Report on the Deliberation Results

November 6, 2014
Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name]	Orfadin Capsules 2 mg, 5 mg, and 10 mg
[Non-proprietary name]	Nitisinone (JAN*)
[Applicant]	Astellas Pharma Inc.
[Date of application]	December 25, 2013

[Results of deliberation]
In the meeting held on October 29, 2014, the First Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The re-examination period is 8 years, the drug substance and the drug product are both classified as powerful drugs, and the product is not classified as a biological product or a specified biological product.

[Conditions for approval]
The applicant is required to:

- Prepare a Risk Management Plan, and implement it appropriately.
- Conduct a post-marketing drug use-results survey covering all patients treated with the product during the re-examination period because clinical experiences in Japan are extremely limited. Based on the survey data, identify characteristics of the patients treated with the product and compile safety and efficacy data for the product in the early post-marketing period, thereby taking necessary measures to ensure proper use of the product.

**Japanese Accepted Name (modified INN)*

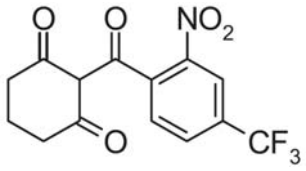
Review Report

October 8, 2014
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Orfadin Capsules 2 mg, 5 mg, and 10 mg
[Non-proprietary name]	Nitisinone
[Applicant]	Astellas Pharma Inc.
[Date of application]	December 25, 2013
[Dosage form/Strength]	Each capsule contains 2 mg, 5 mg, or 10 mg of nitisinone.
[Application classification]	Prescription drug (1) Drug with a new active ingredient

[Chemical structure]



Molecular formula: $C_{14}H_{10}F_3NO_5$
Molecular weight: 329.23
Chemical name: 2-[2-Nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione

[Items warranting special mention]	None
[Reviewing office]	Office of New Drug I

Review Results

October 8, 2014

[Brand name]	Orfadin Capsules 2 mg, 5 mg, and 10 mg
[Non-proprietary name]	Nitisinone
[Applicant]	Astellas Pharma Inc.
[Date of application]	December 25, 2013

[Results of review]

Based on the submitted data, the Pharmaceuticals and Medical Devices Agency (PMDA) has concluded that the efficacy of the product in tyrosinemia type I treatment has been demonstrated and its safety is acceptable in view of its observed benefits. The safety of its long-term use should be further investigated via post-marketing surveillance.

As a result of its regulatory review, PMDA has concluded that the product may be approved for the indication and dosage and administration as shown below, with the following conditions.

[Indication] Tyrosinemia type I

[Dosage and administration] The usual dose is 1 mg/kg/day as nitisinone divided in 2 doses administered orally. The dose may be adjusted according to the patient's condition. The maximum dose is 2 mg/kg/day.

[Conditions for approval] The applicant is required to:

- Prepare a Risk Management Plan, and implement it appropriately.
- Conduct a post-marketing drug use-results survey covering all patients treated with the product during the re-examination period because clinical experience in Japan are extremely limited. Based on the survey data, identify characteristics of the patients treated with the product and compile safety and efficacy data for the product in the early post-marketing period, thereby taking necessary measures to ensure proper use of the product.

Review Report (1)

August 27, 2014

I. Product Submitted for Registration

[Brand name]	Orfadin Capsules 2 mg, 5 mg, and 10 mg
[Non-proprietary name]	Nitisinone
[Applicant]	Astellas Pharma Inc.
[Date of application]	December 25, 2013
[Dosage form/Strength]	Each capsule contains 2 mg, 5 mg, or 10 mg of nitisinone.
[Proposed indication]	Tyrosinemia type I
[Proposed dosage and administration]	The usual dosage is 1 mg/kg as nitisinone divided in 2 doses administered orally daily. The dose may be increased up to 2 mg/kg/day if the response is inadequate.

II. Summary of the Submitted Data and Outline of the Review by Pharmaceuticals and Medical Devices Agency

A summary of the submitted data and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries etc.

Orfadin capsules 2 mg, 5 mg, and 10 mg are capsules (hereinafter collectively referred to as "Orfadin") that contain nitisinone as the active ingredient.

Hereditary tyrosinemia type I (HT-1) is an autosomal recessive genetic disorder caused by a deficiency of fumarylacetoacetate hydrolase (FAH), the last enzyme in the tyrosine catabolic pathway.[1] An FAH deficiency results in the accumulation of toxic intermediate metabolites, maleylacetoacetate (MAA) and fumarylacetoacetate (FAA), and their metabolites, succinylacetone (SA) and succinylacetoacetate (SAA) in the liver and kidneys, causing severe liver dysfunction, clotting disorder, painful neurological crisis, and renal tubular dysfunction, and increasing the risk of hepatocellular carcinoma.[2] Further, SA and SAA lead to an accumulation of 5-aminolevulinate, the substrate of porphobilinogen synthase, in the heme biosynthesis pathway, causing porphyria-like neurological symptoms. The accumulation of toxic metabolites starts at birth and the severity of disease differs depending on the

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1 Lindblad B, et al., *Proc Natl Acad Sci USA*. 1977;74:4641-5

2 Halvorsen S, *Inborn metabolic diseases*. ed. by Fernandes J, et al., Springer Verlag, Berlin, 1990;199-209, Kvittingen EA, *J Inherit Metab Dis*. 1991; 14:554-62, Mitchell G, et al., *The Metabolic and Molecular Bases of Inherited Disease, 7th ed.* ed. by Scriver CR, et al., McGraw Hill Inc., New York, 1995;1077-106, van Spronsen FJ, et al., *Hepatology*. 1994;20:1187-91, Weinberg, et al., *J Pediatr*. 1976;88:434-8

age at onset of symptoms. Patients with acute HT-1, which is the most common form, present with signs of hepatic failure, failure to thrive, vomiting, diarrhea, fever, in the first weeks to months after birth, as well as frequent melena and frequent nosebleed. If untreated, death from hepatic failure commonly occurs within 2 to 8 months after birth.[3] The subacute form of HT-1 is characterized by chronic progressive liver disease and renal tubular dysfunction (the Fanconi syndrome) with hypophosphatemia and rickets, although its clinical course is less rapid and symptoms are less severe than those of the acute form. Approximately 40% of the patients with the subacute form present with recurrent porphyria-like crises with respiratory failure, or hepatocellular carcinoma,[4] both of which are the main causes of death at the age of ≤10 years.[5] In the chronic form of HT-1, the disease progresses slowly, and the most prominent findings are rickets and tubular disorder.

The therapies for HT-1 include dietary restriction of tyrosine and phenylalanine,[6] however, diet therapy alone does not prevent the progression of the disease. Among patients treated with dietary restriction alone, the 2-year and 4-year survival was 29% for patients in whom symptoms developed at the age of <2 months, and 74% and 60%, respectively, for patients in whom symptoms developed at the age of 2 to 6 months. An alternative to diet therapy is liver transplant, and the 1-year survival after transplant was approximately 75% and 80% in patients who underwent liver transplant at the age of 1 year and 2 years, respectively. While the overall 9-year survival after liver transplant was approximately 70%, the 9-year survival without retransplant was reported as approximately 60%.[5] Major concerns about liver transplant include the risk of perioperative morbidity and mortality, potential hazards associated with long-term immunosuppressive therapies, psychological stress of surgery, and difficulty in obtaining compatible donor livers.

The incidence of HT-1 is approximately 1 in 100,000 births in Europe and North America, while it is considerably higher in certain areas, including the province of Quebec, Canada, where it is approximately 1 in 20,000 births.[6] The incidence of HT-1 in Japan is unknown, and only 3 patients with HT-1 in which nitisinone was administered have been reported.[7]

Nitisinone is an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme in the second step of the tyrosine catabolic pathway. Nitisinone is expected to improve the patient's condition by inhibiting the production and accumulation of MAA, FAA, and other toxic intermediate metabolites in the pathway, which is caused by a genetic deficiency in FAH activity in patients with HT-1. An investigator-initiated, NTBC study (compassionate use study) was conducted after the results of a

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3 Goldsmith LA & Laberge C, *The metabolic basis of inherited disease.* ed. by Scriver CR et al., McGraw Hill Inc., New York, 1989;547-62

4 Kvittingen EA, *J Inherit Metab Dis.* 1991;14:554-62

5 van Spronsen FJ, et al., *Hepatology*. 1994;20:1187-91

6 Mitchell G, et al., *The Metabolic and Molecular Bases of Inherited Disease, 7th ed.* ed. by Scriver CR, et al., McGraw Hill Inc., New York, 1995;1077-106

7 In addition to the 3 patients, 1 more case has been reported in which a patient with citrin deficiency, a disease different from HT-1, received nitisinone (Nakabayashi H, et al., *Bulletin on Special Formula: Dietary Management of Inborn Errors of Metabolism*, 2004;40:30-5).

study in patients treated with nitisinone were published by the team from Sahlgrenska University Hospital. The results showed that nitisinone had improved short-term prognosis and health status. Orfadin was approved in January 2002 in the US, in February 2005 in Europe, and as of July 2014, it has been approved in 37 countries including the US and European countries. Today, nitisinone is first-line therapy for HT-1,[8] and the treatment is combined with dietary restriction of tyrosine and phenylalanine.

[redacted][9][redacted]

Given this context, the Study Group on Unapproved and Off-label Drugs of High Medical Need started to review the medical necessity of nitisinone, and concluded, at the second meeting, that a substantial clinical need for the drug existed. In May 2010, following the decision, Japanese companies were encouraged to develop the drug. Nitisinone was granted development subsidies for the drug by the Unapproved Drug Development Grants Program. Astellas Pharma Inc. (the applicant) decided to prepare a regulatory application for Orfadin (nitisinone) in coordination with Swedish Orphan Biovitrum (SOBI).

The applicant has filed a marketing application claiming that the efficacy and safety of Orfadin (nitisinone) in patients with HT-1 have been confirmed by the NTBC and other studies.

2. Data relating to quality

2.A Summary of the submitted data

2.A.(1) Drug substance

2.A.(1).1) Characterization

The drug substance, nitisinone, is a white to yellowish white crystalline powder, and its description, solubility, melting point, particle size distribution, polymorphism, and dissociation constant have been determined.

The chemical structure of the drug substance has been elucidated by elemental analysis, mass spectrometry, nuclear magnetic resonance spectroscopy (^{1}H-, ^{13}C-NMR), and infrared spectrophotometry (IR).

2.A.(1).2) Manufacturing process

[redacted]

8 de Laet, et al., *Orphanet J Rare Dis.* 2013;8:8, McKiernan PJ, *Expert Opinion on Orphan Drugs.* 2013;1:491-7

9 Ito M, et al., *Bulletin on Special Formula: Dietary Management of Inborn Errors of Metabolism.* 2005;41:27-30

[redacted]

2.A.(1).3) Control of drug substance

[redacted]

2.A.(1).4) Stability of drug substance

The stability studies of the drug substance are shown in Table 1. Photostability testing demonstrated that the drug substance was photostable.

Table 1. Stability studies of the drug substance

Study	Primary batch	Temperature	Humidity	Storage container	Storage period
Long-term	3 production batches	25°C	60% RH	Brown glass bottle (with polypropylene screw cap)	60 months
Accelerated	3 production batches	40°C	75% RH		6 months

Based on the above, a retest period of 60 months has been proposed for the drug substance when stored at room temperature in a brown glass bottle (with polypropylene screw cap).

2.A.(2) Drug product

2.A.(2).1) Description and composition of the drug product, and formulation design

The drug product is an immediate-release hard capsule, each containing 2 mg, 5 mg, or 10 mg of nitisinone. The drug product also contains partly pregelatinized starch as an excipient.

2.A.(2).2) Manufacturing process

The drug product is manufactured through the process consisting of the following steps: mixing, encapsulation, filling, and packaging. [redacted].

2.A.(2).3) Control of drug product

The proposed specifications for the drug product consist of content, description, identification (retention time [HPLC], ultraviolet-visible spectrophotometry), purity (related substances [HPLC]), uniformity of dosage units (content uniformity [HPLC]), dissolution (HPLC), microbial limit, and assay (HPLC).

2.A.(2).4) Stability of drug product

The stability studies of the drug product are shown in Table 2. A bracketing design was used. Photostability testing demonstrated that the drug product was photostable.

Table 2. Stability studies of the drug product

Study	Primary batch	Temperature	Humidity	Storage container	Storage period
Long-term	3 production batches	5°C	—	Polyethylene bottle (with polyethylene cap)	36 months
Accelerated	3 production batches	25°C	60% RH		6-36 months

Based on the above, a shelf-life of 24 months has been proposed for the drug product when stored at 2°C to 8°C in a polyethylene bottle (with polyethylene cap).

2.B *Outline of the review by PMDA*

Based on the submitted data and the following reviews, PMDA concluded that the quality of the drug substance and the drug product is adequately controlled.

2.B.(1) Stability of the drug substance

The applicant justified the bracketing designs used in the stability studies of the drug product as follows:

[redacted]. Furthermore, regardless of the strength, the drug product capsules are packed in plastic bottles of the same volume, and each bottle contains 60 capsules. The relative free-space in the plastic bottle is the same for all strengths because the capsule size is the same.

Therefore, the 2-mg and 10-mg strength capsules of the drug product were considered to represent the practical extremes of stability factors, which was the rationale for conducting bracketing stability studies according to "Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products" (PFSB/ELD Notification No. 0731004 dated July 31, 2002).

PMDA asked the applicant to explain whether or not the long-term stability of the 5-mg strength capsule of the drug product should be investigated considering that a different tendency was observed between the 2-mg and 10-mg strength capsules of the drug product in terms of the change in the amount of related substances.

