. The sponsor noted that the abstract contains limited information and that additional data are required to evaluate the significance of the findings. The sponsor also referred to unpublished data from a large Canadian study of HT-1 patients which does not indicate any developmental or cognitive disturbances in patients treated with Orfadin. The sponsor stated that it is closely monitoring the results from this study. No data from this study were submitted.

Eye disorders: Ophthalmic events were reported in 13 patients and all but one were assessed as non-serious. Ophthalmic events may be caused by high tyrosine levels (for example, keratitis, corneal opacities and associated symptoms).

The sponsor continues to monitor hepatic, renal, haematological, neurological, and ophthalmic adverse events. In addition, the sponsor continues to monitor a number of previously identified serious and unexpected adverse events including maculopapular exanthema, mucositis, respiratory distress, neutropenia, seizure, cognitive and attention disorders/disturbances and learning disorders.

Arora et al., 2006 – Cardiomyopathy in HT-1[1]

This study included a retrospective analysis of the incidence and outcome of cardiomyopathy in children with HT-1 referred to the Children's Hospital, Birmingham, UK, between 1986 and 2002. The analysis included 20 consecutively referred children (12 male, 8 female) all of whom had initially been treated with a tyrosine and phenylalanine restricted diet and since 1992 with nitisinone. Prior to 1992 all children were considered for liver transplantation and from 1992 all children were started on nitisinone as soon as HT-1 was diagnosed. In children

treated with nitisinone, liver transplantation was considered if there was no response to nitisinone or if hepatocellular carcinoma was suspected.

On diagnosis of HT-1, all children underwent cardiovascular assessment which included ECG and echocardiography. This assessment was repeated as clinically indicated and on at least one additional occasion if the initial assessment was normal. Cardiomyopathy was detected at the initial examination in 6/20 (30%) children and both localized (n=4) and concentric (n=2) myocardial thickening were observed. The six children with cardiomyopathy were followed-up for a median of 10.4 (range: 2.4-17) years after cardiomyopathy was first recognized. In 5/6 (83%) of these children there was complete resolution of cardiomyopathy after a median of 3 (range: 2.5-5) years. The one child with persistent abnormalities had the most severe disease at presentation. The 14/20 (70%) children with no evidence of cardiomyopathy at initial assessment were followed-up for a median of 8.6 (range: 1.3-11.3) years after the initial cardiac assessment and all remained clinically well. These 14 children had repeat echocardiography on a median of one occasion [range: l-4] and this was always normal. The authors suggest that cardiomyopathy in children with HT-1 may be due to a direct cardiotoxic effect of circulating tyrosine metabolites, and that nitisinone reduces the levels of these metabolites below the threshold at which cardiomyopathy might develop in those who are susceptible.

Koelink et al., 2006 – AFP and Liver Cancer[9]

This study was a prospective analysis of AFP levels in 11 Dutch patients with HT-1 treated with nitisinone. In these 11 patients, 4 developed liver cancer and AFP changes over time in these 4 patients were different from AFP changes in the 7 patients who did not develop liver cancer. The data suggests that, apart from a rise in AFP levels, a slow decrease in AFP levels and a level that never reaches long-standing normalization is of predictive value in distinguishing patients with high risk of developing liver cancer from those with a low risk. No liver cancer was detected in patients with normal AFP levels. The authors recommend that AFP levels "should be monitored carefully" in patients with HT-1. The study demonstrates that although nitisinone reduces the risk of liver cancer it can still occur in some patients despite treatment and that AFP levels are of predictive importance. The study included too few patients to determine if the development of liver cancer is related to age at the time of initiation of treatment and/or the duration of treatment.

Masurel-Paulet et al., 2008 – NTBC Treatment: Long-term Outcome[10]

This study was a retrospective analysis of 46 patients with HT-1 treated with nitisinone in France. Age at initiation of treatment was ≤ 6 months in 67% (31/46), 6-24 months in 22% (10/46), and > 24 months in 11% (5/46). At the time of the study, the mean duration of nitisinone treatment was 4 years and 9 months [range: 3 months to 12 years, 9 months]. The mean daily dose was 0.95 mg/kg [range: 0.5, 1.7]. All patients were given a tyrosine and phenylalanine restricted diet to maintain plasma tyrosine concentrations below 500 µmol/L.

Only the adverse events reported in the study will be described. Transient "leuconeutropenia" was observed in 12 patients and in 5 of these patients this occurred during the first month of treatment. "Thrombopenia" was observed in 9 patients and in 5 of these patients this occurred during the first month of treatment. The observed events were described as "moderate" and "without clinical consequences in all cases". Photophobia occurred in 6 patients, and 4 patients had keratitis (3 of the 4 patients were poorly compliant with the diet and had plasma tyrosine concentrations > 500 µmol/L). Cutaneous lesions such as transient non-specific rash were noted in 5 patients. No adverse events resulted in interruption to nitisinone treatment.

The authors considered it "noteworthy" that schooling difficulties affected 8/23 (35%) of the school age patients and major cognitive disturbances were noted in six patients (memory, concentration difficulties, slowness). The authors postulate that the observed problems might be linked to severe liver failure at presentation. However, the authors note that in the rare condition tyrosinaemia type III caused by 4-hydroxyphenylpyruvate dioxygenase deficiency (the enzyme inhibited by nitisinone) cognitive impairment has been reported in 75% of the cases. The authors also note hypertyrosinaemia has been suggested to be a causal factor for neurological impairment. The authors conclude that it remains to be established whether the high percentage of schooling and cognitive problems observed in the study "result from the disease itself or from some adverse effect of NTBC treatment".

Santra et al., 2008 – Renal Tubular Function[11]

This UK study retrospectively reviewed 21 patients with HT-1 with biochemical evidence of renal tubular dysfunction at presentation treated with nitisinone for 1 year. All children were treated with daily nitisinone of 0.6 mg/kg before 1995 and 1 mg/kg after 1995 with the dose being adjusted according to response (that is, the NTBC Study protocols). In addition, the children were treated with a tyrosine and phenylalanine restricted diet, fat soluble vitamins in the presence of liver dysfunction, and phosphate supplements during hypophosphataemia. At presentation all children had "excessive" proteinuria which reduced to normal values after one year of nitisinone treatment. Of the 21 children, 10 (47%) were hypophosphataemic at presentation, and for 13 children with urinary phosphate data 7 (53%) had abnormally low tubular reabsorption of phosphate. Both mean plasma phosphate concentration and tubular reabsorption of phosphate normalized within one year of treatment. Children (n=4) with rickets at presentation all improved after one year of nitisinone treatment. All three parameters of tubular dysfunction (proteinuria, hypophosphataemia, abnormal tubular reabsorption of phosphate) normalized within the first year of nitisinone therapy combined with dietary control and remained normal at 10 year follow-up.

McKiernan – Nitisinone Treatment of HT-1[2]

This was an excellent review of nitisinone treatment of HT-1, but did not contain new safety information.

Evaluator's Overall Conclusion on Safety

The pre- and post-marketing safety data are limited. This reflects the worldwide rarity of patients with HT-1. The safety data relating to nitisinone prior to the EU birth date of 21 February 2005 was located in the NTBC Study, Safety Addendum and PSURs. The safety data in the *complementary NTBC analysis* is considered to be representative of the safety data collected on nitisinone prior to the EU birth date. The *complementary NTBC analysis* included safety data on 250 HT-1 patients treated with nitisinone between 1 July 1993 and 28 March 2000 with the total treatment period ranging from 0.1 to 80.5 months. In this analysis, 51.2% (128/250) of patients experienced at least one adverse event. The most commonly reported adverse events were visual disorders (10% of patients) including keratitis (3.2%), corneal opacity (2.4%), photophobia (2.4%) and conjunctivitis (2.0%). The probability of developing visual symptoms increased with increasing maximum plasma tyrosine concentration. The data suggest that plasma tyrosine concentrations should be kept as low as possible and certainly below 500 µmol/L. The second most commonly reported adverse events were disorders of the liver and biliary system (9.6% of patients) including liver failure (6.4% of patients). The reported hepatic adverse events appear to be associated with the underlying disease rather than nitisinone treatment. The third most commonly reported adverse events were neoplasms (8.0% of patients) including malignant hepatic neoplasm (4%

of patients) and hepatic neoplasm (3.2%). The reported hepatic neoplasms appear to be related to the underlying disease rather than nitisinone treatment.

In the *complementary NTBC analysis*, the following adverse events were judged by the medical director of SOAB to be possibly related to nitisinone treatment: keratitis (reported by 3% of the patients); corneal opacity (2%); photophobia (2%); conjunctivitis (2%); eye pain (2%); blepharitis (<1%); dermatitis exfoliative (1%); pruritus (l%); rash erythematous (<1%); dry skin (<l%); thrombocytopenia (2%); leukopenia (1%) and granulocytopenia (1%).

The *complementary NTBC analysis* included 68 SAE reports (excluding withdrawals due to death or transplantation) in 64/250 (25.6%) patients. The 68 SAEs included: hepatic failure (16), hepatic neoplasm malignant (11), elective transplantation (10), hepatic neoplasm (8), hepatic cirrhosis (4), convulsion (3), GI haemorrhage (2), porphyria (2), thrombocytopenia (2), multi-organ failure (2), lymphoma malignant (1), hypoglycaemia (1), brain neoplasm benign (1), cyanosis (1), infection (1), retinal disorder (1), complications of prematurity (1), and fever (1).

In the *complementary NTBC analysis*, there were 50 (20%) withdrawals out of 250 patients. Of the 50 withdrawals, 15 were due to death, 34 due to liver transplantation and 1 due to the parents' wishes (died 12 months after discontinuation). No patient was withdrawn from treatment due to an AE considered to have a causal or possibly causal relationship with nitisinone.

The *Bridging PSUR* summarises all the safety data collected on nitisinone from the EU birth date on 21 February 2005 to the data lock on 10 July 2009. The report estimated that 679 patients had been treated with nitisinone in this period (equivalent to 2941 patient years). The safety data in the PSUR was generally consistent with that found in the *complementary NTBC analysis*. However, the Bridging PSUR included a number (n=9) of adverse events associated with impaired cognitive function. Furthermore, in a published study of the French experience of nitisinone treatment in patients with HT-1 the authors noted "major cognitive disturbances" in 6/32 (26%) of school age patients.[10] It was noted that the rare hereditary condition tyrosinaemia III (HT-III) is caused by a deficiency in the enzyme 4-hydroxyphenylpyruvate dioxygenase (the enzyme inhibited by nitisinone). It is possible that the inhibitory effect of nitisinone on 4-hydroxyphenylpyruvate dioxygenase might result in cognitive impairment in some children treated with the drug similar to that seen in patients with HT-III. The Bridging PSUR refers to unpublished data from a large Canadian cohort study which is stated not to indicate developmental or cognitive disturbances in children with HT-1 treated with nitisinone (this study was not submitted). It was considered that ongoing monitoring of the association between nitisinone and cognitive impairment is required. The sponsor should also submit the Canadian study referred to in the Bridging PSUR to the TGA as soon as it becomes available. There were single post-marketing reports of coma/hepatic coma (2 patients), cerebral oedema (1 patient), cerebral vascular haemorrhage (1 patient) and epileptic seizure (1 patient). Neurological adverse events require ongoing monitoring.

Clinical Summary and Conclusions

Clinical Aspects

Pharmacokinetics

The submission included only limited pharmacokinetic data on nitisinone in patients with HT-1. There was no absolute bioavailability study, no formal bioequivalence study, no formal study examining the effect of food on bioavailability, no *in vivo* metabolism study, no

mass balance study, no studies defining terminal half-live, volume of distribution, or renal excretion following IV administration, no PK drug-drug interaction studies, no studies in special populations, and no PK studies in patients with renal or hepatic impairment. Overall, it was considered that the pharmacokinetics of the drug have not been satisfactorily characterised. However, the efficacy and safety data in the NTBC Study are considered to provide sufficient information to allow nitisinone to be used in clinical practice for the treatment of patients with HT-1.

Efficacy

The NTBC Study provides the only adequate efficacy data supporting approval of nitisinone for the treatment of HT-1. In this open-label study, nitisinone was administered with a tyrosine and phenylalanine restricted diet on a compassionate use basis to all patients with HT-1 nominated for treatment, irrespective of country of origin, age, severity and characteristics of the disease, and concomitant illness. There were no randomized, controlled, double-blind data submitted supporting the efficacy of nitisinone for the treatment of HT-1. However, published data from HT-1 "historical controls" assisted interpretation of the primary efficacy outcomes of the NTBC Study.[15] The submission included limited supportive data in the form of a brief "communication" from the Quebec NTBC Study Group which showed that nitisinone and diet (n=34) was significantly superior to diet alone (n=27) on a number of clinically relevant outcomes (that is, hospitalizations for HT-1 complications, neurological crisis, hepatic transplantation, and deaths).

The efficacy and safety data from the NTBC Study were submitted in two separate reports: a main analysis of 207 patients enrolled between 23 February 1991 and 21 August 1997 and a complementary analysis of 250 patients enrolled between 6 July 1993 and 28 March 2000. It was estimated from the provided data that about 290 patients were enrolled in the NTBC study from the beginning of the main analysis (23 February 1991) to the conclusion of the complementary analysis (28 March 2000). It was estimated from the provided data that the main and complementary NTBC analyses shared about 150 patients from the overlapping time period. Consequently, in view of the substantial number of shared patients the reported results for the two NTBC analyses were generally similar whenever the analyses assessed the same outcome.

In the main NTBC analysis, nitisinone treatment was initiated in 207 patients (93 girls and 114 boys) and continued for 1 year (n=149), 2 years (n=95) and 4 years (n=35). The median age at enrollment was 9 months. In the complementary NTBC analysis, nitisinone treatment was initiated in 250 patients and continued for 2 years (n=158), 4 years (n=88) and 6 years (n=16). In the main NTBC analysis, there were data on the age of onset of HT-1 in 69% (n=143) of the 207 patients. In these 143 patients, 98 (69%) presented with symptoms before 6 months of age (considered to be consistent with an acute form of HT-1), and 45 (31%) patients presented after 6 months of age (considered to be consistent with a sub-acute or chronic form of HT-1). The majority of patients in the main NTBC analysis presented with liver disease (69%), followed by failure to thrive (37%), renal disease (27%) and neurological disease (5%). In the complementary NTBC analysis, 123 (49%) patients started treatment before 6 months of age, 69 (28%) patients started treatment between 6-24 months of age, and 58 (23%) started treatment aged $\geq$ 24 months.

The NTBC Study can be considered to provide Level III-3 evidence as defined by the National Health and Medical Research Council (NHMRC) Evidence Hierarchy for interventional studies. It is considered to provide Level III-3 evidence as it is a comparative interventional study without concurrent controls but with historical controls. Level III-3 interventional studies are considered to provide low levels of evidence of efficacy. However,

HT-1 is a rare and serious hereditary condition associated with significant morbidity and mortality with no other therapeutic options apart from treatment with nitisinone and a tyrosine and phenylalanine restriction diet. The data from the NTBC Study and the data from "historical controls" suggests that nitisinone in combination with a restriction diet in patients with HT-1 compared with restriction diet alone reduces the risk of death due to all causes, reduces the risk of death due to liver failure or transplantation due to liver failure, and reduces the risk of developing HCC. In addition, the data from the NTBC Study showed that nitisinone effectively reversed the biochemical abnormalities associated with HT-1 which result in accumulation of toxic metabolites (that is, normalized urinary succinylacetone excretion, normalized plasma succinylacetone concentration, normalized urinary 5-ALA excretion, normalized erythrocyte PBG-synthase activity, and normalized serum AFP concentration). The data from the NTBC Study also showed that nitisinone treatment significantly improved biochemical liver function (serum AST, GGT, bilirubin, prothrombin complex) and clinical liver function (liver failure, hepatomegaly, and ascites).

Safety

The pre- and post-marketing safety data are limited. This reflects the worldwide rarity of patients with HT-1. The safety data relating to nitisinone prior to the EU birth date of the drug on 21 February 2005 was found in the NTBC Study, Safety Addendum and PSURs. The safety data in these sources were similar. Consequently, the safety data in the complementary NTBC analysis was considered to be representative of all the safety data collected on nitisinone prior to the EU birth date of the drug.

The complementary NTBC analysis included safety data on 250 HT-1 patients treated with nitisinone between 1 July 1993 and 28 March 2000 with the total treatment period ranging from 0.1 to 80.5 months. In this analysis, 51.2% (128/250) of patients experienced at least one adverse event. Nitisinone was generally well tolerated. The most commonly reported adverse events were visual disorders (10.0% of patients) including keratitis (3.2%), corneal opacity (2.4%), photophobia (2.4%) and conjunctivitis (2.0%). The probability of developing visual symptoms increased with increasing maximum plasma tyrosine concentration. The data suggest that plasma tyrosine concentrations should be kept as low as possible and certainly below 500 µmol/L. The second most commonly reported adverse events were disorders of the liver and biliary system (9.6% of patients) including liver failure (6.4% of patients), and third most commonly reported adverse events were neoplasms (8.0% of patients) including malignant hepatic neoplasm (4% of patients) and hepatic neoplasm (3.2% of patients). The reported hepatic adverse events appear to be associated with the underlying disease rather than nitisinone treatment. The following adverse events reported in the analysis were judged by the medical director of SOAB to be possibly related to nitisinone treatment: keratitis (reported by 3% of the patients); corneal opacity (2%); photophobia (2%); conjunctivitis (2%); eye pain (2%); blepharitis (<1%); dermatitis exfoliative (1%); pruritus (l%); rash erythematous (<1%); dry skin (<l%); thrombocytopenia (2%); leukopenia (1%) and granulocytopenia (1%).

The *complementary NTBC analysis* included 68 SAE reports (excluding withdrawals due to death or transplantation) in 64/250 (25.6%) patients. The 68 SAEs included: hepatic failure (16), hepatic neoplasm malignant (11), elective transplantation (10), hepatic neoplasm (8), hepatic cirrhosis (4), convulsion (3), GI haemorrhage (2), porphyria (2), thrombocytopenia (2), multi-organ failure (2), lymphoma malignant (1), hypoglycaemia (1), brain neoplasm benign (1), cyanosis (1), infection (1), retinal disorder (1), complications of prematurity (1), fever (1). In the analysis, 50/250 (20%) patients withdrew from the study during the time period covered by the analysis. Of the 50 patients withdrawals, 15 were due to death, 34 due

to liver transplantation and 1 due to the parents' wishes (died 12 months after discontinuation). The majority of the SAEs (including deaths) and withdrawals appear to be related to the underlying disease rather than nitisinone treatment.

The *Bridging PSUR* summarises all the safety data collected on nitisinone from the EU birth date of the drug on 21 February 2005 to the report's data lock on 10 July 2009. It was estimated that 679 patients had been treated with nitisinone in this period (equivalent to 2941 patient years). The safety data in the *Bridging PSUR* were generally consistent with that found in the NTBC Study. However, the *Bridging PSUR* included a small number (n=9) of adverse events associated with impaired cognitive function. Furthermore, in a published study of the French experience of nitisinone treatment in patients with HT-1 the authors noted "major cognitive disturbances" in 6/32 (26%) of school age patients.[10] It is noted that the rare hereditary condition tyrosinaemia III (HT-III) is caused by a deficiency in the enzyme 4-hydroxyphenylpyruvate dioxygenase (the enzyme inhibited by nitisinone). It is possible that the inhibitory effect of nitisinone on 4-hydroxyphenylpyruvate dioxygenase might result in cognitive impairment in some children treated with the drug similar to that seen in patients with HT-III. The *Bridging PSUR* refers to unpublished data from a large Canadian cohort study which is stated not to indicate developmental or cognitive disturbances in children with HT-1 treated with nitisinone. It was considered that ongoing monitoring of the association between nitisinone and cognitive impairment is required. The sponsor should also submit the Canadian study referred to in the Bridging PSUR to the TGA as soon as it becomes available.