The applicant responded as follows:

The amount of Related Substance 1 increased more in the 2-mg strength capsule of the drug product than in the 10-mg strength capsule of the drug product. [redacted]

[redacted]. Therefore, additional testing to confirm the long-term stability of the 5-mg strength capsule of the drug product is not considered necessary.

PMDA accepted the applicant's response.

2.B.(2) New excipient

The drug product contains pregelatinized starch.

2.B.(2).1) Specifications and stability

PMDA concluded that there are no specific problems with the specifications and stability of the pregelatinized starch.

2.B.(2).2) Safety

Based on the submitted data, PMDA concluded that there are no specific problems with the safety of the pregelatinized starch content in the drug product.

Based on the above, PMDA concluded that there are no particular problems with the use of these excipients in the drug product.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

In primary pharmacodynamic studies, inhibitory action against 4-hydroxyphenylpyruvate dioxygenase (HPPD), and effects on the concentration of tyrosine in the plasma and ocular fluid were investigated *in vitro* and *in vivo*. No secondary pharmacodynamic studies or pharmacodynamic interaction studies have been conducted. In safety pharmacology studies, the effects on the central nervous system, cardiovascular system, respiratory system, and peripheral autonomic nervous system were investigated using rats and other animals, although the studies were not conducted in accordance with ICH S7A or S7B guideline. The results of the main studies and published literature are described below.

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1) In vitro studies

Inhibitory action against HPPD derived from rat liver (4.2.1.1-1, 4.2.1.1-2; reference data)

The inhibitory action of nitisinone against HPPD was investigated using male rat liver cytosolic fractions.[10] The results showed competitive inhibition of nitisinone against HPPD activity, with the IC_{50} being approximately 40 nmol/L. The time dependence of HPPD inhibition by nitisinone was investigated by varying the nitisinone-HPPD pre-incubation time before the start of enzyme assay. The

[10] The HPPD activity was defined as the rate of oxygen consumption in the production of homogentisic acid from 4-hydroxyphenylpyruvic acid at 37°C, and HPPD inhibition rate was calculated by comparing HPPD activities at different concentration levels of nitisinone with the HPPD activity in the absence of nitisinone.

HPPD inhibition by 100 nmol/L of nitisinone was ≥50% with a pre-incubation time of 30 seconds, and reached a plateau with a pre-incubation time of ≥1 minute. The reversibility of HPPD activity was investigated using a rat liver cytosolic fraction,[11] which was incubated with nitisinone[12] at 37°C for 15 minutes, dialyzed in a buffer at 0°C for 6 hours to remove nitisinone not bound to HPPD, and then dialyzed at 25°C for 7 hours to allow HPPD-bound nitisinone to dissociate from the enzyme. The HPPD activity after 7 hours of dialysis was 13.7% compared to that in the absence of nitisinone.

3.(i).A.(1).2) In vivo studies

(a) Effects on activities of hepatic HPPD, TAT, and HGO in mice (4.2.1.1-3, 4.2.1.1-4; reference data)

A single oral dose of nitisinone (10 mg/kg) or vehicle[13] was administered to male mice (n = 4/group)[14] to investigate the time course of its effect on the activities of hepatic HPPD, tyrosine aminotransferase (TAT), and homogentisic acid oxidase (HGO). The results showed that HPPD activity was almost completely inhibited 4 hours after administration of nitisinone, with significantly lower activity than in the control group. The HPPD activity in the nitisinone group increased to approximately 10% of that in the control group 4 days or more after administration. The TAT activity was significantly higher in the nitisinone group than in the control group 16 hours and 24 hours after administration of nitisinone, and decreased to about the same levels as the control group 3 days after administration. The HGO activity was significantly lower in the nitisinone group than in the control group 4 days and 5 days after administration of nitisinone.

(b) Effects on activities of hepatic HPPD, TAT, and HGO in rats (4.2.1.1-5, 4.2.1.1-6; reference data)

A single oral dose of nitisinone (0.1, 10 mg/kg) or vehicle[15] was administered to male rats (n = 4/group) to investigate the time course of its effect on the activities of hepatic HPPD, TAT, and HGO. The results showed that the HPPD activity was almost completely inhibited in the nitisinone 0.1 mg/kg group 1 hour after administration; however, the HPPD activity increased to approximately 40% of that in the control group 3 days after administration. In the nitisinone 10 mg/kg group, the HPPD activity was almost completely inhibited 30 minutes after administration, and increased to approximately 25% of that in the control group 7 days after administration. The TAT activity was significantly higher in nitisinone 0.1 mg/kg group than in the control group 1 day after administration, and decreased to about the same level as that in the control group 3 days after administration. In the nitisinone 10 mg/kg group, the TAT activity was significantly higher than in the control group 24 hours and 2 days after

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11 HPPD activity is obtained by measuring $^{14}CO_2$ produced from 1-[^{14}-C]-4-hydroxyphenylpyruvic acid. The recovery rate of enzyme activity was calculated by comparing the HPPD activity with the activity of HPPD prepared in the absence of nitisinone as the control.

12 The final concentration was 25 μmol/L

13 5 or 10 mL/kg of corn oil or water.

14 The means of the parameters for 3 mice in the nitisinone treatment group were calculated only at 3 days after administration.

15 5 mL/kg of corn oil or water.

administration, and decreased to about the same level as that in the control group 4 days after administration. The HGO activity was significantly higher in the nitisinone 0.1 mg/kg group than in the control group 24 hours and 2 days after administration, whereas no effect of nitisinone administration was observed in the nitisinone 10 mg/kg group.

(c) Effects on the plasma and ocular fluid concentrations of tyrosine in mice (4.2.1.1-3, 4.2.1.1-4; reference data)

A single oral dose of nitisinone (10 mg/kg) or vehicle[13] was administered to male mice to investigate the time course of its effect on the concentrations of tyrosine in the plasma and ocular fluid up to 2 days after administration. The results showed that the concentrations of tyrosine in the plasma and ocular fluid reached maximum levels 16 hours after administration, and these concentrations were significantly higher than in the control group. The tyrosine concentrations in the plasma and ocular fluid remained significantly higher than in the control group 2 days after administration. The plasma tyrosine concentration was repeatedly examined for up to 5 days after administration, and the concentrations remained significantly higher than in the control group for up to 3 days[16] after administration.

(d) Effects on the plasma and ocular fluid concentrations of tyrosine in rats (4.2.1.1-5, 4.2.1.1-6; reference data)

A single oral dose of nitisinone (0.1, 10 mg/kg) or vehicle[15] was administered to male rats to investigate the time course of its effect on the concentrations of tyrosine in the plasma and ocular fluid. The results showed that the concentrations of tyrosine in the plasma and ocular fluid in the nitisinone 0.1 mg/kg group reached maximum levels 24 hours after administration, and these concentrations were significantly higher than in the control group. The concentrations of tyrosine in the plasma and ocular fluid decreased to the same levels as the control group 2 days and 3 days after administration, respectively. The concentrations of tyrosine in the plasma and ocular fluid in the nitisinone 10 mg/kg group reached plateaus anywhere from 16 hours to 2 days after administration, showing significantly higher levels than in the control group. The plasma tyrosine concentration remained significantly higher than in the control group for up to 3 days after administration, and then decreased to about the same levels as the control group. The ocular fluid tyrosine concentration still remained significantly higher than in the control group 5 days after administration.

(e) Effects on FAH-deficient mice (4.2.1.1-7; reference data)

Male and female heterozygous fumarylacetoacetate hydrolase (FAH)-deficient mice[17] were mated, and on Gestation Day 15, dams started receiving 3 mg/kg of nitisinone once daily by subcutaneous

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16 It is not known whether or not there was significant difference in the plasma tyrosine concentration 8 hours after administration.

17 A replacement vector was constructed in which pol2neo and herpes simplex virus thymidine kinase expression cassettes were inserted into the region containing exon 5 of the mouse FAH gene, and the vector was electroporated into mouse ES cells, which were injected into C57BL/6j blastocysts, to derive heterozygous FAH-deficient mice (Grompe M, et al., *Genes Dev*. 1993;7:2298-307).

injection. After birth, all neonatal pups received 1 mg/kg of nitisinone orally once daily until weaning, and 5 days a week after weaning. As a result, while all homozygous FAH-deficient mice born to dams that had not received nitisinone died within 24 hours after birth, none of homozygous FAH-deficient mice born to dams that had received nitisinone died during the neonatal period. On the day of birth, hepatic mRNA expression levels in homozygous FAH-deficient mouse pups were measured. Pups delivered from nitisinone-treated dams had higher levels of TAT and phosphoenolpyruvate carboxykinase (PEPCK) and lower levels of CHOP-10, a DNA damage- response factor, and NMO-1, oxidative stress-response factor, than those from non-treated dams. While the plasma succinylacetone (SA) levels were higher in homozygous FAH-deficient mice that received nitisinone continuously than in wild type mice and heterozygous FAH-deficient mice, no difference was observed in the plasma levels of aspartate aminotransferase (AST) or conjugated bilirubin between these mice. In some of the homozygous FAH-deficient mice that received nitisinone continuously, the hepatic mRNA expression levels of TAT and PEPCK were elevated to and that of CHOP-10 were decreased to almost the same levels as the hepatic mRNA expression levels in wild or heterozygous FAH-deficient mice. In contrast, the hepatic mRNA expression levels of NMO-1 and H19, a hepatocyte proliferation index, remained high, regardless of the administration of nitisinone.

3.(i).A.(2) Safety pharmacology (4.2.1.3-1; reference data)

3.(i).A.(2).1) Effects on central nervous system and autonomic nervous system

(a) Clinical signs and behavior

A single oral dose of nitisinone (50, 200, 500 mg/kg) or vehicle[18] was given to male rats (weighing 250-301 g, n = 2/group) after 16- to 20-hour fasting, and clinical signs were observed. One of the rats in the nitisinone 500 mg/kg group exhibited reflex inhibition, labored breathing, etc., and was sacrificed in a moribund condition on the day of administration. The other rat exhibited signs including hypoactivity and hunchback position, etc., up to 2 days after administration, but these signs were not observed 5 days after administration.

(b) Muscle relaxant activity

Muscle relaxant activity was studied in male rats (weighing 272-360 g, n = 10/group) which, after overnight fasting, received a single oral dose of nitisinone (200, 350, 500 mg/kg) or vehicle,[18] and underwent pull-up tests[19] 1 hour later. The results showed that, compared with the control group, the time required for the rats in the nitisinone 500 mg/kg group to pull themselves up was significantly prolonged, and 2 rats from this group could not pull themselves up within 30 seconds.

(c) Effects on halothane-induced sleeping time

The effects on halothane-induced sleeping time were investigated in male rats (weighing 220-269 g, n = 5/group) which, after overnight fasting, received a single oral dose of nitisinone (200, 350,

[18] 10 mL/kg of corn oil

[19] The time taken by a rat to pull itself up was recorded.

500 mg/kg) or vehicle,[18] and were anesthetized with halothane gas[20] 1 hour later.[21] The results showed that, compared with the control group, sleeping time was significantly prolonged in the nitisinone 500 mg/kg group.

(d) Effects on β2 receptor

The effects of 10 μmol/L of nitisinone or vehicle[22] on isoprenaline-induced relaxation of the carbachol-induced contraction of the isolated guinea pig trachea were investigated. The results showed no effect of nitisinone on isoprenaline-induced relaxation.

(e) Effects on α1 and α2 receptors

The effects of 10 μmol/L of nitisinone or vehicle[22] on methoxamine-induced contraction in isolated rat vas deferens were investigated. The results showed no effect of nitisinone on methoxamine-induced contraction.

The effects of 10 μmol/L of nitisinone or vehicle[22] on clonidine-induced relaxation of the field stimulation-induced contraction of the isolated rat vas deferens to were investigated. The results showed no effect of nitisinone on clonidine-induced relaxation.

3.(i).A.(2).2) Effects on cardiovascular and respiratory systems

A single oral dose of 350 mg/kg of nitisinone (n = 3) or vehicle[18] (n = 2) was given to anesthetized male rats (weighing 245-305 g) to investigate the effects on blood pressure, heart rate, cardiac contractility (QA interval and systolic index), and respiration rate. The results showed no effect of nitisinone on these parameters.

3.(i).B Outline of the review by PMDA

3.(i).B.(1) Mechanism of action

PMDA asked the applicant to explain the mechanism of action of nitisinone in the context of the mechanism of liver injury and renal injury caused by the accumulation of fumarylacetoacetate (FAA), maleylacetoacetate (MAA), SA, and succinylacetoacetate (SAA) in patients with hereditary tyrosinemia type I (HT-1), taking into account the species differences in tyrosine catabolic pathway.

The applicant responded as follows:

It has been reported that L-tyrosine in the rat liver is oxidized, via the production of 4-hydroxyphenylpyruvic acid (HPPA) and homogentisic acid, to acetoacetate, and the tyrosine catabolic pathway is considered to involve TAT, HPPD, HGO, maleylacetoacetate isomerase (MAAI), and FAH.[23] Further, enzymes involved in the tyrosine catabolic pathway have specific tissue distribution

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20 4% in oxygen

21 The time to recovery of the righting reflex after the rat was taken out from the anesthetizing box was measured.

22 Dimethyl sulfoxide (DMSO)

23 Knox WE & LeMay-Knox M, *Biochem J*. 1951;49:686-93, Knox WE, *Methods Enzymol*. 1955;2:287-300

in mice, rats, and humans: TAT is found specifically in the liver; and HPPD, HGO, MAAI,[24] and FAH are distributed in the liver and kidney in these species.[25] Consequently, the applicant considers that mice, rats, and humans have the same tyrosine catabolic pathway.