Benefit Risk Assessment

Benefits

In order to assess the benefits of nitisinone treatment it is necessary to compare the outcomes observed in the open-label NTBC Study with the same outcomes in "historical controls". There are comparative data on survival, death due to liver failure, transplantation due to liver failure and HCC, all liver transplantation and HCC.

In the complementary NTBC analysis, survival at 2, 4, and 6 years of nitisinone treatment was 94% at each of the three time points when all cause death was assessed. The probability of survival at each time point was greater in children starting treatment at > 6 months of age compared with children starting treatment at ≤ 6 months of age. There were 15 deaths in patients treated with NTBC during the period covered by the analysis: 8 due to liver failure, 2 due to HCC, and 5 due to other reasons (2 from multi-organ failure, 1 from GI bleeding, 1 from complications of prematurity and 1 from death not specified). For children starting NTBC treatment aged ≤ 2 months all deaths occurred during the first month of treatment. In the "historical controls", the risk of death within 6 months of the onset of symptoms in children treated with dietary restriction alone were 44% (0-2 months of age), 23% (2-6 months of age) and 4% (> 6 months of age). It is not possible to undertake a formal statistical comparison between the NTBC and "historical control" data due to differences in study design. However, comparison of the results strongly suggest that nitisinone treatment has a marked survival benefit in children with HT-1, particularly in those in whom treated is started at ≤ 6 months of age.

In the complementary NTBC analysis, the probability of survival without death due to liver failure or transplantation due to liver failure in all patients at 2, 4, and 6 years of nitisinone treatment was 94%, 94%, and 92%, respectively. The probability of experiencing these events was greater at each time point in patients aged 6-24 months at the start of treatment compared with patients aged ≤ 6 months. In the period covered by the complementary analysis there were 8 deaths due to liver failure and 8 patients were transplanted due to liver failure. All but 1 of the 16 events occurred in patients aged ≤ 24 months at the start of

treatment. In the complementary NTBC analysis, the probability of survival without liver transplantation in all patients at 2, 4, and 6 years of nitisinone treatment was 84%, 79%, and 75%, respectively. The probability of surviving without a liver transplantation at each time point was greater in patients aged ≤ 6 months at the start of treatment compared with patients aged > 6 months.

In the period covered by the complementary NTBC analysis a total of 34 patients underwent transplantation due to liver failure (8 patients), suspicion of HCC (16 patients, confirmed in 8), or as elective transplantation (10 patients). The overall frequency of liver transplant was 13.6% (34/250) in the complementary NTBC analysis compared with 24.1% (26/108) in the "historical controls". In the "historical controls", there were 83 patients with the acute form of the disease (i.e. presenting with severe liver disease before 6 months of age). Of these 83 patients, 35 (42.2%) died of liver failure (n=20) or recurrent bleeding (n=8) or a combination of liver failure and bleeding (n=7). In the complementary NTBC analysis there were 8/250 (3.2%) deaths due to liver failure in the total population compared with 20/83 (24.1%) deaths due to liver failure in "historical controls" presenting with severe liver disease before 6 months of age. In the "historical controls" transplantation due to liver failure occurred in 6.4% (6/108) of all patients in the survey compared with 3.2% (8/250) of patients in the complementary NTBC analysis. The corresponding figures for death due to liver failure were 27/108 (25%) in the "historical controls" (liver failure plus liver failure/bleeding), and 8/250 (3.2%) in the complementary NTBC analysis. The results suggest that nitisinone in combination with a restriction diet significantly reduces the risk of death due to liver failure and transplant due to liver failure compared with restriction diet alone.

In the complementary NTBC analysis, the probability of no occurrence of HCC in all patients at 2, 4, and 6 years of nitisinone treatment was 98%, 94%, and 91%, respectively. The probability of no occurrence of HCC was higher at each of the three time points in children aged ≤ 24 months at the start of treatment compared with children aged > 24 months. In children who started treatment aged ≤ 24 months, the 6-year probability of developing a HCC was 1% compared with 25% in children aged > 24 months at start of treatment. In the period covered by the complementary analysis, 10 patients developed HCC and only 1 of these patients was younger than 24 months at the start of treatment. Of the 10 patients with HCC, 8 underwent liver transplantation and there were 2 deaths due to verified HCC (1 occurring in a patient while undergoing liver transplantation). In the complementary NTBC analysis, HCC occurred in 10/250 (4%) of all patients while in the "historical controls" HCC occurred in 18% of patients who had lived for at least 2 years. In the main NTBC analysis, HCC was reported in 6% (9/146) of all patients who reached at least 2 years of age during the study period. Overall, the results suggest that nitisinone in combination with a restriction diet reduces the risk of developing HCC compared with restriction diet alone. In the complementary NTBC analysis, starting nitisinone treatment at ≤ 24 months of age markedly reduced the risk of developing HCC compared with starting treatment at > 24 months of age.

In the complementary NTBC analysis, there were only 2 cases of porphyric crises in the period covered by the analysis. In the main NTB analysis, renal tubular dysfunction improved in a small number of patients with this condition pre-treatment. This effect was confirmed in a larger published case series of patients with pre-treatment renal tubular dysfunction with the condition significantly improving after 1 year of nitisinone treatment.[11] Nitisinone treatment appears to decrease the risk of cardiomyopathy.[1] Other benefits observed in the NTBC Study comparing pre-treatment with post-treatment values included normalization of urine succinylacetone concentration, plasma succinylacetone concentration, erythrocyte PBG-synthase activity, urine 5-ALA activity and serum AFP concentration. These results indicate that nitisinone can prevent the accumulation of toxic metabolites through its inhibitory action

on 4-hydroxyphenylpyruvate dioxygenase. The NTBC study also showed that nitisinone significantly improved biochemical and clinical liver function.

Risks

Nitisinone treatment was generally well tolerated in patients with HT-1. However, the safety data for nitisinone are limited due to the rarity of patients with HT-1. The majority of deaths and SAEs (for example, liver failure, liver cell cancer, liver transplantation) reported in nitisinone treated patients appear to be related to the underlying condition rather than nitisinone treatment. Adverse reactions related to the visual system occurred commonly in patients treated with nitisinone and were related to plasma tyrosine concentration. In order to minimise adverse reactions involving the visual system the maximum plasma tyrosine concentration should be kept below 500 µmol/L. Slit-lamp examination is recommended prior to treatment and if visual symptoms or signs develop during treatment. The post marketing data showed an association between nitisinone treatment and cognitive dysfunction but a causal relationship has not been established. Nevertheless, there are theoretical reasons for a potential causal relationship between nitisinone treatment and cognitive delay. Consequently, it is recommended that that all patients treated with nitisinone should undergo systematic developmental assessment. Transient haematological (thrombocytopenia, leukopenia) and dermatological adverse reactions have been linked with nitisinone treatment. Persistently elevated serum AFP levels and levels which only slowly reduce despite nitisinone treatment have been identified as predictors for the development of liver cancer in nitisinone treated patients.[9]

Risk-Benefit

The evaluator considered that the benefits of nitisinone treatment in patients with HT-1 outweigh the risks. The recommended initial daily dose is 1 mg/kg given in two divided doses. Nitisinone should be administered with a tyrosine and phenylalanine restriction diet. There is a predictable increase in plasma tyrosine concentrations if nitisinone is administered without a restriction diet. Plasma tyrosine concentration should be kept below 500 µmol/L to minimise the probability of eye disorders. The initial nitisinone dose should be adjusted based on body weight gain and on biochemical response to nitisinone treatment. The NTBC investigators consider that a patient who is well treated with nitisinone is "characterised by a plasma tyrosine concentration between 200 and 500 µmol/L, no detectable succinylacetone in urine and plasma and a normalised porphyrin metabolism, that is, normal Erc-PBG synthase activity and 5-ALA urine excretion". In patients with a nitisinone serum concentration of 30 µmol/L the probability of recurrence of detectable concentrations of succinylacetone in the urine and erythrocyte PBG-synthase activity below the reference range was estimated to be 6% for both variables, while the probability of recurrence of detectable plasma concentrations of succinylacetone was about 40%.

The evaluator recommended that nitisinone in combination with dietary restriction of tyrosine and phenylalanine be approved for the treatment of patients with hereditary tyrosinaemia type 1.

V. Pharmacovigilance Findings

The sponsor submitted a Risk Management Plan which was reviewed by the TGA's Office of Medicines Safety Monitoring (OMSM). The sponsor identified the following important identified and potential risks:

- Identified:

- Haematological events including thrombocytopenia, leukopenia and granulocytopenia.
- Eye disorders including conjunctivitis, corneal opacity, keratitis, photophobia, eye pain and blepharitis.
- Skin and subcutaneous tissue disorders including exfoliative dermatitis, pruritus.

· Potential:
- Hypertyrosinaemia: Development of hypertyrosinaemia may cause eye disorders, skin disorders and has been suggested to have a role in development of neurological disorders.
- Insufficient treatment: Insufficient treatment increases the risk of developing symptoms related to the underlying disease of HT-1.

The sponsor's overall view was that, "Compared to the consequences of non-treatment of HT-l, there are very few important identified or potential risks."

As regards important missing information, the sponsor noted that:

· There are limited data in an adult population, and no information on the treatment of elderly.
· There is no information on the treatment of pregnant women and effects of breastfeeding.
· Long term effects of nitisinone treatment have not been studied.

Routine pharmacovigilance (PhV) and risk minimisation activities were proposed.[16,17] An additional PhV activity comprising inclusion of Australian patient data in an active surveillance program, the "ORFADIN Active Surveillance Program" was also proposed.

The OMSM reviewer noted that the RMP was not presented according to the EU Template. Additionally, the information provided was incomplete and a number of issues were identified. The key concern was that no information was provided on how the identified and potential risks and adverse events (AEs) were ascertained. Hence, the proposed PhV and risk minimisation plans cannot be evaluated.

Also, in the clinical evaluation report, it was noted that the post marketing data showed a possible association between nitisinone treatment and cognitive dysfunction. It is recommended that that all patients treated with nitisinone should undergo systematic developmental assessment.

It was also recommended by OMSM that an updated RMP, presented in accordance with the EU Template, be provided, and that this include particular attention to the following:

· Information on pre-authorisation clinical trials;
· Use in pregnancy or lactation;
· How the list of AEs or the identified and potential risks were ascertained;

[16] Routine pharmacovigilance practices involve the following activities:
- All suspected adverse reactions that are reported to the personnel of the company are collected and collated in an accessible manner;
- Reporting to regulatory authorities;
- Continuous monitoring of the safety profiles of approved products including signal detection and updating of labeling;
- Submission of PSURs;
- Meeting other local regulatory agency requirements.

[17] Routine risk minimisation activities may be limited to ensuring that suitable warnings are included in the product information or by careful use of labelling and packaging.

- Reports of cognitive dysfunction associated with nitisinone treatment in post marketing data;
- The recommendation of the clinical evaluator for all patients treated with nitisinone to undergo systematic developmental assessment;
- The potential for transmission of infectious agents;
- Justification for proposing routine PhV;
- Consideration of obtaining patient parent / guardian consent for data on Australian patients to be included in the active surveillance program, and, patient confidentiality;
- Reporting of active surveillance data to the TGA;
- Information on ongoing and completed pharmacoepidemiological studies and clinical trials;
- The need for risk minimisation activities to mitigate the potential for medication errors;
- Consideration of CYP 3A4 inhibitors or inducers and the consequences of their co-administration with nitisinone in identified and potential interactions; and,
- The need for additional information in the PI about CYP 3A4 inhibitors or inducers and the associated requirements for dose adjustment and patient metabolic monitoring when co-administered with nitisinone.

VI. Overall Conclusion and Risk/Benefit Assessment

The submission was summarised in the following Delegate's overview and recommendations:

Quality

Details of this submission were presented at the 131st meeting of the Pharmaceutical Sub-committee of ACPM (PSC) in March 2010. The PSC:

- concluded that an absolute bioavailability study was not required for this product,
- concluded that it should be recommended that all dosing occur with food, and
- made recommendations in relation to information included in the draft PI which were subsequently made.

The Delegate endorsed these recommendations.

Approval of the application was recommended with respect to chemistry and quality control. Taking into account the relevant justification statements submitted with respect to bioavailability, the quality evaluator was of the opinion that the application was approvable with respect to this parameter also.

Nonclinical

Summary points of nonclinical evaluation

- A maximum recommended human dose (MRHD) is 2 mg/kg/day, orally, for all patients.
- A single safety pharmacology study in rats showed evidence of systemic toxicity and CNS effects at high oral doses (500 mg/kg, 45-fold the MHRD, based on BSA).
- PK studies were limited. Nitisinone was shown to be, at least in part, metabolised by CYP3A4 *in vitro*. Based on *in vitro* studies in human hepatic microsomes, nitisinone is not expected to significantly inhibit the clearance of drugs that are metabolised via the CYP450 system.
- The acute oral toxicity of nitisinone was low. Acute IV toxicity was not investigated.

- Repeat-dose toxicity studies conducted in the mouse, rat, rabbit, dog and monkey were largely deficient in terms of study design and parameters assessed. Due to the lack of adequate PK studies, the adequacy of the animal models for toxicity testing could not be established. However, the animal species were responsive to the pharmacological action of nitisinone, as indicated by elevated plasma tyrosine levels. As well, the studies performed did enable identification of the primary target organs.
- The eyes, liver, kidney and nervous system were identified as the primary target organs of toxicity. A NOAEL was not established in the majority of studies but the NOAELs that were determined in a limited number of studies were low (0.1 – 2 times the MRHD based on BSA). Elevated tyrosine levels were observed following HPPD inhibition by nitisinone in all species examined. In the absence of adequate PK profiling, the relevance to humans remains unclear.
- There was some limited evidence of genotoxic potential for nitisinone *in vitro* and *in vivo*.
- No carcinogenicity studies were completed for nitisinone, nor any adequately conducted non-rodent chronic toxicity study. The non-clinical evaluator considered this a major deficiency for a product intended for long-term human use.
- Reproductive toxicity studies in mice, rats and rabbits highlighted fertility, embryofetal, pre- and post-natal effects of nitisinone at maternotoxic doses which often provided little to no apparent safety margin, suggesting its use in pregnancy or during lactation should be avoided.

Conclusions and recommendations of the nonclinical evaluator

Given the rare occurrence, seriousness of the disease and the lack of therapeutic alternatives, a reduced toxicology program and limited safety margin may be considered acceptable. The significant shortcomings of the nonclinical investigations should not preclude a recommendation for registration under these circumstances, provided the therapeutic benefit is adequately demonstrated by the clinical data and patients are regularly examined for plasma tyrosine levels and eye, liver, kidney and nervous system disorders.

Significant reproductive effects of nitisinone were observed in animal species exposed prior to mating, *in utero* or through lactation. Its use in pregnancy and during lactation should be avoided.

Clinical

The clinical evaluator's final recommendation was that the data adequately support the slightly amended indication as follows:

Orfadin capsules (nitisinone) in combination with dietary restriction of tyrosine and phenylalanine are indicated for the treatment of patients with hereditary tyrosinaemia type 1

The clinical data provided comprised the following:

- one pivotal clinical efficacy and safety study, NTBC
- limited PK data on nitisinone as follows:
    - one study in 10 healthy young males assessing administration of capsule and liquid formulations (including BE of the two formulations)
    - one study in 7 patients with HT-1

- one subgroup study involving patients from the NTBC study assessing changes in serum nitisinone concentration over 6 years of treatment
- one subgroup crossover analysis and one parallel group analysis assessing BE of an early nitisinone + lactose formulation and a later nitisinone + pre-gelatinised starch formulation (both studies retrospective and involved patients from NTBC)

- in addition to the safety data from the NTBC study, the submission also included a number of safety reports prepared prior to the EU birth date of the drug on 21 February 2005, PSURs 1-6 and a PSUR Summary Bridging Report and 5 studies with safety information published since 2005

Clinical Pharmacology

Pharmacokinetics

There was only limited PK data on nitisinone in patients with HT-1. There was no absolute bioavailability study, no formal study examining the effect of food on bioavailability, no PK drug-drug interaction studies, no studies in special populations, including patients with renal or hepatic impairment. Overall, the clinical evaluator considered that the pharmacokinetics of the drug have not been satisfactorily characterised.

A study in 10 young, healthy males showed that following administration of a single oral nitisinone capsule (1 mg/kg), the mean (SD) AUC was 599 (153) µg.h/mL, the C_{max} was 7.69 (0.95) µg/mL and the terminal half-life was 54.5 (13.0) hours while the median t_{max} [range] was 2.84 hours [1.66 – 11.0]. This study showed that the capsule and liquid formulations were bioequivalent but that the liquid formulation was absorbed more rapidly than the capsule formulation.

In 6 children with HT-1 the mean terminal half-life was 25 hours compared with 21 hours in one adult with HT-1. The mean volume of distribution was 0.3 L/kg in 3 children with HT-1 and 0.07 L/kg in one adult with HT-1. The terminal half-life of nitisinone in patients with HT-1 was significantly shorter than that in healthy, young, adult males

Information provided by one of the principal investigators in the NTBC study indicated that that it was likely that nitisinone had been added to food or formula in most patients treated with this drug.

There was no formal BE study comparing the nitisinone + lactose formulation used in many of the patients in the NTBC study with the nitisinone + pre-gelatinised starch formulation proposed for registration. However, retrospective data in patients with HT-1 from the NTBC study showed that the two formulations are likely to be bioequivalent.

Data from the NTBC study showed that serum nitisinone concentrations increased over time, reaching a plateau after about 3 years of treatment in patients with a starting age of at least 24 months and after about 4 years in patients with starting ages less than 24 months.

Pharmacodynamics

There were no formal stand-alone PD studies in humans. However, the PD effects of nitisinone were reflected in efficacy effects of the NTBC study and have been reviewed under *Efficacy.*

The data from the NTBC study showed that nitisinone at the dose proposed for registration rapidly reduced urinary excretion of succinylacetone and 5-ALA, rapidly normalised erythrocyte PBG-synthase activity and slowly reduced plasma succinylacetone concentration.

These results indicate that nitisinone can effectively inhibit 4-hydroxyphenylpyruvate dioxygenase at the dose proposed for registration.

Efficacy

There were no formal dose-response studies. The first clinical study of NTBC in HT-1 was a Phase II pilot study in 5 patients on daily doses ranging from 0.1 to 0.6 mg/kg body weight with dose adjustment based on biochemical response. On the basis of this pilot study, the initial dose in the NTBC study was 0.6 mg/kg body weight. However, with increasing clinical experience the initial recommended dose was increased to 1.0 mg/kg body weight per day during the course of the NTBC study.