In patients with HT-1, a reduced activity of FAH, the last enzyme in the tyrosine catabolic pathway, leads to production and accumulation of highly reactive intermediate metabolites, FAA, MAA, SA, and SAA, which cause hepatic and renal disorders. Among the intermediate metabolites, FAA and MAA have been reported to be able to alkylate sulfhydryl (SH) groups, amino groups, and other functional groups of intracellular molecules, and to thereby cause toxic effects.[6] In addition, the formation of glutathione adducts by FAA and MAA results in a decrease in the concentrations of reduced glutathione in the liver and erythrocytes of patients with HT-1 to approximately half the levels of healthy adults.[26] It is reported that SA nonenzymatically binds to amino acids and proteins to form adducts, and that SA is excreted in the urine of patients with HT-1 in the form of amino acid adducts or protein adducts.[27] These findings suggest that FAA, MAA, and SA affect SH groups, thus enhancing oxidative stress, which may cause damage to cells and tissues. In fact, in homozygous FAH-deficient mice as a potential animal model for human HT-1, increased hepatic mRNA-expression levels of NMO-1 and glutamate-cysteine ligase modifier subunit, molecular markers of the oxidative stress response, have been reported.[28] Although data that directly indicate the toxicity of SAA have not been obtained, the similarity of the structure suggests that SAA also shows comparable toxicity to that of FAA and MAA. Based on the mechanisms described above, hepatic and renal disorders in patients with HT-1 are attributable to the accumulation of FAA, MAA, SA, and SAA.

Nitisinone is expected to improve the condition of patients with HT-1 by directly inhibiting HPPD, an enzyme in the second step of the tyrosine catabolic pathway, and by indirectly limiting the production and accumulation of the toxic intermediate metabolites mentioned above, i.e., FAA, MAA, SA, and SAA. Although data from literature that indicate a decrease in accumulated hepatic or renal FAA, MAA, and SAA concentrations after administration of nitisinone have not been obtained, a decrease in urinary SA and SAA concentrations in humans after administration of nitisinone has been reported.[29]

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24 In rats, it is not known whether MAAI is distributed in the kidneys, whereas it is found in the liver.

25 Hargrove JL & Mackin RB, J Biol Chem. 1984;256:386-93, Griffiths JA & Oliveira DBG, Scand J Immunol. 1988;27:357-60, Neve S, et al., Cell Bio Int. 2003;27: 611-24, Fellman JH, et al., Biochim Biophys Acta. 1972;284:90-100, Rüetschi U, et al., Genomics. 1997;44:292-9, Schmidt SR, et al., Mamm Genome. 1997;8:168-71, Coufalik A & Monder C, Biol Neonate. 1978;34:161-6, Chen J, et al., Hum Exp Toxicol. 2011;30:1616-25, Fernández-Cañón JM, et al., Nat Genet. 1996;14:19-24, Lim CE, et al., Am J Pathol. 2004;165:679-93, Fernández-Cañón JM, et al., Genomics. 1999;58:263-9, Guo X, et al., Drug Metab Dispos. 2006;34:36-42, Labelle Y, et al., Gene. 1991;104:197-202, Kvittingen EA, et al., Pediatr Res. 1983;14:541-4, Berger R, et al., Pediatr Res. 1987;22:394-8

26 Seltzer S & Lin M., *J Am Chem Soc*. 1979;101:3091-7, Stoner E, et al., *Pediatr Res*. 1984;18:1332-6

27 Manabe S, et al., *J Exp Med*. 1985;162:1060-74

28 Grompe M, et al., *Nat Genet*. 1995;10:453-60, Dieter MZ, et al., *Free Radic Biol Med*. 2003;221:73-9

29 Lindstedt S, et al., *Lancet*. 1992;340:813-7

PMDA concluded that the submitted data demonstrates the *in vitro* and *in vivo* inhibitory effects of nitisinone against HPPD, and nitisinone can be expected to be effective based on the explained mechanism of action, and accepted the applicant's response [see "4.(iii).B.(2).1) Efficacy" for clinical efficacy].

3.(i).B.(2) Safety pharmacology

PMDA asked the applicant to explain the effects of nitisinone on the central nervous system, cardiovascular system, and respiratory system.

The applicant responded as follows:

With regard to the effects on the central nervous system, nitisinone is reported to have low blood-to-brain permeability in rats (4.2.1.1-5, 4.2.1.1-6), and based on the results of the studies on the cerebrospinal fluid concentrations of nitisinone after its administration to patients with HT-1,[30] its blood-to-brain permeability is considered low in humans, too. The expected plasma C_{max} of protein-unbound nitisinone at the no-effect dose (200 mg/kg) obtained from the study on the effects on the central nervous system in rats (4.2.1.3-1) was 39.2 μg/mL,[31] which was approximately 23-fold higher than the estimated concentration of plasma protein unbound nitisinone (1.72 μg/mL)[32] in human plasma at the maximum clinical dose (2 mg/kg/day). In the NTBC clinical study in patients with HT-1, the following events were reported: convulsions (3 of 291 subjects), hyperkinesia (2 of 291 subjects), headache (1 of 291 subjects), hypokinesia (1 of 291 subjects), and somnolence (1 of 291 subjects). Convulsions, coma, hypotonia, and polyneuropathy (1 to 3 events each) were also reported in overseas post-marketing surveillance reports; however, it is not known if nitisinone has direct effects on these events. Based on the above data on the permeability to central nervous system, the relationship between the exposure levels investigated in non-clinical studies and those for humans, clinical study results, and overseas post-marketing surveillance, it is unlikely that nitisinone will have direct effects on the central nervous system.

The effects of nitisinone on the cardiovascular system and respiratory system were investigated in rats. The results showed no differences between the control group and the nitisinone group for any of the parameters examined (4.2.1.3-1). The plasma C_{max} of protein-unbound nitisinone at the no-effect dose

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30 Thimm E, et al., *Mol Genetic Metabolism*. 2011;102:122-5

31 Calculated in the following manner: C_{max} value in rats at a dose of 200 mg/kg was estimated to be 438 μg/mLbased on the observed C_{max} value of 6.57 μg/mL after a single oral dose of nitisinone (3 mg/kg) (4.2.2.2-1, 4.2.2.2-2), assuming that plasma nitisinone concentrations increase proportionally (linearly) to the administered dose; the estimated C_{max} value of 438 μg/mL is multiplied by the protein-unbound nitisinone concentration in rat plasma of 8.96% (4.2.2.3-1, 4.2.2.3-2).

32 Calculated in the following manner: the serum nitisinone concentration in humans at the maximum clinical dose (2 mg/kg) was estimated to be 120 μmol/L based on the mean serum concentrations of nitisinone of 60 μmol/L at most after administration of nitisinone 1 mg/kg/day in the NTBC study (5.3.3.3-1, 5.3.5.2-1) assuming that plasma nitisinone concentrations increase proportionally (linearly) to the administered dose; the estimated maximum serum nitisinone concentration in humans (120 μmol/L) is multiplied by protein-unbound concentration of nitisinone in human plasma of 4.36% (4.2.2.3-1, 4.2.2.3-2).

(350 mg/kg) in the above study was 68.7 μg/mL,[33] which was approximately 40-fold higher than the estimated concentration of protein-unbound nitisinone (1.72 μg/mL)[32] in human plasma at the maximum clinical dose (2 mg/kg). In the NTBC clinical study in patients with HT-1, cyanosis (1 of 291 subjects) was reported. Aortic valve stenosis, atrial fibrillation, cardiac arrest, cardiomyopathy, cardio-respiratory arrest, cyanosis, myocardial infarction (1 event each) were also reported in overseas post-marketing surveillance reports. However, the incidence of these events is low, and events that clearly suggest the effect of nitisinone on the cardiovascular system or respiratory system have not been observed. Based on the above, it is unlikely that nitisinone will have effects on the cardiovascular system or respiratory system.

PMDA generally accepted the applicant's response concerning the effects of nitisinone on the central nervous system, cardiovascular system, and respiratory system noted in non-clinical studies. Considering that the safety margin was calculated based on the estimate, PMDA will further discuss its safety in humans in the clinical section [see "4.(iii).B.(2).2) Safety" for clinical safety].

3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

The pharmacokinetics of nitisinone and ^{14}C-nitisinone after a single intravenous or oral administration to mice and rats was analyzed. The pharmacokinetics of nitisinone after repeated administration was also analyzed based on toxicokinetic findings from repeated dose toxicity studies in mice. The plasma nitisinone concentration in mice and rats was measured using high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) and high performance liquid chromatography with ultraviolet detection (HPLC-UV), with their lower limits of quantification being 0.005 μg/mL and 0.08 μg/mL, respectively. Thin layer chromatography (TLC) was used to identify metabolites. The radioactivity in biological samples was measured by liquid scintillation counting. The results of the main studies and published literature are described below.

3.(ii).A.(1) Absorption (4.2.3.2-1) (4.2.2.2-1, 4.2.2.2-2; reference data)

The pharmacokinetic parameters of nitisinone after a single intravenous or oral administration to male rats were as shown in Table 3.

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[33] Calculated in the following manner: C_{max} value in rats at a dose of 350 mg/kg was estimated to be 767 μg/mL based on the observed value of 6.57 μg/mL after a single oral dose of nitisinone (3 mg/kg) (4.2.2.2-1, 4.2.2.2-2) assuming that plasma nitisinone concentrations increase proportionally (linearly) to the administered dose; the estimated C_{max} value of 767 μg/mL is multiplied by the protein-unbound concentration of nitisinone in rat plasma of 8.96 % (4.2.2.3-1, 4.2.2.3-2).

Table 3. Pharmacokinetic parameters of nitisinone after a single intravenous or oral administration

Administration route	Dosage form	Dose (mg/kg)	Number of subjects	C_{max} (μg/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{inf} (μg·h/mL)	BA (%)
i.v.	Aqueous solution[a]	2.85 ± 0.38 (2.30, 3.15)	7	–	–	9.3 ± 0.4	172 ± 30	–
p.o.	Suspension[b]	2.61 ± 0.14 (2.52, 2.80)	6	6.57 ± 1.77	5.49 ± 2.63	7.3 ± 1.9	136 ± 21	90.6
	Aqueous solution[c]	3.03 ± 0.04 (2.97, 3.09)	7	10.10 ± 2.49	3.57 ± 3.28	9.1 ± 1.2	180 ± 16	101

Mean or mean ± standard deviation; dose, mean ±standard deviation (minimum, maximum);–, not calculated;

C_{max}, maximum plasma concentration; t_{max}, time to maximum plasma concentration; $t_{1/2}$, elimination half-life; AUC_{inf}, area under the plasma concentration-time curve (extrapolated to t = infinity); BA, absolute bioavailability

a) NaOH/HCl/phosphate buffer aqueous solution (pH 7.0)

b) 0.5 % carboxymethyl cellulose suspension

c) Xylitol/phosphate buffer/NaOH aqueous solution (pH 7.0)

The pharmacokinetics of nitisinone following the administration of the last dose was investigated in male and female mice that received repeated oral administration of nitisinone at 3, 30, or 100 mg/kg/day for 4 weeks, 200 mg/kg/day for 3 weeks, or 300 mg/kg/day for 1 week. The results showed that both the maximum plasma concentration of nitisinone (C_{max}) and the area under the concentration-time curve from time zero to 24 hours (AUC_{24h}) increased with increase in dose, in a less than dose-proportional manner. The time to maximum plasma concentration (t_{max}) (mean value) was 0.5 to 1.0 hour, and elimination half-life ($t_{1/2}$) was 2.24 to 2.98 hours. No significant sex differences in pharmacokinetic parameters were noted in mice.

3.(ii).A.(2) Distribution (4.2.2.3-1, 4.2.2.3-2) (4.2.1.1-3 to 4.2.1.1-6; reference data)

After a single oral dose of 30 μmol/kg (10 mg/kg) of ^{14}C-nitisinone[34] was administered to male mice (n = 4/time point), the concentration of radioactivity peaked at the first measuring time point, 2 hours after administration, in the plasma, eyes, Harderian glands, liver, kidneys, and lungs. Radioactivity remained in the liver and kidneys, and was still detectable 168 hours after administration at 67-fold and 5.7-fold higher concentrations than in plasma, respectively. In contrast, the concentrations of radioactivity in the eyes and Harderian glands at 168 hours after administration were less than the lower limits of quantification.

After a single oral dose of 0.3 μmol/kg (0.1 mg/kg) of ^{14}C-nitisinone[34] was administered to male rats (n = 4/time point), radioactivity was detected in the plasma, eyes (except the corneas), extraorbital lacrimal glands, Harderian glands, liver, kidneys, and lungs. The concentration of radioactivity peaked 4 hours after administration in the kidneys, 24 hours after administration in the liver, and 8 hours after administration in other tissues. Radioactivity remained in the liver, kidneys, and Harderian glands, and was still detectable in the Harderian glands 72 hours after administration, and in the liver and kidneys 96 hours after administration. The concentration of radioactivity in the liver, kidneys, and Harderian glands 8 hours after administration were 34-fold, 10-fold, and 2.0-fold higher than in plasma,

34 Phenyl-labeled ^{14}C-nitisinone

respectively. In contrast, the concentration of radioactivity in the eyes (except the corneas) and extraorbital lacrimal glands 24 hours or more after administration were less than the lower limit of quantification.

After a single oral dose of 30 μmol/kg (10 mg/kg) of ^{14}C-nitisinone[34] was administered to male rats (n = 4/time point), radioactivity was detected in all tissues including the plasma (i.e., plasma, corneas, eyes [except the cornea], extraorbital lacrimal glands, intraorbital lacrimal glands, Harderian glands, liver, kidneys, lungs, and brain) with a lower level in the brain. The concentration of radioactivity peaked 4 hours after administration in the corneas, and 2 hours after administration in the other tissues. Radioactivity remained in the liver, kidneys, and Harderian glands, and was still detectable 168 hours after administration at 61-fold, 13-fold, and 1.3-fold higher concentrations than in plasma, respectively. The concentration of radioactivity at 168 hours after administration in the corneas, eyes (except the corneas), extraorbital lacrimal glands, and intraorbital lacrimal glands were below the lower limits of quantification.[35] Cytosolic fractions were prepared from the liver and eye homogenates 4 hours and 24 hours after administration, and the results of their analysis showed that 90% or more of the radioactivity was distributed in the cytosolic fractions for the liver and eyes.

The fractions of protein-unbound nitisinone (50, 500 μmol/L) in mice were 23.4% and 59.3% (mean value; equilibrium dialysis method), respectively, and the fraction of protein-unbound nitisinone (50 μmol/L) in rats was 8.96%.

3.(ii).A.(3) Metabolism (4.2.2.4-1; reference data)

A single oral dose of 100 mg/kg of ^{14}C-nitisinone[36] was administered to male and female rats (n = 2/sex), and 3.3% to 6.7% of the administered radioactivity was recovered in the urine 6 hours after administration.[37] The majority of the administered radioactivity was observed in the following tissues 6 hours after administration: the liver (6.6%-7.9% of the administered radioactivity), kidneys (0.9%-1.2%), organs other than the liver and kidneys[38] (22.5%-39.9%), skin (11.4%-14.4%), blood (4.5%-9.1%), eye (0.16%-0.31%), and carcass (27.7%-43.0%). There were no significant sex differences or differences due to ^{14}C labeling sites. Urine components in male rats (n = 2) during the first 6 hours after nitisinone administration were analyzed using TLC. The results showed that the component identified by TLC as having the same mobility as the reference standard of ^{14}C-labeled nitisinone accounted for 1.7% to 2.3% of the urine radioactivity.[39] Most of the urine radioactivity was accounted for by 2 polar metabolites (51.9%-55.3% and 31.1%-31.3% of the urine radioactivity), which were considered to be the metabolites formed by hydroxylation at positions 4 and 5 of the cyclohexanedione

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35 Radioactivity was not measured in the intraorbital lacrimal gland 72 hours after administration or thereafter.

36 One of the following 2 types of ^{14}C-nitisinone was administered: one type was phenyl-labeled ^{14}C in the benzene ring; the other type was cyclohexanedione-labeled ^{14}C-nitisinone.

37 The radioactivity in the feces was not measured because samples from 2 of 4 rats were not obtained 6 hours after administration, and only small amount of the samples were available from the other 2 rats.

38 Other organs including the small intestine, large intestine, heart, lungs, spleen, and mesentery.

39 The radioactivity in each band relative to the total radioactivity in the sample.

ring, respectively. A metabolite was detected in the urine of rats that received phenyl-labeled ^{14}C-nitisinone; however, this metabolite was not detected in the urine of rats that received cyclohexanedione-labeled ^{14}C-nitisinone, and this was considered to be 2-nitro-4-trifluoromethylbenzoic acid.

A single oral dose of 100 mg/kg of ^{14}C-nitisinone[34] was administered to 1 female rat to investigate the components of the cornea and the other tissues of eye at 6 hours after administration using TLC. The results showed that the component identified by TLC as having the same mobility as the reference standard of ^{14}C-labeled nitisinone accounted for 85.0% and 50.1% of the total concentration of radioactivity in the respective tissues mentioned above. At least 8 additional metabolites were identified as components corresponding to ^{14}C-nitisinone, and these accounted for 15.0% and 49.9% of the total concentration of radioactivity in the corneal and non-corneal tissues, respectively.

3.(ii).A.(4) Excretion (4.3-7; published literature[40])

A single oral dose of 10 mg/kg of ^{14}C-nitisinone[34] was administered to rats, and 45% each of the administered radioactivity was excreted in urine and feces during the first 4 days after administration.

3.(ii).B Outline of the review by PMDA

PMDA asked the applicant to explain the effects of nitisinone *in vivo* by comparing and discussing the following data: the data obtained from studies on the distribution and metabolic profiles of nitisinone; and the safety data obtained from toxicology and clinical studies of nitisinone.

The applicant responded as follows:

A single oral dose of 0.3 μmol/kg (0.1 mg/kg) of ^{14}C-nitisinone[34] was administered to rats, and a single oral dose of 30 μmol/kg (10 mg/kg) of ^{14}C-nitisinone[34] was administered to mice and rats in a tissue distribution study. The results showed that the liver, kidneys, and Harderian glands all had tissue-to-plasma radioactivity ratios of greater than 1, while, in the cornea and other eye tissues, no radioactive retention was found. In a study to analyze metabolic profiles in rats, more than 5% of administered radioactivity was recovered in the following tissues 6 hours after a single oral dose of 100 mg/kg of ^{14}C-nitisinone[36]: the liver, skin, blood, and internal organs including small intestine, large intestine, heart, lungs, spleen, and mesentery. Toxicity studies showed various compensatory and secondary changes in the liver presumably due to drug-metabolizing enzymes induced by nitisinone, and hematological findings of decreases in platelet, white blood cell, and red blood cell counts [see "3.(iii).A.(2) Repeat-dose toxicity"]. In the NTBC clinical study in patients with HT-1, the most commonly reported adverse event by system organ class (SOC) was eye disorders (a total of 69 events in 29 of 291 subjects: 12 events of keratitis in 10 of 291 subjects; 17 events of corneal opacity in 7 of 291 subjects; 8 events of conjunctivitis in 6 of 291 subjects; 8 events of photophobia in 6 of 291 subjects; 18 events of eye pain in 4 of 291 subjects; 3 events of cataract in 3 of 291 subjects; 2 events

40 Lock EA, et al., *J Inher Metab Dis*. 1998;21:498-506

of blepharitis in 2 of 291 subjects; and 1 event of retinal disorder in 1 of 291 subjects). For most of the events, a causal relationship to nitisinone could not be ruled out, and all cases were non-serious except for 1 case (retinal disorder), in which a causal relationship to nitisinone was ruled out [see "4.(iii).B.(2).2).(a) Eye disorders"]. A review of 250 patients included in the complementary analysis of the NTBC study showed that development of eye symptoms was caused by an increase in plasma tyrosine concentration. It has been known that increased tyrosine concentration caused by the mechanism of action of nitisinone is involved in the formation of tyrosine crystals.[41] Therefore, in order to lower the risk of eye symptoms caused by an elevation in tyrosine concentrations, adherence to dietary restriction to prevent a significant elevation in tyrosine concentrations is important. Patients who exhibit vision problems during treatment with nitisinone should consult an ophthalmologist, and patients with a plasma tyrosine concentration above 500 μmol/L should have a diet in which tyrosine and phenylalanine intake is more strictly restricted. Other common adverse events reported in the NTBC study include the following hepatic adverse events: hepatic neoplasm malignant (15 events in 15 of 291 subjects), hepatic neoplasm (12 events in 12 of 291 subjects), hepatic failure (23 events in 23 of 291 subjects), and liver transplant (15 events in 15 of 291 subjects). Patients with HT-1 are at high risk of developing severe hepatic failure, impaired coagulation, painful neurological crisis, renal tubular disorder, and hepatocellular carcinoma. Given the wide range of symptoms in HT-1 patients, it is difficult to evaluate hepatic disorder and other adverse events during treatment with nitisinone separately from the progression of the underlying disease.

PMDA accepted the above response, and will further discuss the safety of nitisinone in humans in the clinical section [see "4.(iii).B.(2).2) Safety" for clinical safety].

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

The submitted evaluation data of nitisinone toxicity include a repeated dose study (1 study), genotoxicity studies (6 studies), and reproductive and developmental toxicity studies (4 studies). Many of the submitted results of toxicity studies, including single dose toxicity studies, repeated dose toxicity study, genotoxicity studies, and reproductive and developmental toxicity studies, were conducted at the time nitisinone was being developed as an herbicide. In addition, the submitted reports have been newly produced based on raw data and simple reports prepared by the study director; therefore, these studies were used as reference data in the evaluation by PMDA. The results from the main studies are described below.

3.(iii).A.(1) Single-dose toxicity (4.2.3.1-1, 4.2.3.1-2; reference data)

The median lethal dose (LD_{50}) levels for mice that received a single oral dose of nitisinone were determined to be 637 mg/kg for males and 796 mg/kg for females.

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[41] Lock EA, et al., *J Inher Metab Dis*. 1998;21:498-506, Robinson M, *Ocular Toxicology.* ed. by Weisse I, et al., Plenum Press, New York, 1995;327-34.

No deaths were observed when a single oral dose (100 mg/kg) of nitisinone was administered to male and female rats (n = 10/sex), while 8 of 10 rats died when a single oral dose (1000 mg/kg) of nitisinone was administered to female rats (n = 10; the dose of 1000 mg/kg was administered to female rats only).

3.(iii).A.(2) Repeat-dose toxicity

3.(iii).A.(2).1) Four-week repeated oral dose toxicity study in mice (4.2.3.2-1)

Oral doses of 0 (vehicle[42]), 3, 30, 100, or 300 mg/kg/day of nitisinone were administered to male and female C57BL/6 mice once daily for 4 weeks. Because of the rapid deterioration in the clinical condition (e.g., emaciation, irregular breathing, ataxia, and cold to touch) of mice in the 300 mg/kg/day group, on Day 2, 1 of 6 male mice, and on Day 6, 1 of 6 male mice and 1 of 6 female mice were sacrificed moribund. For this reason, the 200 mg/kg/day group was introduced, and the doses were administered from Day 8 for 22 days.

Although hepatocyte hypertrophy and increased liver weight were observed in the ≥30 mg/kg/day groups and in the ≥100 mg/kg/day groups, respectively, these findings were not accompanied by changes in biochemical parameters indicating degeneration, necrosis, and inflammation of hepatocytes, or hepatic cell lesion; therefore, they were considered to be of little toxicological significance.

Based on the above results, the no-observed-adverse-effect level (NOAEL) was determined to be 200 mg/kg/day.

3.(iii).A.(2).2) Six-week dietary administration toxicity study in mice (4.2.3.2-2; reference data)

Male and female ICR mice were fed a diet containing nitisinone at concentration levels of 0, 300, 1000, 3000, 7000, or 10,000 ppm[43] for 6 weeks. Because 1 of 6 female mice in the 10,000 ppm group exhibited decreased activity and pallor, it was sacrificed moribund.

The following findings were noted: elevated 5'-nucleotidase (5'-NT) and sorbitol dehydrogenase (SDH) in the ≥300 ppm groups; increased liver weight and liver discoloration in the ≥1000 ppm groups; suppression of weight gain, centrilobular or midlobular hepatocyte hypertrophy, Wallerian degeneration in the sciatic nerve in the ≥3000 ppm groups; and hindlimb weakness, tremor, weight decreased, and decreased white blood cells counts and platelets counts in the ≥7000 ppm groups.

Based on the above results, the NOAEL was determined to be <300 mg/kg/day.

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42 Phosphate buffer solution (0.1 mol/L; pH 7.1 ± 0.1) containing 1% carboxymethyl cellulose.

43 Based on the food consumption, the concentration levels are equivalent to 0, 43, 136, 507, 1199, and 2037 mg/kg/day for male mice; and 0, 36, 196, 673, 1722, and 1692 mg/kg/day for female mice.

3.(iii).A.(2).3) Twenty-eight-week dietary administration toxicity studies in mice (4.2.3.2-2, 4.2.3.2-3; reference data)

Male and female ICR mice were fed a diet containing nitisinone at concentration levels of 0, 10, 350, 1500, or 3500 ppm[44] for 28 weeks.

The following findings were noted: increased liver weight, centrilobular hepatocyte hypertrophy, and karyomegaly in the ≥350 ppm groups; suppression of weight gain, and demyelination in the sciatic nerve in the ≥1500 ppm groups; decreased feed intake, elevated alanine transaminase (ALT), hyperplasia of the hepatic portal areas, and circumscribed hepatic necrosis in the 3500 ppm group.

Based on the above results, the NOAEL was determined to be 10 ppm.

3.(iii).A.(2).4) Six-week dietary administration toxicity study in rats (4.2.3.2-4; reference data)

Male and female SD rats were fed a diet containing nitisinone at concentration levels of 0, 500, 2000, or 4000 ppm[45] for 6 weeks.

The following findings were noted: suppression of weight gain, unkempt fur, abnormalities in the cornea (dystrophy, granular degeneration, and neovascularization), elevated inorganic phosphorus levels and cholesterol in the ≥500 ppm groups; hyperactivity, soiled fur, decreased platelets, and elevated potassium levels in the ≥2000 ppm groups; weight decreased, decreased white blood cell counts, elevated SDH, and Wallerian degeneration in the sciatic nerve in the 4000 ppm group.

Based on the above results, the NOAEL was determined to be <500 ppm.