The NTBC study was multinational, multi-centre, open-label, uncontrolled and included patients with HT-1 treated with nitisinone on a compassionate use basis. It was co-ordinated from Sweden. In view of the improvements observed in the Phase II pilot study, the NTBC study did not include a placebo control or a diet alone control as this would have been unethical. However, for selected clinical outcomes, the NTBC study did include a comparison with an international survey of 108 patients treated only with a tyrosine and phenylalanine restricted diet. Thus the subjects in this international survey formed historical controls.

The main NTBC analysis was conducted from 23 Feb 1991 to 21 Aug 1997 and included 207 patients in 25 countries, including one patient from Australia. There was also a complementary analysis conducted from 1 Jul 1993 to 28 Mar 2000 and this included 250 patients from 30 countries, including 2 patients from Australia. It is estimated that the two analyses shared about 150 patients. Patients who had undergone liver transplantation were not included in the study but there were no other exclusion criteria.

The primary clinical efficacy variables of the NTBC study were death, survival without liver transplantation, death due to liver failure or transplantation due to liver failure, hepatocellular carcinoma and porphyric crisis. Other primary efficacy evaluations were the effect of NTBC on urine and plasma succinylacetone concentrations, erythrocyte PBG-synthase activity, urine excretion of 5-ALA and the relationship between serum NTBC concentration and these biochemical effects.

In the summary of the efficacy data, the focus is on the complementary analysis as the latter included more patients than the main analysis and patients were followed up for 6 years rather than for 4 years..

In the complementary NTBC analysis, the probability of survival in all patients at 2, 4 & 6 years of nitisinone treatment was 94% at each of the three time points. In the same analysis, the probability of survival after 2 years of treatment was 94% for all patients, 93% for those who started treatment below 2 months of age, 93% for those who started treatment below 6 months of age and 96% for those who started treatment after 6 months of age (Table 7).

Historical control results cannot be directly compared with the above results. Subjects were classified by age of onset of symptoms rather than by age of start of treatment. However, the two are reasonably comparable. Also the historical data only goes out to 6 months. Table 16 comprises the relevant data from the clinical evaluation (Table 8) but with an important difference. The Delegate has shown the results as probability of survival (100% - risk of death), rather than as risk of death, simply for ease of comparison with Table 7.

Table 16: Historical controls – probability of survival within a specified time after onset of symptoms

Age at onset of symptoms	Within 1 month	Within 2 months	Within 6 months
0-2 months	82%	69%	56%
2-6 months	96%	91%	77%
> 6 months	100%	100%	96%

As noted by the clinical evaluator, comparison of results indicates that nitisinone has a marked survival benefit in children with HT-1, particularly in those in whom treatment is started at ≤ 6 months of age. The same conclusion is reached on comparing the results from the main NTBC analysis (Table 6) with the results of the historical controls.

In the complementary NTBC analysis, the probability of survival without death due to liver failure or transplantation due to liver failure in all patients at 2, 4 and 6 years was 94%, 94% and 92%, respectively. The probability of experiencing these events was greater at each time point in patients aged 6-24 months at start of treatment compared with patients aged ≤ 6 months. As noted by the clinical evaluator, the results indicate that increased benefits as regards liver failure come from starting treatment before 6 months of age.

The percentages of each overall population dying because of liver failure were 3.2% (8/250) in the complementary NTBC analysis, 3.4% (7/207) in the main NTBC analysis and 25% (27/108) in the historical controls. In the historical controls, there were 83 patients with severe liver disease before 6 months of age, of whom 35 (42.2%) died of liver failure (n = 20) or recurrent bleeding (n = 8) or a combination of liver failure and bleeding (n = 7). In the complementary NTBC analysis, transplantation due to liver failure occurred in 3.2% (8/250) of patients, compared with 6.4% (6/108) of historical controls. These results indicate that nitisinone treatment significantly reduces the risk of death due to liver failure and transplant due to liver failure compared with diet alone.

In the complementary NTBC analysis, hepatocellular carcinoma occurred in 4% (10/250) of all patients while in the historical controls, it occurred in 18% of patients who had lived for 2 years. The results indicate that nitisinone treatment reduces the risk of developing hepatocellular carcinoma compared with diet alone. In the complementary analysis, starting nitisinone treatment before the age of 24 months markedly reduced the risk of developing hepatocellular carcinoma compared with starting treatment after the age of 24 months,

In the complementary analysis, there was evidence of early normalisation of the following parameters: plasma succinylacetone, erythrocyte PBG-synthase activity & urinary 5-ALA excretion. Serum AFP was normalised in about 50% of patients after 1 year of treatment and in about 90% of patients after 2 years. The prothrombin complex normalised after a median of 1.1 months of treatment [range 0.2 – 16] and serum AST, GGT and bilirubin concentrations normalised during treatment while serum ALT concentrations increased. Improvements in RBC count, Hb concentration and platelet count were generally seen in patients aged 0-24 months at start of treatment but not in those aged over 24 months at start of treatment.

Plasma tyrosine and valine concentrations increased with nitisinone treatment in all patients after 1 year of treatment but there was little change in plasma phenylalanine concentration.

Safety

A total of 406 patients were treated with nitisinone from February 1991 until April 2001. The usual daily dose before mid 1993 was about 0.6 mg/kg and after that time it was usually about 1 mg/kg.

The complementary NTBC analysis included safety data on 250 HT-1 patients treated with nitisinone between 1 July 1993 and 28 March 2000 with the total treatment period ranging from 0.1 to 80.5 months.

In the complementary NTBC analysis, 51.2% (128/250) of patients experienced at least one AE. The most commonly reported AEs were visual disorders (10.0% of patients) including keratitis (3.2%), corneal opacity (2.4%), photophobia (2.4%) and conjunctivitis (2.0%).

The probability of developing visual symptoms increased with increasing maximum plasma tyrosine concentration. The data indicate that plasma tyrosine concentrations should be kept as low as possible and certainly below 500 µmol/L.

The second most commonly reported AEs were disorders of the liver and biliary system (9.6% of patients) including liver failure (6.4% of patients). The reported hepatic AEs appeared to be associated with the underlying disease rather than with nitisinone treatment.

The third most commonly reported AEs were neoplasms (8.0% of patients) including malignant hepatic neoplasm (4% of patients) and hepatic neoplasm (3.2%). The hepatic neoplasms appeared to be related once again to the underlying disease rather than to nitisinone treatment.

In the complementary NTBC analysis, the following AEs were judged to be possibly related to nitisinone treatment: keratitis (reported by 3% of the patients), corneal opacity (2%), photophobia (2%), conjunctivitis (2%), eye pain (2%), blepharitis (< 1%), dermatitis exfoliative (1%), pruritus (1%), rash erythematous (< 1%), dry skin (< 1%), thrombocytopenia (2%), leukopenia (1%) and granulocytopenia (1%).

In the complementary NTBC analysis there were 68 SAE reports (excluding withdrawals due to death or transplantation) in 25.6% (64/250) of patients. The 68 SAEs included: hepatic failure (16), hepatic neoplasm malignant (11), elective transplantation (10), hepatic neoplasm (8), hepatic cirrhosis (4), convulsion (3), GI haemorrhage (2), porphyria (2), thrombocytopenia (2), multi-organ failure (2), lymphoma malignant (1), hypoglycaemia (1), brain neoplasm benign (1), cyanosis (1), infection (1), retinal disorder (1), complications of prematurity (1) and fever (1).

In the complementary analysis, there were 50 (20%) withdrawals out of 250 patients. Of the 50 withdrawals, 15 were due to death, 34 due to liver transplantation and 1 due to the parents' wishes (this patient died 12 months after discontinuation). No patient was withdrawn from treatment due to an AE considered to have a causal or possibly causal relationship with nitisinone.

Post-marketing experience

The Bridging PSUR summarised all the safety data collected on nitisinone from the EU birth date on 21 February 2005 to the data lock point on 10 July 2009. The report estimated that 679 patients had been treated with nitisinone in this period (equivalent to 2941 patient years). The safety data in the PSUR were considered by the clinical evaluator to be generally consistent with those found in the complementary NTBC analysis.

The sponsor has been closely monitoring reports of learning difficulties and/or disturbances of cognitive function in patients treated with nitisinone. The Bridging PSUR included a

number (n = 9) of adverse events associated with impaired cognitive function. It should be noted that in none of these have progressive decreases in cognitive/developmental function been observed despite continued treatment with Orfadin. A published study of the French experience of nitisinone treatment in patients with HT-1 was in the submission and evaluated. It was a retrospective analysis of 46 patients with HT-1 treated with nitisinone. Schooling difficulties affected 35% (8/23) of the school age patients and major cognitive disturbances were noted in 6 patients (memory, concentration difficulties and slowness). The authors of this study postulated that the inhibitory effect of nitisinone on the enzyme 4-hydroxyphenylpyruvate dioxygenase may be contributory. However, they concluded that it remains to be established whether the high percentages of these problems observed in their study result from the disease itself or from nitisinone treatment. The Bridging PSUR also referred to unpublished data from a large Canadian cohort study the results of which are that there is no indication of either developmental or cognitive disturbances in children with HT-1 treated with nitisinone. The clinical evaluator has recommended that the sponsor submit for evaluation to the TGA the report of the latter study as soon as it becomes available. The Delegate concurred and indicated this should be a specific condition of registration.

Risk Management Plan

The Delegate noted the OMSM review of the RMP (version 1) submitted by the sponsor. The sponsor indicated the following important identified and potential risks:

Identified

- haematological events including thrombocytopenia, leukopenia & granulocytopenia
- eye disorders including conjunctivitis, corneal opacity, keratitis, photophobia, eye pain and blepharitis
- skin and subcutaneous tissue disorders including exfoliative dermatitis & pruritus

Potential

- hypertyrosinaemia – the development of hypertyrosinaemia may cause eye disorders, skin disorders and has been suggested to have a role in development of neurological disorders
- insufficient treatment – insufficient treatment increases the risk of developing symptoms related to the underlying disease of HT-1.