3.(iii).A.(2).5) Thirteen-week and 1-year dietary administration toxicity studies in rats (4.2.3.2-4, 4.2.3.2-5; reference data)

Male and female SD rats were fed a diet containing nitisinone at concentration levels of 0, 1, 5, 40, 120, 320, or 800 ppm[46] for 1 year. At Week 13, 10 rats per sex per group were necropsied. At Week 25, all the rats in the 5 ppm and 120 ppm groups were removed from the study.[47] After 1 year of administration, 10 rats per sex per group (0, 1, 40, 320, or 800 ppm groups) were necropsied. Among the animals that were not necropsied after 1 year of administration, 45 male rats in which ocular lesions were observed in the ophthalmological examination were re-classified into groups based on the severity of ophthalmological findings. Normal feed were given to these rats for 13 weeks to

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44 Based on the food consumption, the concentration levels are equivalent to 0, 1.3, 45, 205, and 496 mg/kg/day for male mice; and 0, 1.7, 56, 257, and 561 mg/kg/day for female mice.

45 Details of food consumption are unknown.

46 Based on the food consumption, the concentration levels are equivalent to 0, 0.05, 0.31, 2.4, 8.0, 18, and 45 mg/kg/day for male rats; and 0, 0.06, 0.31, 2.3, 7.6, 18, and 45 mg/kg/day for female rats.

47 It was specified in the study design that the doses for continued administration would be determined based on the toxicity findings after 13-week administration; therefore, the number of groups assigned was more than normally required.

investigate the reversibility of ocular lesions. Five male rats in the 320 ppm group, which served as controls, were continuously fed a diet containing nitisinone for the subsequent 13 weeks.

During administration, 2 male rats in the 320 ppm group and 1 male and 2 female rats in the 800 ppm group died.

The following findings were noted: increases in liver and kidney weights, corneal opacity, keratitis (e.g., inflammatory cell infiltration, corneal epithelial hyperplasia, and neovascularization) in the ≥1 ppm groups; decreased red blood cell counts in the ≥40 ppm groups; suppression of weight gain and decreased blood glucose levels in the ≥320 ppm groups; centrilobular hepatocyte hypertrophy in the 800 ppm.

At the end of the 13-week recovery period, corneal opacity improved, although transparency was not fully restored.

Based on the above results, the NOAEL was determined to be <1 ppm.

3.(iii).A.(2).6) Four-week repeated oral dose toxicity study in dogs (4.2.3.2-6; reference data)

Oral doses of 0 (vehicle), 1, 5, 10, 50, 100, or 150 mg/kg/day of nitisinone[48] were administered to male and female beagle dogs once daily for 4 weeks. In the 10 mg/kg/day group, 3 of 4 dogs died or were sacrificed moribund on Days 10 or 21, and nitisinone was discontinued in the remaining 1 of 4 dogs on Day 21. In the 50 mg/kg/day group, 3 of 4 dogs died or were sacrificed moribund on Days 6, 8, or 9, and nitisinone was discontinued in the remaining 1 of 4 dogs on Day 9. In the 100 mg/kg/day group, 3 of 4 dogs died or were sacrificed moribund on Days 2 or 3, and nitisinone was discontinued in the remaining 1 of 4 dogs on Day 3. In the 150 mg/kg/day group, 2 of 4 dogs were sacrificed moribund on Day 2, and nitisinone was discontinued to the remaining 2 of 4 dogs.

The following findings were noted: keratitis (corneal epithelial hyperplasia, corneal epithelial disorganization) in the 1 mg/kg/day group; ataxia, emaciation, dehydration, and elevated SDH in the 5 mg/kg/day group; vomiting, diarrhoea, weight decreased, tremor, hindlimb weakness, gait disturbance, postural and spinal reflex inhibition, lenticular opacities, and red lesions in the stomach, colon, and rectum in the 10 mg/kg/day group; convulsions, delayed muscle activity, increase in rectal temperature, decreased pupillary response, hindlimb ataxia, gait ataxia, wide-based stance in the hindlimbs, and elevated ALT and AST in the 50 mg/kg/day group; and pale colored lesions in the liver in the 100 mg/kg/day group.

Based on the above results, the NOAEL was determined to be <1 mg/kg/day.

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[48] Nitisinone was administered in gelatin capsules. Empty capsules were given to the control group.

3.(iii).A.(2).7) Twenty-two-week repeated oral dose toxicity study in dogs (4.2.3.2-6; reference data)

This study was originally intended to investigate the toxicity in male and female beagle dogs that received oral doses of 0 (vehicle), 0.1, 0.5, 1.5, or 5 mg/kg/day of nitisinone[48] once daily for 3 months. However, the objective for the study was changed to the investigation of corneal lesions because corneal opacity was observed in the lowest dose group at and after Week 7. At Week 19, 21 dogs that had not exhibited corneal lesions were sacrificed and the tyrosine concentrations in their plasma and ocular fluid were measured, and the remaining 19 dogs were assigned to the groups shown in Table 4.

Table 4. Study group structure under new study objective

Group under new study objective	Number of animals	Group at the start of the study	Ocular lesion	Treatment
Control group	6	3 males and 3 females in the control group	Absent	Sacrificed on Week 33
22-week administration group	5	1 male in the 0.1 mg/kg/day group 2 males in the 0.5 mg/kg/day group 1 male in the 1.5 mg/kg/day group 1 male in the 5.0 mg/kg/day group	Absent	After 22 weeks of nitisinone administration at the initial dose levels, tyrosine concentration in the ocular fluid was measured.
Continued administration group	4	1 female in the 0.1 mg/kg/day group 1 male in the 0.5 mg/kg/day group 1 male and 1 female in the 1.5 mg/kg/day group	Present	After 32 weeks of nitisinone administration at the initial dose levels, pathologic eye examination was performed.
Reversibility study group	4	1 male and 1 female in the 0.1 mg/kg/day group 1 female in the 0.5 mg/kg/day group 1 male in the 1.5 mg/kg/day group	Present	After 22 weeks of nitisinone administration at the initial dose levels and subsequent 9-week washout period, pathologic eye examination was performed

Corneal opacity was observed in the 0.1, 0.5, and 1.5 mg/kg/day groups by Week 7, but not in the 5 mg/kg/day group to which administration continued up to Week 22. Eye examination by scanning electron microscopy (SEM) revealed the following findings in the corneal epithelium in the continued administration group: loss of stratification, intracellular vacuoles, increased cell proliferation, necrosis, inflammatory cell infiltration, keratohyalin granules, uneven cellular electron density, and epithelial basement membrane changes. In contrast, these conditions tended to improve in the reversibility study group. The tyrosine concentrations in the plasma and ocular fluid were approximately 1800 nmol/mL and 1400 nmol/mL, respectively, on Day 127 or 129 [see "3.(iii).A.(7).6) Study on development of ocular lesions and tyrosinemia in dogs, rabbits, and rhesus monkeys"].

3.(iii).A.(2).8) Two- and 13-week repeated oral dose toxicity study in rhesus monkeys (4.2.3.2-7; reference data)

During the dose-finding study period, oral doses of 0 (vehicle[49]), 0.1, 1, or 10 mg/kg/day of nitisinone were administered to male rhesus monkeys 5 days a week for 2 weeks. In the main study period following a 2-week washout period, animals that were given vehicle in the dose-finding study period received 0 mg/kg/day (vehicle) of nitisinone, and animals that were given nitisinone received 10 mg/kg/day of nitisinone, 5 days a week for 13 weeks, followed by a 4-week washout period.

[49] Phosphate buffer solution (pH 7.4)

No toxicity findings were observed in clinical signs, weight, food consumption, or ophthalmological examination during the study, hematology or blood chemistry test results at the ends of the dose-finding or main study periods, or fecal examination results at the end of the main study period.

Based on the above results, the NOAEL was determined to be 10 mg/kg/day.

3.(iii).A.(3) Genotoxicity

3.(iii).A.(3).1) Bacterial reverse mutation assay (4.2.3.3.1-1 to 4.2.3.3.1-3) (4.2.3.3.1-4; reference data)

The results of bacterial reverse mutation assays showed that an unpurified batch of, or technical grade[50] nitisinone was mutagenic; whereas a purified batch of, or clinical grade[50] nitisinone was not mutagenic.

3.(iii).A.(3).2) Gene mutation assay in mammalian cells (4.2.3.3.1-5) (4.2.3.3.1-6; reference data)

The results of gene mutation assays using mouse lymphoma cells showed that technical grade nitisinone and clinical grade nitisinone[50] were mutagenic.

3.(iii).A.(3).3) Chromosome aberration assay in mammalian cells (4.2.3.3.1-7; reference data)

The results of chromosome aberration assays using mouse lymphoma cells showed increases in chromosome aberration and sister chromatid exchange in the presence of a metabolic activation system, and thus technical grade[50] nitisinone was clastogenic.

3.(iii).A.(3).4) Micronucleus assay in mice (4.2.3.3.2-1) (4.2.3.3.2-2; reference data)

In micronucleus assays of clinical grade[50] nitisinone, a single oral dose of 0 (vehicle[51]), 125, 250, or 500 mg/kg of nitisinone was administered to male ICR mice; however, deaths occurred in the 500 mg/kg group, which made it difficult to perform an evaluation. Accordingly, the 62.5 mg/kg group was introduced (initial study). An increase in the incidence of micronucleated polychromatic erythrocytes observed in the nitisinone administration group was assessed not to be a change of biological significance, based on the following reasons: the incidence of micronucleated polychromatic erythrocytes was not dose-dependent; and although a significant decrease in the rate of polychromatic erythrocytes was observed 48 hours after administration in the 250 mg/kg group, the incidence of micronuclei did not increase.

Next, an additional study was conducted in which a single oral dose of 0, 5, 25, 62.5, or 250 mg/kg of nitisinone was administered to male ICR mice. An increase in the incidence of micronuclei formation was observed in the lowest dose group (5 mg/kg) to an extent that still remained within the laboratory historical values.

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[50] "Technical grade nitisinone" refers to a test substance containing 90% to 92% nitisinone, in which the content of impurities, Related Substance 2 and Related Substance 3, was not determined; "clinical grade" refers to a test substance that was manufactured by the same chemical route and processes as the technical grade, but contained 98% or more nitisinone, less than [redacted]% of Related Substance 2, and less than [redacted]% of Related Substance 3. "Purified batch" refers to a test substance batch containing 99.3% nitisinone, and the total content of impurities, Related Substances 2 and 3, is less than [redacted]%; and "unpurified batch" refers to a test substance batch containing 92.8% nitisinone, and content of impurities, Related Substances 2 and 3, was not determined.

[51] One percent aqueous solution of carboxymethyl cellulose

The results of a study in which a single oral dose of 0 (vehicle[52]), 125, 250, or 500 mg/kg of technical grade[50] nitisinone was administered to male and female ICR mice showed no increase in the incidence of micronucleated polychromatic erythrocytes.

Given the above results that the increased incidence of micronuclei in the initial study with clinical grade nitisinone was of little biological significance, and not reproduced in the additional study, and the incidence of micronuclei did not increase in the study with technical grade nitisinone, it was assessed that nitisinone does not induce micronuclei.

3.(iii).A.(3).5) Other genotoxicity

(a) Morphological transformation assay in mammalian cells (4.2.3.3.1-8, 4.2.3.3.1-9; reference data)

The results of the study of morphological transformation of mouse BALB/3T3 cells showed that morphological transformation was not induced regardless of the presence or absence of a metabolic activity system. Based on the results, it was assessed that technical grade[50] nitisinone did not induce morphological transformation.

(b) DNA damage and repair assay in mammalian cells (4.2.3.3.1-10; reference data)

The results of the study of DNA damage and repair using human fibroblasts showed no induction of strand breaks or repair in DNA of fibroblasts. Based on the results, it was assessed that technical grade[50] nitisinone did not have the ability to damage DNA.

(c) Unscheduled DNA synthesis assay in mice (4.2.3.3.2-3)

A single oral dose of 0 (vehicle[51]), 62.5, or 250 mg/kg of nitisinone was administered to male ICR mice in the unscheduled DNA synthesis assay. The results showed no increase in the number of cells with repaired DNA in hepatocytes, and it was assessed that clinical grade[50] nitisinone did not have the ability to damage DNA.

3.(iii).A.(4) Carcinogenicity

No carcinogenicity studies have been performed with nitisinone.

3.(iii).A.(5) Reproductive and developmental toxicity

3.(iii).A.(5).1) Study on fertility and early embryonic development to implantation in mice (4.2.3.5.1-1)

Oral doses of 0 (vehicle[53]), 5, 50, or 250 mg/kg/day of nitisinone were administered to male and female ICR mice. Male mice were treated for 4 weeks prior to mating, during mating, and until the day before necropsy; and female mice were treated for 2 weeks prior to mating, during mating, and until Gestation Day 7, and underwent cesarean section on Gestation Day 14.

A prolonged period prior to mating was observed in the ≥50 mg/kg/day groups, and suppression of weight gain and a decrease in food consumption (only in male mice), and increased post-implantation loss were observed in the 250 mg/kg/day group.

Next, male mice in the 50 or 250 mg/kg/day groups were mated with non-treated female mice to investigate whether the prolonged period prior to mating was attributable to administration of nitisinone to male mice or to female mice. The results showed no effects on the length of period prior to mating or post-implantation loss, and therefore, it was considered that these were attributable to nitisinone administration to female mice.

Based on the above results, the NOAEL for general toxicity was determined to be 50 mg/kg/day for males and 250 mg/kg/day for females, and the NOAEL for reproductive toxicity was determined to be 250 mg/kg/day for males and 5 mg/kg/day for females.

3.(iii).A.(5).2) Study on fertility and early embryonic development to implantation in rats (4.2.3.5.1-2; reference data)

Oral doses of 0 (vehicle[52]) or 100 mg/kg/day of nitisinone were administered to male and female SD rats. Male rats were treated for 60 days prior to mating and during mating, and female mice were treated for 14 days prior to mating, during mating, and until 21 days postpartum. Although a decrease in the number of pups born, decreased 4-day survival rate, and decreased body weight at birth were observed, the fertility rate was the same as the control group.