As regards important missing information, it was noted that:

- there is very limited data in the adult population and no information on the treatment of elderly
- there is no information on the treatment of pregnant women and effects of breast feeding
- long-term effects of nitisinone treatment have not been studied

The RMP evaluation report recommended that an updated RMP, presented in accordance with the EU template should be provided and that it should include particular attention to the following:

- information on pre-authorisation clinical trials
- use in pregnancy or lactation
- how the list of adverse events or the identified and potential risks were ascertained

- reports of cognitive dysfunction associated with nitisinone treatment in the post-marketing data
- the recommendation of the clinical evaluator for all patients treated with nitisinone to undergo systematic developmental assessment
- the potential for transmission of infectious agents
- justification for proposing routine pharmacovigilance
- consideration of obtaining patient parent/guardian consent for data on Australian patients to be included in the active surveillance program and patient confidentiality
- reporting of active surveillance data to the TGA
- information on ongoing and completed pharmacoepidemiological studies and clinical trials
- the need for risk minimisation activities to mitigate the potential for medication errors
- consideration of CYP3A4 inhibitors or inducers and the consequences of their co-administration with nitisinone in identified and potential interactions, and
- the need for additional information in the PI about CYP3A4 inhibitors or inducers and the associated requirements for dose adjustment and patient metabolic monitoring when co-administered with nitisinone.

The Delegate noted that many of the above concerns have already been raised in the other evaluations, particularly those of the non-clinical and clinical evaluators and arise from the limitations of the data, for example the limited PK data, in particular the absence of specific drug-drug interaction studies and studies in special populations, the limited clinical efficacy and safety data and the limited post-marketing data. Some of these deficiencies arise from the fact that there is a quite small patient population due to the rarity of the disease.

The sponsor responded to the above issues raised in the RMP evaluation report. Overall, it was considered by OMSM that the updated RMP was well presented and comprehensive with the majority of information addressing the issues identified in the RMP evaluation. In particular, the proposed approach to monitoring potential developmental delay was accepted.

However, it was noted that the response referred to the annual follow up form for the Active Surveillance Program, details of which were not provided. Also, there was no reference to aggregate reporting of the Active Surveillance Program.

It was recommended that the updated RMP should be accepted and that if Orfadin is approved for registration, the following should be provided prior to registration:

- the annual follow up form for the Active Surveillance Program; and
- Information on the how regular and aggregate reporting of the Active Surveillance Program will be undertaken.

Risk-Benefit Analysis

Delegate Considerations

The pharmacokinetics of the drug have not been satisfactorily characterised.

The Delegate agreed that the NTBC study can be considered to provide Level III-3 evidence as defined by the NH&MRC. However, HT-1 is a rare and very serious hereditary condition associated with significant morbidity and mortality with no other therapeutic options apart from treatment with nitisinone and a tyrosine and phenylalanine restricted diet.

The data from both NTBC analyses and that from the historical controls in patients with HT-1 indicate that nitisinone in combination with a restricted diet compared with the restricted diet alone reduces the risk of death due to all causes, reduces the risk of death due to liver failure or transplantation due to liver failure and reduces the risk of developing hepatocellular carcinoma. The data from both analyses of the NTBC study also showed that nitisinone effectively reversed the biochemical abnormalities associated with HT-1 which result in accumulation of toxic metabolites. As well the data showed that nitisinone treatment significantly improved both biochemical and clinical indices of liver function.

The pre- and post-marketing safety data are limited, once again reflective of the worldwide rarity of patients with HT-1. Nitisinone was generally well tolerated in patients with HT-1. The majority of deaths and SAEs (for example, liver failure, liver cell cancer, liver transplantation) reported in nitisinone-treated patients appeared to be related to the underlying condition rather than nitisinone treatment.

Adverse reactions related to the visual system occurred commonly in patients treated with nitisinone and were related to plasma tyrosine concentration.

The Delegate noted the concerns of the clinical evaluator with regard to the post-marketing reports of cognitive dysfunction. The investigators of the French study concluded that it remained to be established whether cognitive dysfunction results from the disease itself or from some adverse effect of the nitisinone treatment. The sponsor is actively monitoring the reports of cognitive dysfunction in patients being treated with nitisinone and both the clinical evaluator and the delegate have requested that the sponsor submit the results of the large Canadian cohort study mentioned in the PSUR Bridging Report when these are formally available or published.

In the Committee for Medicinal Products for Human Use (CHMP) Scientific Discussion of Orfadin, specifically the last paragraph of the benefit/risk assessment, it was noted that 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 HT-1, include the monitoring of liver, renal, haematological, neurological and ophthalmic status.[18]

At the time of the last renewal of marketing authorisation in the EU (19/01/2010), the comment was made that Orfadin remains the only therapeutic treatment so far available for the treatment of the rare disease tyrosinaemia type 1 and the risk/benefit of Orfadin in the treatment of the approved indication continues to be favourable.

In the approval letter from the US FDA, the US sponsor of the product was encouraged to establish a voluntary registry of hereditary tyrosinaemia type 1 patients treated with nitisinone to collect information on clinical outcomes with long-term use of nitisinone. The sponsor was also reminded of a post-marketing commitment to the FDA to perform standard reproductive toxicity studies, according ICH guidance (which has also been adopted by the TGA).[19] The final study report was to be submitted by 30 March 2003. The sponsor confirmed that these studies have been done and that they were part of the submission to the TGA.

The Delegate agreed with the clinical evaluator that the benefits of nitisinone treatment in patients with HT-1 outweigh the risks. Nitisinone should be administered with a diet restricted in tyrosine and phenylalanine content. There is a predictable increase in plasma

[18] available at http://www.ema.europa.eu/humandocs/PDFs/EPAR/Orfadin/3202905en6.pdf .

[19] pp. 25 - 44 of Rules Governing Medicinal Products in the European Union - EudraLex - Medicinal products for human use, 1998 Edition: Volume 3B - Safety and the Environment1998 (3B) - 3BS4a, Detection of Toxicity to Reproduction for Medicinal Products.

tyrosine concentrations if nitisinone is administered without a restricted diet with an associated increased risk of eye disorders.

Therefore the Delegate proposed to recommend approval of the submission for the slightly amended indications as recommended by the clinical evaluator, the wording which incorporates reference to the need for concurrent dietary restrictions.

Orfadin (nitisinone) is indicated for the treatment of patients with hereditary tyrosinaemia type 1, in combination with dietary restriction of tyrosine and phenylalanine

The Delegate intended to impose the following as a specific condition of registration:

- the submission, as evaluable data within the context of a category 1 submission, of the final study report of the large Canadian cohort study mentioned in the PSUR Bridging Report

The sponsor should address the following issues in their Pre-ACPM response:

- An up-to-date summary of the post-marketing experience in both the EU and the USA & elsewhere
- It was recommended that the updated RMP should be accepted and that if Orfadin is approved for registration, the following should be provided prior to registration:
    - the annual follow up form for the Active Surveillance Program; and
    - Information on the how regular and aggregate reporting of the Active Surveillance Program will be undertaken.
- The sponsor was requested to provide a commitment in its pre-ACPM response that the information/documents requested under the second point above will be provided to OMSM.

The Delegate also asked three questions of the ACPM:

- Does the ACPM agree with the Delegate and the clinical evaluator that the PI should include some information about cognitive impairment observed in post-marketing data?
- Does the ACPM agree with the Delegate that, in the absence of suitable long-term data on treatment with nitisinone, there should be a statement in the PI recommending regular and systematic developmental assessment, including neuro-cognitive development.
- Does the ACPM agree with the proposed condition of registration recommended by the Delegate?

Sponsor Response

In its response, the sponsor made the following points.

None of these ongoing analyses has yet identified sufficient data to address whether the cognitive and developmental disturbances are caused by the disease itself, the medication or a combination of the two. Disturbances in cognitive and developmental function are therefore identified as an important potential risk. This has been addressed in the updated Risk Management Plan (RMP). As it is unclear if this important potential risk is directly related to treatment with nitisinone, the sponsor believed that a statement relating to observation of cognitive impairment during postmarketing surveillance should not be added to the PI at the current time and that the routine pharmacovigilance activities and the additional

pharmacovigilance activities described in the RMP are presently considered sufficient to monitor this important potential risk.

A statement regarding developmental assessment has been added under the Precautions section of the proposed PI.

The sponsor indicated that they will not be able to submit the final study report of the large Canadian cohort study. The chief investigator has no affiliations with the product licensor, the study was conducted totally independently of the sponsor and although it has made many requests for the study report and other information he has indicated that he prefers to be independent of the product licensor. He has referred to a publication in preparation and the sponsor has committed to provide a copy of the published paper to the TGA when available.

The sponsor provided the latest PSUR for the period 21 February 2009 to 20 February 2010 which contained a summary of the patient exposure and safety data.

The sponsor also confirmed that the documents requested to be provided to the OMSM had been provided.

Advisory Committee Considerations

The ACPM, having considered the evaluations and the Delegate's overview, as well as the sponsor's response to these documents, recommended approval of the submission for the indication:

For the treatment of patients with hereditary tyrosinaemia type 1, in combination with dietary restriction of tyrosine and phenylalanine.

In making this recommendation, the ACPM considered that an overall positive risk benefit profile for the amended indication was demonstrated for the small target population.

Outcome

Based on a review of quality, safety and efficacy, TGA approved the registration of Orfadin hard capsules containing nitisinone 2 mg, 5 mg and 10 mg for the indication:

The treatment of patients with hereditary tyrosinaemia type 1 in combination with dietary restriction of tyrosine and phenylalanine.

Approval was subject to a number of conditions including:

- The full implementation of the updated Risk Management Plan identified as Version 2, dated 22 June 2010.