3.(iii).A.(5).3) Study on embryo-fetal development in mice (4.2.3.5.2-3) (4.2.3.5.2-2; reference data)

In the dose-finding study, oral doses of 0 (vehicle[53]), 50, 100, or 200 mg/kg/day of nitisinone were administered to pregnant ICR mice from Gestation Days 7 to 16, and no abnormalities were observed in dams, embryos, or fetuses.

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52 Corn oil

53 Phosphate buffer solution (pH 6.8) containing 1% aqueous solution of carboxymethyl cellulose

In the main study, oral doses of 0 (vehicle[53]), 5, 50, or 250 mg/kg/day of nitisinone were administered to pregnant ICR mice from Gestation Days 7 to 16, and cesarean sections were performed on Gestation Day 19.

Delayed ossification was observed in fetuses in the 5 mg/kg/day group, and decreased food consumption in dams in the 250 mg/kg/day group were observed. An increase in early post-implantation embryonic deaths was observed in the ≥5 mg/kg/day groups, but not in a dose-dependent manner, and within laboratory historical values. Therefore, the finding was considered to be an accidental change.

Based on the above results, the NOAEL was determined to be 50 mg/kg/day for dams, and <5 mg/kg/day for embryo-fetal development.

3.(iii).A.(5).4) Study on embryo-fetal development in rats (4.2.3.5.2-4, 4.2.3.5.2-5; reference data)

In the preliminary study, oral doses of 0 (vehicle[52]) or 200 mg/kg/day of nitisinone were administered to pregnant SD rats from Gestation Days 8 to 15. On Gestation Day 21, 1 of 10 animals was sacrificed moribund, and 4 of 10 animals had total resorption of embryos, and only the remaining 5 of 10 animals gave birth. The following findings were noted: decreased activity, unkempt fur, colored nasal discharge, convulsions, tremor, suppression of weight gain, and decreased food consumption in the dams; and decreased number of live births, decreased pup birth weight, and skeletal variations (extra ribs, incomplete ossification of the fifth sternebra) in the pups.

In the dose-finding study, oral doses of 0 (vehicle[52]), 20, 50, or 125 mg/kg/day of nitisinone were administered to pregnant SD rats from Gestation Days 8 to 20. Effects on dams included decreased food consumption in the ≥20 mg/kg/day groups; suppression of weight gain in the ≥50 mg/kg/day groups; and 1 death (Gestation Day 19), colored nasal discharge, weight decreased, and increased kidney weight in the 125 mg/kg/day group. Effects on pups included decreased 4-day pup survival in the ≥50 mg/kg/day groups; and decreased birth weight, suppression of weight gain, and decreased live births in the 125 mg/kg/day group.

3.(iii).A.(5).5) Study on embryo-fetal development in rabbits (4.2.3.5.2-7) (4.2.3.5.2-6; reference data)

In the dose-finding study, oral doses of 0 (vehicle[53]), 5, 12, 25, or 50 mg/kg/day of nitisinone were administered to pregnant New Zealand White (NZW) rabbits from Gestation Days 7 to 19, and cesarean sections were performed on Gestation Day 30. Decreased weight, and decreased food consumption were observed in dams, and 2 of 8 animals died in the 50 mg/kg/day group. Increased early embryonic deaths and decreased fetal weight were also observed in this group.

Oral doses of 0 (vehicle[53]), 5, 12, or 25 mg/kg/day of nitisinone were administered to pregnant NZW

rabbits from Gestation Days 7 to 19, and cesarean sections were performed on Gestation Day 30. One of 20 animals in the 25 mg/kg/day group exhibited anorexia, no feces, and weight decreased, therefore, it was sacrificed moribund.

The following findings were noted: decreased fetal weight, and increased incidence of fetal skeletal abnormalities in the ≥5 mg/kg/day groups; inhibition of maternal weight increase, decreased maternal weight, decreased food consumption, and decreased feces in the ≥12 mg/kg/day groups; increased incidences of fetal umbilical hernia and gastroschisis in the 25 mg/kg/day group.

Based on the above results, the NOAEL was determined to be 5 mg/kg/day for general toxicity in dams, and <5 mg/kg/day for embryo-fetal development.

3.(iii).A.(5).6) Study on pre- and postnatal development, including maternal function in mice (4.2.3.5.3-1)

Oral doses of 0 (vehicle[53]), 5, 50, or 250 mg/kg/day of nitisinone were administered to pregnant ICR mice from Gestation Day 7 to Lactation Day 21.

The following findings were noted: prolonged pregnant period in dams, suppression of weight gain, prolonged swimming time in the Y-maze test in the ≥50 mg/kg/day groups; decreased weight and decreased food consumption in postpartum dams, decreased survival rate, decreased olfactory discrimination ability in pups in the 250 mg/kg/day group. No evidence of toxicity were found in the examinations of the sensory function and reflex functions of pups (surface righting reflex, auditory startle reaction, functional observation battery, and tail flick test), behavioral tests (locomotive activity), or reproductivity (fertility).

Based on the above results, the NOAEL was determined to be 5 mg/kg/day for both dams and pups.

3.(iii).A.(5).7) Study on pre- and postnatal development, including maternal function in rats (4.2.3.5.3-2; reference data)

Oral doses of 0 (vehicle[52]) or 100 mg/kg/day of nitisinone were administered to pregnant SD rats from Gestation Day 8 to Lactation Day 20. Twenty-nine of the animals in each of the control and nitisinone groups gave birth. Fifteen dams per group fostered natural pups (Groups I and IV in Table 5), and 14 dams per group cross-fostered pups between the control and nitisinone groups within 24 hours after birth (Groups II and III in Table 5). One week after weaning, pups that were fostered by dams of the control group (Group I and III in Table 5) were fed a diet containing 0 or 80 ppm of nitisinone to investigate the effects of exposure to nitisinone at a young age.

Table 5. Exposure to nitisinone in the embryonic, lactation, and postweaning periods in Group I to IV

	Administration to dams		Administration to pups
Group	Embryonic period	Lactation period	Postweaning period
I	Control	Control	Control feed or feed containing nitisinone
II	Control	Nitisinone 100 mg/kg/day	
III	Nitisinone 100 mg/kg/day	Control	Control feed or feed containing nitisinone
IV	Nitisinone 100 mg/kg/day	Nitisinone 100 mg/kg/day	

The following effects on dams were noted: corneal opacity, suppression of weight gain, and decreased food consumption during pregnancy (Groups III and IV); and suppression of weight gain (Groups II and IV) and decreased food consumption (Groups II, III, and IV) during the lactation period.

The following effects on pups were noted: decreased birth weight, decreased 4-day survival rate, and delayed eyelid opening (Groups III and IV), and corneal opacity (Groups II and IV) during the lactation period. Pups in Groups II and IV weighed less than those in Groups I and III. The ophthalmological examination after weaning revealed the following findings: occurrence of lenticular opacities in animals in Group III regardless of whether or not they were fed a diet containing nitisinone; and corneal opacity in animals in Groups I and III that were fed a diet containing nitisinone.

3.(iii).A.(6) Local tolerance

No local tolerance studies have been performed for nitisinone.

3.(iii).A.(7) Other toxicity study

3.(iii).A.(7).1) Study on morphological changes in ocular lesions in rats and potential for reversibility (4.2.3.7.7-1; reference data)

Oral doses of 0 (vehicle[52]), 2, 10, or 40 mg/kg/day of nitisinone were administered to male Wistar rats for 14 weeks, and at Week 14, an ophthalmological examination was performed on rats in the 0, 10, and 40 mg/kg/day groups. In the 2 mg/kg/day group, rats with ocular lesions were divided into the continued administration group and the reversibility study group, and underwent ophthalmological examinations until Week 21.

Corneal opacity and corneal neovascularization were noted in the nitisinone treatment group with the following histopathological changes: keratitis with polymorphonuclear leucocyte and eosinophil infiltration, epithelial hyperplasia, and stromal vascularization.

In the reversibility study group, corneal lesions tended to improve at Week 21.

3.(iii).A.(7).2) Study on morphological aspects of development of ocular lesions in rats (4.2.3.7.7-2; reference data)

Repeat oral doses of 10 mg/kg/day of nitisinone were administered to male Wistar rats, and rats with corneal lesions were sacrificed 1 to 15 days after onset, and histopathological examinations were performed.

Forty-eight of 70 animals exhibited corneal lesions (corneal opacity, neovascularization) by Day 95, and the histopathological examination indicated focal epithelial disorganization in the cornea, and polymorphonuclear leucocyte infiltration in the stroma 1 or 2 days after onset. These lesions spread extensively over time, and anterior uveitis and inflammation in the Descemet's membrane were also observed.

It has been reported that administration of nitisinone increased plasma tyrosine concentrations, and corneal lesions were caused by feeding of a low protein diet with 5% L-tyrosine [see "3.(iii).A.(7).3) Study on effects of L-tyrosine-supplemented low protein diet in rats"]. To rats that had not exhibited corneal lesions even with nitisinone treatment, further nitisinone was continuously administered while the low protein diet was given. As a result, corneal lesions were observed in 2 of 21 rats, with no significant change in incidence.

3.(iii).A.(7).3) Study on effects of L-tyrosine-supplemented low protein diet in rats (4.2.3.7.7-3; reference data)

A low protein diet (control group) or a 5% L-tyrosine-supplemented low protein diet (study group) was given to male Wistar rats for 7 days. Rats in the study group showed markedly elevated plasma tyrosine levels (2786 nmol/mL)[54] compared to the control group (182 nmol/mL). In the study group, the following findings were observed: corneal opacity on Day 1; adhesion of iris on Day 3; and scab formation and eyelids covered with materials caused by lacrimation with excess mucus production by Day 7. Histopathological findings include keratitis and anterior uveitis. These ocular findings were similar to the findings induced by administration of nitisinone.

3.(iii).A.(7).4) Study on development of ocular lesions and administration routes in rats (4.2.3.7.7-4; reference data)

A study was conducted in male SD rats by administering nitisinone via different routes: intravenous (1 mg/kg/day), intraperitoneal (1 mg/kg/day), subcutaneous (1 mg/kg/day), oral (1 mg/kg/day), or eye drops (1 mg/mL, 20 μL/eye, twice daily). Also weekly ophthalmological examinations were performed. A group given a diet containing nitisinone (20 ppm) and control diet group (0 ppm) were also included as positive and negative controls, respectively.

Administration was discontinued after 2 months when 5 of 20 rats in the subcutaneous administration group developed corneal lesions with neovascularization, and the lesions were evaluated. The incidences of corneal opacity for other administration routes were as follows: 1 of 20 rats in the intravenous administration group; 2 of 20 rats in the intraperitoneal group and the group administered a diet containing nitisinone; 3 of 20 rats in the oral administration group; and no lesions in the eye-

[54] The mean plasma tyrosine concentrations at Day 7 after starting feeding (n = 10/group). The data for the study group include the data for the animals sacrificed moribund on Days 2 and 4 (n = 2/day).

drop and control feed groups.

3.(iii).A.(7).5) Three-month oral administration ocular toxicity study in rabbits (4.2.3.7.7-5; reference data)

Oral doses of 50 or 250 mg/kg/day of nitisinone were administered to male NZW rabbits, and ophthalmological examinations were performed at Months 1, 2, and 3.

Three of 4 rabbits died in the 250 mg/kg/day group (1 rabbit each on Days 5, 6, and 7), and 1 of 4 rabbits were sacrificed moribund (Day 5). None of the animals showed signs of ocular toxicity.

Three of 4 rabbits died during the administration period in the 50 mg/kg/day group (1 rabbit each on Days 14, 24, and 43), and administration continued to the remaining 1 rabbit until Day 102. No corneal opacity was observed, nor were any abnormalities found in the ophthalmological examinations.

3.(iii).A.(7).6) Study on development of ocular lesions and tyrosinemia in dogs, rabbits and rhesus monkeys (4.2.3.7.7-6; reference data)

Repeat oral doses of 0 (vehicle[48]), 0.1, 0.5, 1.5, or 5 mg/kg/day of nitisinone were administered to male and female beagle dogs for 22 weeks. The results showed that on Day 127 or on Day 129, tyrosine concentrations in plasma and ocular fluid reached approximately 1800 nmol/mL, and approximately 1400 nmol/mL, respectively, in the nitisinone groups, regardless of different dose levels. In this study, corneal lesions were observed in the 0.1, 0.5, and 1.5 mg/kg/day groups. In a study in which repeat oral doses of 0 or 10 mg/kg/day of nitisinone were administered to male NZW rabbits for 6 weeks, the plasma tyrosine levels were maintained at approximately 1500 nmol/mL from Week 1 to Week 6, with no evidence of corneal lesions. In another study with male rhesus monkeys to which repeat oral doses of 0 or 10 mg/kg/day of nitisinone were administered 5 days a week for 13 weeks, the plasma tyrosine levels were elevated to 1550 to 1700 nmol/mL from Week 4 to Week 12; however, no corneal lesions were observed.

In summary, oral administration of nitisinone produced a marked increase in plasma tyrosine levels in dogs, rabbits, and rhesus monkeys; however, corneal lesions were observed only in dogs, and not in rabbits or rhesus monkeys.

3.(iii).B Outline of the review by PMDA

PMDA considered that, although most of the submitted repeat-dose toxicity studies consisted of reference data, these studies were conducted in compliance with the GLP regulations when nitisinone was being developed as an herbicide, and can therefore serve as the basis, albeit with limitations, for its toxicological evaluation.

PMDA also considers that the applicant's explanation of the carcinogenicity, ocular toxicity, and use in pregnant women of nitisinone from a toxicological standpoint is acceptable, based on the discussion in

the following sections (1) to (3). PMDA will discuss the ocular toxicity and use in pregnant women in the clinical sections because these issues should be decided in consideration of the risk-benefit balance including warning statements [see "4.(iii).B.(2).2).(a) Ocular lesions," and "4.(iii).B.(5) Special populations: women who are pregnant or may be pregnant, or are breastfeeding"].

3.(iii).B.(1) Genotoxicity and carcinogenicity

Given that technical grade nitisinone was assessed to be genotoxic by bacterial reverse mutation assays, gene mutation assays and chromosome aberration assays using mouse lymphoma cells, PMDA asked the applicant to explain the genotoxic and carcinogenic risk of nitisinone.

The applicant responded as follows:

Technical grade nitisinone showed positive response but clinical grade nitisinone showed negative response in reverse mutation assays. Therefore technical grade nitisinone may have contained mutagenic impurities. At this point, identification of the potential impurities has not been performed; therefore, it is not known whether the clinical grade nitisinone is completely free of the potential impurities. However, based on the results of 2 *in vivo* genotoxicity studies using mice (4.2.3.3.2-1, 4.2.3.3.2-2, 4.2.3.3.2-3), which showed no genotoxicity, PMDA considers that there is no particular concern regarding genotoxicity in clinical use of nitisinone.

Assuming that clinical grade nitisinone also contains mutagenic impurities, the potential genotoxic risk is considered as follows. The tolerance for impurity content in clinical grade nitisinone is specified such that each peak is not more than 0.1%. When a person weighing 60 kg is given the maximum clinical dose (2 mg/kg/day) of nitisinone, 120 μg/day would be the largest amount of impurities that could be consumed. Based on the acceptable limits of mutagenic impurities corresponding to a 10^{-5} lifetime risk of cancer, 1.5 μg/day, as specified in ICH M7 Guidelines, if consuming 120 μg/day of mutagenic impurities contained in nitisinone over a lifetime, the resultant risk of cancer can be estimated to be 10^{-3}. This value represents the result of an estimation which was calculated erring on the side of safety using a maximum mutagenic impurity content of 0.1%. However, even an impurity that is present at high levels and the structure of which has been identified, it will not exceed a content of 0.1%. Therefore, the actual risk of cancer should be less than 10^{-3}. It is known that in patients with HT-1, the target population for nitisinone, reactive metabolites derived from tyrosine accumulate locally due to FAH deficiency, and cause hepatocellular carcinoma [see "4.(iii).B.(2).2).(b) Incidence of hepatic failure and hepatocellular carcinoma, and clinical laboratory values"], and the risk is considered to be higher than 10^{-3}. Clinical studies have shown that the risk of developing cancer is lowered by administration of nitisinone compared to a regimen of dietary restriction alone. Patients who have started nitisinone treatment earlier have a lower risk of cancer, and the risk can be almost eliminated in patients who started treatment before 24 months of age [see "4.(iii).B.(2).2).(b) Incidence of hepatic failure and hepatocellular carcinoma, and clinical laboratory values"].

As described above, PMDA considered there is no particular concern regarding genotoxicity in clinical grade nitisinone. Even if mutagenic impurities were to be contained, the benefits of nitisinone for patients with HT-1 outweigh the potential cancer risks that may be associated with the ingestion of the impurities concerned.

3.(iii).B.(2) Ocular toxicity

The applicant explained the ocular toxicity of nitisinone as follows:

After oral administration of nitisinone, keratitis and other corneal lesions were observed in rats and dogs, but not in other animal species studied (mice, rabbits, rhesus monkeys). Because corneal lesions due to nitisinone administration resembled ocular lesions induced by a low-protein diet supplemented with L-tyrosine, the likely cause of the eye disorders is tyrosine crystallization in tear fluid resulting from an increase in plasma tyrosine concentration due to the pharmacological action of nitisinone.[41]

After rats were given Mesotrione, an agricultural chemical which inhibits HPPD activity, corneal lesions were observed, and it has been shown that the frequency of occurrence and severity of lesions are correlated with plasma tyrosine concentrations (4.3-8). When HPPD is completely inhibited *in vivo*, the rate limiting step for tyrosine catabolism is the conversion from tyrosine to HPPA catalyzed by TAT. The TAT activity and tyrosine *in vivo* pharmacokinetics in humans and mice are somewhat similar to each other; however, TAT activity in rats is significantly lower than that in humans and mice, therefore, suppression of elevated tyrosine levels is difficult in rats, causing toxicity (4.3-9). Because of species difference in corneal lesions, which is also attributable to species difference in tyrosine *in vivo* pharmacokinetics, it is considered that the corneal lesions observed in rats are not relevant to humans. In a study on pre- and postnatal development, including maternal function in rats, the ophthalmological examination after weaning revealed lenticular opacities in pups that were exposed to nitisinone during the prenatal period. Lenticular opacities are often caused by reduced solubility of the lens protein. One of the mechanisms that has been suggested that could explain the cause of lowered lens protein solubility is that crystallin, a lens protein, is denatured and rendered insoluble through bonding with quinoids, which are produced by a metabolic disorder of amino acids such as tyrosine and tryptophan.[55] It is possible that, in this study, normal tyrosine catabolism was disrupted by elevated fetal tyrosine levels induced by nitisinone which had transferred via the placenta of dams, leading to the formation of quinoids, which bonded with lens proteins to render it insoluble, and resulted in the development of lenticular opacities.

In the NTBC study, however, eye disorders including keratitis, corneal opacity, conjunctivitis, photophobia, and eye pain were reported; therefore, the occurrence of ocular toxicities in patients with high plasma tyrosine levels cannot be ruled out [see “4.(iii).B.(2).2) Eye disorders”]. Further,

[55] Ogino S, *Japan Medical Journal*. 1957;1732:13-22

lenticular opacities (cataracts) have not been reported in patients with hereditary tyrosinemia type II,[56] which is characterized by elevated blood tyrosine levels, higher than other types of tyrosinemia, with symptoms including skin lesions and corneal lesions (corneal erosion, ulcers) due to precipitation of needle-shaped tyrosine crystals. It cannot be ruled out that the use of nitisinone in a pregnant woman with HT-1 may lead to subsequent development of lenticular opacities in newborns, albeit rare. It is therefore considered that when nitisinone is used clinically, plasma tyrosine levels should be monitored to ensure they remain at a level associated with a low risk of the toxicity.

3.(iii).B.(3) Use in pregnant women

PMDA asked the applicant to explain the possibility of teratogenic effects caused by nitisinone in light of the findings obtained in the study on embryo-fetal development in rabbits, which showed increased incidences of fetal skeletal abnormalities, umbilical hernia, and gastroschisis.

The applicant responded as follows:

The following findings from the study on embryo-fetal development in rabbits were noted: a dose-dependent increase in the incidence of fetal skeletal abnormalities in the ≥5 mg/kg/day groups; increased incidence of external abnormalities (umbilical hernia, gastroschisis) in the 25 mg/kg/day group. The NOAEL was determined to be less than 5 mg/kg/day for embryo-fetal development. This study collected no toxicokinetic data, and therefore the safety margin in terms of exposure ratio is unknown. However, given that fetal skeletal abnormalities in rabbits occurred at 5 mg/kg/day (human equivalent dose on a body surface area basis, 1.6 mg/kg/day), which was 2.5-fold (0.8-fold on a human equivalent dose basis) higher than the maximum clinical dose (2 mg/kg/day), it is considered that there is no acceptable safety margin. Therefore, the possibility of teratogenic effects caused by nitisinone in humans cannot be ruled out. Given that there is insufficient data to determine the safety of nitisinone in pregnant women, the applicant will add a precautionary statement that nitisinone should be given to a pregnant woman only if clearly needed.

4. Clinical data

4.(i) Summary of biopharmaceutic studies and associated analytical methods

4.(i).A Summary of the submitted data

Two different immediate-release capsule formulations, the initial and final formulations, were used in the clinical development of Orfadin (hereinafter referred to as nitisinone).[57]

Blood concentrations of nitisinone were determined using the high performance liquid chromatography with ultraviolet detection (HPLC-UV) method and enzyme inhibition assay method[58]

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56 Endo F, *Bulletin on Special Formula*. 2005;41:11-3

57 [redacted]. Formulations manufactured in June 2001 or later are identical to the proposed commercial formulations (2, 5, and 10 mg capsules).

58 The blood nitisinone concentration was determined by enzyme inhibition assay, a method which was established based on the measurement of $^{14}CO_2$ released from the 4-hydroxy[1-^{14}C]phenylpyruvate by the enzyme activity in the

with the lower limits of quantification being 0.2 μg/mL and 5.1 μg/mL, respectively.

The submitted biopharmaceutic data (reference data) included the results of the comparison of blood nitisinone concentrations between the formulations in the Phase I single dose study (Study CCT/96/001) and the clinical study in patients with HT-1 (NTBC study).

4.(i).A.(1) Phase I single dose study (5.3.1.2-1, Study CCT/96/001 [from May 1996 to June 1996]; reference data)

A randomized, open-label, two-period crossover study was conducted in foreign healthy adult male subjects (target number of subjects, 10) to study the pharmacokinetics after a single dose of nitisinone (liquid or capsule formulation[59]).

Subjects received a single oral dose of 1 mg/kg of nitisinone, liquid or capsule formulation, in the fasted state, in each treatment period, with a ≥14-day washout period between the 2 study periods.

All 10 treated subjects were included in the pharmacokinetic and safety analysis sets.

The pharmacokinetic parameters after administration of nitisinone (liquid and capsule) were as follows: the plasma C_{max} (mean ± standard deviation) values were 7.8 ± 1.4 μg/mL and 7.7 ± 1.0 μg/mL, respectively; AUC values were 598 ± 142 μg·h/mL and 599 ± 153 μg·h/mL, respectively ; $t_{1/2}$ values were 53.6 ± 8.2 hours and 54.5 ± 13.0 hours, respectively; and t_{max} (median, [minimum, maximum]) were <0.25 (<0.25, 1.95) and 2.8 (1.7, 11.1) hours, respectively. The percentage of protein-bound nitisinone in plasma (mean value; ultrafiltration) studied using plasma samples from 0.25 to 24 hours after administration of nitisinone (liquid and capsule [n = 3/dosage form]) was 96.2%.

As for safety, the following events occurred: 5 adverse events in 4 of 10 subjects after administration of the liquid formulation (1 event each of hay fever, rash pruritic, facial incision, hot flush/fatigue); 3 adverse events in 3 of 10 subjects after administration of the capsule formulation (2 events of headache; 1 event of photosensitivity). The photosensitivity was determined to be an adverse event for which a causal relationship with the study drug could not be ruled out (adverse drug reaction). There were no deaths, serious adverse events, or adverse events leading to discontinuation of treatment.

4.(i).A.(2) Comparison of the initial and final formulations in terms of blood nitisinone concentrations (5.3.1.2-2, NTBC study [from May 1997 to April 1999]; 5.3.1.2-3, NTBC study [from January 1996 to March 2000]; reference data)

In patients with hereditary tyrosinemia type I (HT-1), the initial and final formulations of nitisinone were compared in terms of blood nitisinone concentrations and clinical laboratory values [see

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presence of nitisinone, by taking advantage of the fact that nitisinone is a powerful inhibitor of 4-hydroxyphenylpyruvate dioxygenase

[59] Initial formulation

"4.(iii).A.(2) NTBC study" for the details of study design, and efficacy and safety results].

4.(i).A.(2).1) Comparison of blood nitisinone concentrations and clinical laboratory values before and after switching from the initial formulation to the final formulation

In patients with HT-1 (n = 47),[60] blood nitisinone concentrations, erythrocyte porphobilinogen (PBG) synthase activity, urine 5-aminolevulinate (5-ALA) level, and urine and plasma succinylacetone (SA) levels were investigated at one time point each before and after switching from the initial formulation to the final formulation. Both formulations were administered twice daily with the mean dose of approximately 1.0 mg/kg/day. More than half of the patients (approximately 67%) received nitisinone at doses of 0.8 to 1.2 mg/kg/day,[61] and no significant changes of the dose between the initial and the final formulations were noted.

The blood nitisinone concentrations following administration of the initial and final formulations were 45.7 ± 22.4 μmol/L and 46.3 ± 23.1 μmol/L (mean value ± standard deviation), and the ratio of the geometric mean blood nitisinone concentration of the initial formulation to that of the final formulation [95% confidence interval (CI)] was 1.02 [0.935, 1.103].

The erythrocyte PBG synthase activities (mean value ± standard deviation) following administration of the initial and final formulations were 1.00 ± 0.26 nkat[62]/g hemoglobin and 1.01 ± 0.26 nkat/g hemoglobin, respectively, and urine 5-ALA levels were 10.0 ± 19.3 mmol/mol creatinine and 7.5 ± 6.0 mmol/mol creatinine following administration of the initial and final formulations, indicating no significant difference between the formulations. The urine and plasma SA levels following administration of the initial and final formulations were within reference ranges[63] in most of the patients, and no significant differences were observed between the initial and final formulations.

4.(i).A.(2).2) Comparison of the initial and final formulations in terms of blood nitisinone concentrations and clinical laboratory values

The initial and final formulations were administered in patients with HT-1 (n = 53 and n = 55, respectively) to compare blood nitisinone concentrations and clinical laboratory values.