Attachment 1. Product Information

The following Product Information was approved at the time this AusPAR was published. For the current Product Information please refer to the TGA website at www.tga.gov.au.

PRODUCT INFORMATION

Orfadin

2 mg, 5 mg and 10 mg hard capsules

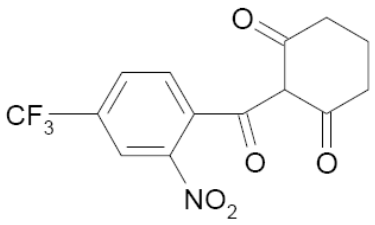
NAME OF MEDICINE

Nitisinone, 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione

Molecular Formula: $C_{14}H_{10}F_3NO_5$

Molecular Weight: 329.23

Chemical Structure:



CAS Registry Number: 104206-65-7

DESCRIPTION

Orfadin® capsules are white, opaque, hard gelatine capsules, imprinted with "NTBC" and the strength "2 mg", "5 mg" or "10 mg" in black.The active substance is a weak acid and it is highly soluble in the pH range 4.5 - 7.2 according to the Biopharmaceutics classification system. Partition coefficient for nitisinone in octanol/water at pH 6.5 is 0.432.

Each capsule contains 2 mg, 5 mg or 10 mg nitisinone, plus pregelatinised starch (maize). The capsule shell is gelatine and titanium dioxide. The imprint is either Opacode monogramming ink S-1-27794 Black (ARTG ID: 12104) or TekPrint SW-9008 Black Ink (ARTG ID: 2328).

PHARMACOLOGY

Pharmacotherapeutic group: Other alimentary and metabolism products.
ATC code: A16A X04.

The biochemical defect in hereditary tyrosinaemia type 1 (HT-1) is a deficiency of fumarylacetoacetate hydrolase, which is the final enzyme of the tyrosine catabolic pathway. Nitisinone is a competitive inhibitor of 4-hydroxyphenylpyruvate dioxygenase, an enzyme which precedes fumarylacetoacetate hydrolase in the tyrosine catabolic pathway. By inhibiting the normal catabolism of tyrosine in patients with HT-1, nitisinone

prevents the accumulation of the toxic intermediates maleylacetoacetate and fumarylacetoacetate. In patients with HT-1, these intermediates are converted to the toxic metabolites succinylacetone and succinylacetoacetate. Succinylacetone inhibits the porphyrin synthesis pathway leading to the accumulation of 5-aminolevulinate.

Pharmacokinetic Properties

Formal absorption, distribution, metabolism and elimination studies have not been performed with nitisinone. In 10 healthy male volunteers, after administration of a single dose of nitisinone capsules (1 mg/kg body weight) the terminal half-life (median) of nitisinone in plasma was 54 hours. Population pharmacokinetic analysis has been conducted on a group of 207 HT-1 patients. The clearance and half-life were determined to be 0.0956 l/kg body weight/day and 52.1 hours respectively. In a small study in 6 children with HT-1 the mean terminal half –life was 25 hours compared with 21 hours in one adult with HT-1. The mean volume of distribution was 0.3 L/kg in 3 children with HT-1 and 0.07 L/kg in one adult with HT-1.

In vitro studies using human liver microsomes and cDNA-expressed P450 enzymes have shown limited CYP 3A4-mediated metabolism.

CLINICAL TRIALS

An open-label study of the use of nitisinone in patients with HT-1 was conducted by 96 investigators at 87 hospitals in 25 countries. The data presented in the main analysis were obtained over a period covering more than six years and are derived from 207 patients with a diagnosis of HT-1 verified by the presence of succinylacetone in the urine or plasma. The median age of patients at enrolment was 9 months (range birth to 21.7 years, see Table 1).

Table 1. Characteristics of the Study Population

	N	**Treatment time in months (Median)**
Total population	207	22
Females	93	23
Males	114	21
Age at start of nitisinone therapy		
0-24 months	142	20
> 24 months	65	28

The median duration of treatment was 22 months with a range of 0.1 months to 78 months.

Biochemical Effects of Nitisinone Treatment

The efficacy of nitisinone as an inhibitor of 4-hydroxy-phenylpyruvate dioxygenase was inferred by the effects of treatment on the following biochemical parameters: urine succinylacetone, plasma succinylacetone and erythrocyte porphobilinogen synthase (PBG) activity. For all 186 patients for whom data are available, the excretion of succinylacetone in urine was reduced to a level below the reference limit, which represents the sensitivity of the analytical procedure. The median time to normalization was 0.3 months. For most patients for whom data are available (150/172=87%) the plasma concentration of succinylacetone

decreased to a level below the reference. The median time to normalization was 3.9 months. For all 180 patients for whom data are available, the porphobilinogen synthase activity of erythrocytes increased to within reference limits. The median time to normalization was 0.3 months. The differences in these indices compared to the start of nitisinone treatment were statistically significant ($p<0.001$).

Effects on Overall Survival

When compared to data for historical controls treatment with nitisinone together with dietary restriction results in a better survival probability in all HT-1 phenotypes than dietary restriction alone. This is seen in the following tables from the main analysis, complementary analysis and the historical control group:

Survival probability: Main analysis of the study conducted during 1991-1997 includes 207 patients.

Patients	Number of patients				Probability of survival % (95% CI) Main analysis (N= 207)		
	Start	1 year	2 years	4 years	1 year	2 years	4 years
All	207	149	95	35	96	96 %	93 %
Start 0-2 m	16	12	7	3	88 %	88 %	88 %
Start 0-6 m	80	55	30	11	94 %	94 %	94 %
Start > 6 m	127	94	65	24	97 %	97 %	93 %

Survival probability: Complementary analysis of the same study conducted during 1993-2000 includes 250 patients and share approx 150 patients with the main analysis above.

Patients	Number of patients				Probability of survival (95% CI) Complementary analysis (N=250)		
	Start	2 years	4 years	6 years	2 years	4 years	6 years
All	250	158	88	16	94 %	94 %	94 %
Start 0-2 m	60	32	16	2	93 %	93 %	93 %
Start 0-6 m	128	75	38	6	93 %	93 %	93 %
Start ≥ 6 m	122	83	50	10	96 %	95 %	95 %

Survival probability in control group with dietary restriction alone. (From figure 1, Van Spronsen et al., 1994).

Age at onset of symptoms	Survival probability with dietary control (%)	
	5 years	10 years
0-2 months	28	---
2-6 months	51	34
> 6 months	93	59

Nitisinone treatment leads to normalised porphyrin metabolism with normal erythrocyte PBG- synthase activity and urine 5-ALA, decreased urinary excretion of succinylacetone, increased plasma tyrosine concentration and increased urinary excretion of phenolic acids. Available data from a clinical study indicates that in more than 90% of the patients urine succinylacetone was normalized during the first week of treatment. Succinylacetone should not be detectable in urine or plasma when the nitisinone dose is properly adjusted.

Treatment with nitisinone was also found to result in reduced risk for the development of hepatocellular carcinoma (2.3 to 3.7-fold) compared to historical data on treatment with dietary restriction alone. It was found that the early initiation of treatment resulted in a further reduced risk for the development of hepatocellular carcinoma (13.5-fold when initiated prior to the age of 12 months).

INDICATION

Orfadin® (nitisinone) is indicated for the treatment of patients with hereditary tyrosinaemia type 1 in combination with dietary restriction of tyrosine and phenylalanine.

CONTRAINDICATIONS

Hypersensitivity to the active substance or to any of the excipients.

Mothers receiving nitisinone should not breast-feed.

PRECAUTIONS

High Plasma Tyrosine Concentrations
There is a predictable increase in plasma tyrosine concentrations if nitisinone is administered without a diet restricted in tyrosine and phenylalanine content. Inadequate restriction of tyrosine and phenylalanine intake can result in elevations in plasma tyrosine. Plasma tyrosine levels should be kept below 500 μmol/L in order to avoid toxic effects to the eyes (corneal ulcers, corneal opacities, keratitis, conjunctivitis, eye pain, and photophobia), skin (painful hyperkeratotic plaques on the soles and palms) and nervous system (variable degrees of mental retardation and developmental delay).

Diet compliance and Monitoring of plasma tyrosine levels
To avoid side effects that can occur due to high plasma tyrosine levels as described above, it is important to establish that the patient adheres to the dietary regimen and to monitor plasma tyrosine concentrations regularly. A more restricted tyrosine and phenylalanine diet should be implemented if the plasma tyrosine level goes above 500 micromole/l. It is not recommended to lower the plasma tyrosine concentration by reduction or discontinuation of nitisinone, since the metabolic defect may result in deterioration of the patient's clinical condition.

General Development
Cognitive and developmental disturbances have been observed in the patient population. On-going analysis has yet not identified whether these are caused by the disease itself, the medication or other contributing factors. In the view of the limited data on the long-term effects of nitisinone treatment, it is essential that all patients treated with nitisinone undergo regular and systematic developmental assessment, including neuro-cognitive development.

Eye monitoring
It is recommended that a slit-lamp examination of the eyes is performed before initiation of nitisinone treatment. A patient displaying visual disorders during treatment with nitisinone should without delay be examined by an ophthalmologist.

Liver monitoring
The liver function should be monitored regularly by liver function tests and liver imaging. It is recommended also to monitor serum alpha-fetoprotein concentration. Increase in serum alpha-fetoprotein concentration may be a sign of inadequate treatment. Patients with increasing alpha-fetoprotein or signs of nodules in the liver should always be evaluated for hepatic malignancy.

Platelet and white blood cell (WBC) monitor
It is recommended that platelet and white cell counts are monitored regularly, as a few cases of reversible thrombocytopenia and leucopenia were observed during clinical evaluation.

Monitoring visits should be performed every 6 months; shorter intervals between visits are recommended in case of adverse events.

Effects on Fertility
Prolonged mating period and increased post-implantation loss were observed following treatment of female mice prior to mating through early embryogenesis at 50 mg/kg/day per oral (2 times the maximum clinical dose based on body surface area). No effects were observed at 5 mg/kg/day (less than the maximum clinical dose based on body surface area).

Carcinogenicity
The carcinogenic potential of nitisinone has not been studied in animals.

Genotoxicity
There is limited evidence of genotoxic potential for nitisinone *in vitro* and *in vivo*. Nitisinone was not mutagenic in the bacterial reverse mutation test but was genotoxic in the mouse lymphoma cell forward mutation test *in vitro*. *In vivo* nitisinone was weakly positive in the mouse bone marrow micronucleus test but negative in the mouse liver unscheduled DNA synthesis (UDS) test.

Use in Pregnancy (Category B3)
There are no adequate data from the use of nitisinone in pregnant women. Nitisinone should not be used during pregnancy unless clearly necessary.

Gestation length was increased in pregnant mice given nitisinone at oral doses from 50 mg/kg/day (2 times the maximum clinical dose based on body surface area).

In pregnant mice and rabbits, embryotoxicity (decreased fetal weights, increased early intra-uterine deaths and increased post-implantation loss) and fetal abnormalities (incomplete skeletal ossification in mice, umbilical hernia, gastroschisis, reduced or absent lung, increased skeletal malformations and variations in rabbits) were observed at oral nitisinone doses from 5 mg/kg/day during organogenesis (less than the maximum clinical dose based on body surface area). In a preliminary study in pregnant rats, embryotoxicity (increased stillbirths, reduced live births, birth weights and survival after birth) and fetal abnormalities (increased skeletal variants) were observed at maternally toxic oral doses from 50 mg/kg/day (4 times the maximum clinical dose based on body surface area).

Use in lactation
It is not known whether nitisinone is excreted in human breast milk. Animal studies have shown adverse postnatal effects via exposure of nitisinone in milk (see below). Therefore, mothers receiving nitisinone should not breast-feed, since a risk to the suckling child cannot be excluded.

Maternal treatment of mice at oral doses from 5 mg/kg/day (less than the maximum clinical dose based on body surface area) during organogenesis through weaning was associated with reduced pup survival, weight gain and developmental delays. In rats, lactational exposure of naïve pups to nitisinone from treated dams given 100 mg/kg/day orally was associated with reduced pup weight and the development of corneal opacities (9 times the maximum clinical dose based on body surface area).

Use in adult population
There is very limited data in the adult population and no information on the treatment of the elderly.

Effects on ability to drive and use machine
No studies on the effects on the ability to drive and use machines have been performed.

Interaction with other medicines (and other forms of interaction)
No formal interaction studies with other medicinal products have been conducted.

Nitisinone is metabolised *in vitro* by CYP 3A4 and dose-adjustment may therefore be needed when nitisinone is co-administered with inhibitors or inducers of this enzyme. Based on *in vitro* studies, nitisinone is not expected to inhibit CYP 1A2, 2C9, 2C19, 2D6, 2E1 or 3A4-mediated metabolism.

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.

ADVERSE EFFECTS

Orfadin was studied in one open-label, uncontrolled main study of 207 patients with HT-1, from ages 0 to 21.7 years at enrolment (median age 9 months), who were diagnosed with HT-1 by the presence of succinylacetone in the urine or plasma. The starting dose of nitisinone was 0.6 to 1 mg/kg/day, and the dose was increased in some patients to 2 mg/kg/day based on weight, biochemical, and enzyme markers. Median duration of treatment was 22.2 months (range 0.1 to 80 months). A complementary analysis was performed on 250 patients.

Patients with HT-1 are at increased risk of developing porphyric crises, hepatic neoplasm, and liver failure requiring liver transplantation. Regular monitoring of these complications by hepatic imaging (ultrasound, computerized tomography, and magnetic resonance imaging) and laboratory tests, including serum alpha-fetoprotein concentration is recommended. Patients with increasing alpha-fetoprotein levels or development of liver nodules during treatment with nitisinone should be evaluated for hepatic malignancy.

Additional Adverse Events, **regardless of causality assessment,** reported in the complementary analysis of 250 patients, are presented in Table 2.

The adverse reactions considered at least possibly related to treatment are listed below, by body system organ class, and absolute frequency. Frequencies are defined as common (≥1/100, <1/10) or uncommon (≥1/1,000, <1/100). Within each frequency grouping, undesirable effects are presented in order of decreasing seriousness.

Blood and lymphatic system disorders
Common: thrombocytopenia, leucopenia, granulocytopenia

Eye disorders
Common: conjunctivitis, corneal opacity, keratitis, photophobia, eye pain
Uncommon: blepharitis

Skin and subcutaneous tissue disorders
Uncommon: exfoliative dermatitis, rash, pruritus

Nitisinone treatment is associated with elevated tyrosine levels. Elevated levels of tyrosine have been associated with corneal opacities and hyperkeratotic lesions. Restriction of tyrosine and phenylalanine in the diet should limit the toxicity associated with this type of tyrosinaemia.

Table 2. Adverse Events, **regardless of causality assessment,** reported in the complementary analysis of 250 patients.

WHO Body System Class	**WHO Preferred Term**	**Total frequency (n=250)**
Body as a whole, general disorders	death	1.6%
	elective transplantation	4.0%
Cardiovascular disorders, general	cyanosis	0.4%
Central and peripheral nervous system disorders	convulsions	0.8%
	headache	0.8%
	hyperkinesia	0.8%
	hypokinesia	0.4%
Gastro-Intestinal system disoders	abdominal pain	0.4%
	constipation	0.4%
	enanthema	0.4%
	gastroenteritis	0.8%
	GI haemorrhage	0.8%
	melaena	0.4%
	tooth discoloration	0.4%
Liver and biliary system disorders	hepatic cirrhosis	0.8%
	hepatic enzymes increased	0.8%
	hepatic failure	6.4%
	hepatic function abnormal	0.4%
	hepatomegaly	0.4%
	porphyria	0.8%
Metabolic and nutritional disorders	dehydration	0.4%
	hypoglycaemia	0.4%

Neoplasm	brain neoplasm benign	0.4%
	hepatic neoplasm	3.2%
	hepatic neoplasm malignant	4.4%
	lymphoma malignant	0.4%
Platelet, bleeding and clotting disorders	epistaxis	0.4%
Psychiatric disorders	nervousness	0.8%
Red blood cell disorders	anaemia	0.4%
Reproductive disorders, female	amenorrhoea	0.4%
Resistance mechanism disorders	infection	1.2%
	otitis media	0.4%
Skin and appendages disorders	alopecia	0.8%
	skin dry	0.4%
Urinary system disorders	haematuria	0.4%
	cataract	0.8%
	retinal disorder	0.4%

DOSAGE AND ADMINISTRATION

Nitisinone treatment should be initiated and supervised by a physician experienced in the treatment of HT-1 patients. Treatment of all genotypes of the disease should be initiated as early as possible to increase overall survival and avoid complications such as liver failure, liver cancer and renal disease. Adjunct to the nitisinone treatment, a diet deficient in phenylalanine and tyrosine is mandatory. The patient should be provided with clear instructions on the restricted diet and on the importance of adherence to the restricted diet. The patient's compliance to the diet should be checked regularly by monitoring plasma tyrosine levels.

The dose of nitisinone should be adjusted individually.
The recommended initial dose is 1 mg/kg body weight/day divided in 2 doses administered orally.

Orfadin should be administered with food. In the case of paediatric patients, the capsules may be opened and the content suspended in a small amount of water or formula diet immediately before intake.

Dose adjustment
During regular monitoring, it is appropriate to follow urine succinylacetone, liver function test values and alpha-fetoprotein levels if urine succinylacetone is still detectable one month after the start of nitisinone treatment, the nitisinone dose should be increased to 1.5 mg/kg body weight/day divided in 2 doses. A dose of 2 mg/kg body weight/day may be needed based on the evaluation of all biochemical parameters. This dose should be considered as a maximal dose for all patients.

If the biochemical response is satisfactory, the dose should be adjusted only according to body weight gain.

However, in addition to the tests above, during the initiation of therapy or if there is a deterioration, it may be necessary to follow more closely all available biochemical

parameters (i.e. plasma succinylacetone, urine 5-aminolevulinate (ALA) and erythrocyte porphobilinogen (PBG)-synthase activity).

OVERDOSAGE

For advice on the management of overdosage, please contact the Poisons Information Centre (telephone 13 11 26).

No case of overdose has been reported. Accidental ingestion of nitisinone by individuals eating normal diets not restricted in tyrosine and phenylalanine will result in elevated tyrosine levels. Elevated tyrosine levels have been associated with toxicity to eyes, skin, and the nervous system. Restriction of tyrosine and phenylalanine in the diet should limit toxicity associated with this type of tyrosinaemia. No information about specific treatment of overdose is available.

PRESENTATION AND STORAGE CONDITIONS

Storage

Store refrigerated at 2-8 °C.
After first opening, store bottle below 25°C for not more than 3 months, after which the product must be discarded.

Presentation

Orfadin® capsules are white and marked in black with "NTBC" and indentified as 2 mg, 5 mg or 10 mg strengths of nitisinone. The capsules are packed in a high density (HD) polyethylene bottle with a tamper proof low density (LD) polyethylene cap. Each bottle contains 60 capsules.

Orfadin® is available as:
2 mg white capsules imprinted "NTBC 2 mg" in black ink.
5 mg white capsules imprinted "NTBC 5 mg" in black ink.
10 mg white capsules imprinted "NTBC 10 mg" in black ink.

NAME AND ADDRESS OF THE SPONSOR

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POISON SCHEDULE OF THE MEDICINE

S4

DATE OF APPROVAL

Product Information approved by the TGA on 14 October 2010

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