Table 6 shows trends in dose-corrected blood nitisinone concentrations in all patients enrolled who

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60 The median age (minimum, maximum) at the start of treatment with nitisinone in patients with HT-1 (n = 47; 25 males, 22 females) was 10.7 (0.2, 171) months; the mean nitisinone treatment period prior to the first evaluation (prior to the administration of the initial formulation) of the data used in the analysis was 3.5 years or more (shortest treatment period was approximately 1.5 years); and the mean interval between the first evaluation (administration of the initial formulation) and the second evaluation (administration of the final formulation) was approximately 6 months.

61 Majority of the patients (approximately 67%) were treated at doses between 0.8 and 1.2 mg/kg/day; approximately 20% of the patients received lower doses than the above range and approximately 13% of the patients received higher doses.

62 kat is the abbreviation for katal, a unit of measurement to express enzyme activity (mol/second).

63 The data for urine SA following the administration of the initial and final formulation nitisinone were from 40 of 41 subjects, and 41 of 41 subjects, respectively; and data for plasma SA following the administration of the initial and final formulation nitisinone were from 44 of 47 subjects, and 42 of 47 subjects, respectively.

were followed up for ≥12 months of nitisinone administration. The difference in dose-corrected blood nitisinone concentrations after 12 months of administration was approximately 5% between the initial and final formulation groups, indicating no major difference between the two formulations.

Table 6. Trends in dose-corrected blood nitisinone concentrations after administration

Time point of evaluation after start of administration	1 week	1 month	2 months	4 months	6 months	8 months	10 months	12 months
Initial formulation	25.7 ± 9.21 (12)	26.4 ± 9.14 (15)	28.5 ± 7.65 (16)	30.9 ± 7.38 (16)	33.7 ± 6.63 (17)	37.8 ± 7.22 (17)	37.9 ± 8.69 (17)	37.9 ± 7.80 (17)
Final formulation	35.7 ± 16.8 (11)	33.8 ± 8.23 (20)	31.8 ± 4.81 (21)	28.4 ± 4.37 (25)	34.4 ± 3.88 (26)	36.7 ± 4.13 (28)	37.6 ± 3.76 (28)	35.9 ± 3.62 (29)

Mean value ± 2 standard deviations (number of cases); unit, μmol/L per mg/kg

The clinical laboratory values were as follows: the urine SA levels returned to reference range within 13 days of administration both in the initial and final formulation groups (30 of 33 subjects and 29 of 32 subjects, respectively). In contrast, the plasma SA levels did not return to the reference range for a prolonged period, and abnormal values were observed in some subjects even after 6 months of administration. After 12 months of administration, all subjects in both groups had laboratory values in reference ranges (23 of 23 subjects and 16 of 16 subjects in the initial and final formulation groups, respectively). The erythrocyte PBG synthase activities and urine 5-ALA levels returned to reference ranges within a short time after administration in both groups, with most of the subjects exhibiting laboratory values in reference ranges within weeks of administration.

4.(i).B Outline of the review by PMDA

[redacted]

Bioequivalence of the initial and final formulations was investigated with two types of analyses performed in patients enrolled in the NTBC study. In the first analysis, blood nitisinone concentrations and clinical laboratory values for HT-1-specific biochemical parameters of 47 subjects were evaluated before and after switching from the initial formulation to the final formulation. The results showed no significant difference between the formulation groups. The second analysis was a comparison of the following two groups: 53 subjects who received the initial formulation from the treatment initiation; and 55 subjects who received the final formulation from the treatment initiation. The results showed no significant differences in blood nitisinone concentrations, or clinical laboratory values for HT-1-specific biochemical parameters between the two groups. On the basis of the above results, the initial and final formulations used in the NTBC study were considered bioequivalent.

The applicant also explained the effects caused by differences in formulation and strength as follows:

[redacted]

[redacted]

PMDA considers as follows:

Although the applicant's explanation on the bioequivalence of the initial and final formulations is not based on the "Partial Revision of the Guideline for Bioequivalence Studies of Generic Drugs" (PFSB/ELD Notification No. 0229-10 dated February 29, 2012), there are no particular problems with evaluating the efficacy and safety of nitisinone on the basis of the results of the NTBC study in which two different formulations, the initial and final formulations, were used, based on the following grounds: (1) the blood nitisinone concentrations and clinical laboratory values (urine SA, plasma SA, erythrocyte PBG synthase activity, and urine 5-ALA) were about the same before and after switching from the initial to final formulations; and (2) although the blood nitisinone concentrations after administration showed variations early on, both formulations showed similar trends later, and after 12 months, no difference was found between the initial and final formulation groups in terms of blood nitisinone concentrations and clinical laboratory values.

[redacted] In principle, bioequivalence between formulations with different strengths should be demonstrated in accordance with the above guidelines before filing a regulatory application; however, all the formulations with three strengths are acceptable for approval based on the following grounds and in consideration of the need for formulations with several different strengths: (1) [redacted]; (2) nitisinone has been approved in foreign countries for more than 10 years, and there have been no particular problems due to unestablished bioequivalence between formulations with different strengths; (3) at present no other drugs for treatment of HT-1 are available in Japan; and (4) in the clinical trials and in actual use in clinical practice up until now, formulations with all different strengths have been used, and dose adjustment according to body weight is necessary.

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

The results of blood nitisinone concentrations in the clinical trial (NTBC study) in patients with HT-1

were submitted as reference data. Other submitted clinical pharmacology results are from studies in which human biomaterials were used. The results from the main studies are described below.

4.(ii).A.(1) Studies using human biomaterials (4.2.2.3-1, 4.2.2.3-2, 5.3.2.2-1 to 5.3.2.2-3)

The fraction of protein-unbound nitisinone (50 μmol/L) concentrations in human plasma (mean value; equilibrium dialysis method) was 4.36%. Varying concentrations of human serum albumin (15 to 45 mg/mL) were added to 0.75 mg/mL of human α1-acid glycoprotein solutions, and fractions of protein-unbound nitisinone in the prepared solutions were studied. The results showed that the fraction of protein-unbound nitisinone decreased (8.81% to 2.33%) with increase in the concentration of human serum albumin, indicating that serum albumin is the main binding protein for nitisinone in plasma.

Incubation of 100 μmol/L of ^{14}C-nitisinone with human liver microsomes in the presence of NADPH resulted in the production of 1 metabolite, but the rate of metabolism was low: the amount of the metabolite produced at a microsomal protein concentration of 1 mg/mL accounted only for less than 2% of total radioactivity. In an attempt to increase the rate of metabolism, a lower concentration (10 μmol/L) of ^{14}C-nitisinone was incubated at higher microsomal protein concentrations (2 and 5 mg/mL), but with no marked increase in the metabolite. Incubation of 5 μmol/L of ^{14}C-nitisinone with freshly isolated human liver cells did not yield the metabolites.

Metabolism of 10 μmol/L of ^{14}C-nitisinone in the expression systems of the cytochrome P450 (CYP) isoforms (CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4) in the presence of NADPH was studied, and the results showed production of 1 metabolite only in the expression system of CYP3A4.

The structure of the nitisinone metabolites was estimated using metabolic reaction solution samples obtained by incubating ^{14}C-nitisinone with human liver microsomes, or ^{14}C-nitisinone with the CYP3A4 expression system. Based on the results, the metabolite observed in the metabolic reaction solution sample of the CYP3A4 expression system was estimated to be a monohydroxylated metabolite of nitisinone. The metabolite observed in the metabolic reaction solution sample of the human liver microsomes was also suspected to be a monohydroxylated metabolite based on the HPLC retention time; however structural information was not obtained due to the extremely small quantity of the metabolite produced.

The inhibitory effects of nitisinone (0.1 to 100 μmol/L) on CYP isoforms (CYP1A2, 2C9, 2C19, 2D6, 2E1, 3A4) were evaluated with human liver microsomes. The results showed that IC_{50} value of nitisinone against CYP2C9 was 46 μmol/L, and that no inhibitory effects were observed in the rest of the CYP isoforms.[64]

4.(ii).A.(2) Studies in patients (5.3.3.2-1, NTBC study [from December 1991 to January 1995]; reference data)

At an early stage of the NTBC study in patients with HT-1, pharmacokinetics were analyzed based on blood nitisinone concentrations [see "4.(iii).A.(2) NTBC study" for the details of study design, and efficacy and safety results].

The pharmacokinetics were studied based on blood nitisinone concentrations after administration of nitisinone to 4 patients (3 pediatric patients, 1 adult patient), using one-compartment model analysis. The results showed that the volume of distribution was 0.07 to 0.55 L/kg, with $t_{1/2}$ being 16 to 26 hours (Table 7, subjects 1 to 4). The $t_{1/2}$ was estimated to be 22 to 37 hours based on the blood nitisinone concentrations after discontinued administration in 3 pediatric subjects to whom administration of nitisinone was stopped following 10 to 25 months of administration (Table 7, subjects 5 to 7).

64 In published literature (Neat JN, et al., *Drug Metab Rev,* 2010; 42 Suppl 1: 115-6), the inhibitory effects of nitisinone on CYP2B6 and 2C8, which were not evaluated in the study, were investigated. The literature reported that nitisinone had weak inhibitory effects on CYP2B6 and 2C8, with the IC_{50} values >100 μmol/L for both isoforms. The literature also examined the inhibitory effects of nitisinone on the metabolic activities of CYP1A2, 2C9, 2C19, 2D6, and 3A4/5, and reported the following results: IC_{50} value for CYP2C9 was 11 μmol/L, and IC_{50} values for other CYP isoforms were all greater than 100 μmol/L. Furthermore, time-dependent or metabolism-dependent inhibitory effects were minimal in the isoforms. The enzyme inducing effects of nitisinone on CYP1A2, 2B6, and 3A4/5 were also investigated using primary human hepatocytes. The results showed that the enzyme activities and mRNA levels of CYP2B6 and 3A4/5 were not significantly influenced (less than 2-fold) by 1, 10, or 100 μmol/L of nitisinone, and the enzyme activities and mRNA levels of CYP1A2 were increased by 100 μmol/L of nitisinone, with the inducing ability accounting for only 9% of the positive control (omeprazole).

Table 7. Pharmacokinetic parameters in individual subjects

Subject	Age[h)]	Body weight[h)] (kg)	k_a (h^{-1})	V_d (L/kg)	K_{el} (h^{-1})	$t_{1/2}$ (h)
1[a)]	2 months	5.7	0.35	0.10	0.027	26
2[b)]	9 months	7.9	0.46	0.55	0.043	16
3[c)]	27 months	15.4	0.17	0.25	0.030	23
4[d)]	21 years	70.0	0.35	0.07	0.033	21
5[e)]	4 years	21.6	–	–	0.025	28
6[f)]	6 years	22.9	–	–	0.031	22
7[g)]	6 years	27.0	–	–	0.019	37

k_a, Absorption rate constant; V_d, distribution volume; K_{el}, elimination rate constant;
$t_{1/2}$, elimination half-life; –, not calculated

a) Pharmacokinetic parameters after 0.56 mg/kg of nitisinone was administered twice daily for 9 days
b) Pharmacokinetic parameters after 0.20, 0.20, and 0.61 mg/kg of nitisinone were each administered once daily for 3 days
c) Pharmacokinetic parameters after 0.21, 0.21, and 0.62 mg/kg of nitisinone were each administered once daily for 3 days
d) Pharmacokinetic parameters after the following series of nitisinone administration: first 0.57 mg/kg, and then 0.29 mg/kg each at 17.4, 30, and 41.5 hours later
e) Pharmacokinetic parameters after the last administration in the discontinued 2-year treatment with 0.22 mg/kg twice daily
f) Pharmacokinetic parameters after the last administration in the discontinued 25-month treatment with 0.21 mg/kg twice daily
g) Pharmacokinetic parameters after the last administration in the discontinued 10-month treatment with 0.5 mg/kg twice daily
h) Age and body weight at the time of administration of nitisinone

4.(ii).B Outline of the review by PMDA

Relationship between blood nitisinone concentrations and efficacy

The applicant explained the relationship between the blood nitisinone concentration and efficacy as follows:

The relationship between the blood nitisinone concentration and the recurrence rate of abnormal HT-1-specific biochemical indicators (urine SA, plasma SA, erythrocyte PBG synthase activity, and urine 5-ALA) in a 2-year period was studied using logistic regression analysis of 207 patients enrolled in the NTBC study from February 1991 to August 1997. As shown in Figure 1, the blood nitisinone concentration was inversely correlated with HT-1-specific biochemical indicators with statistical significance.

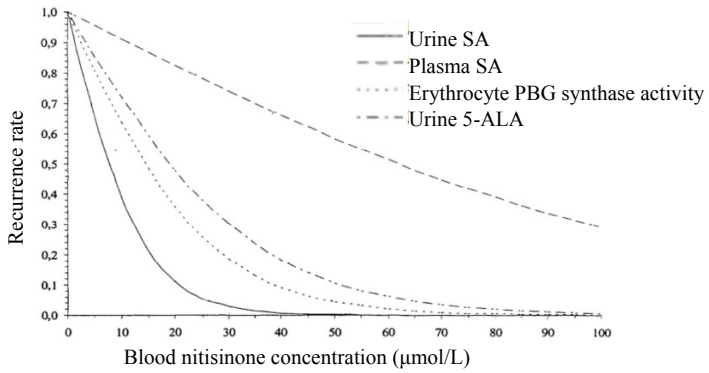

Figure 1. Blood nitisinone concentration and recurrence rate of abnormalities after normal clinical laboratory values were observed

The blood nitisinone concentration that gives a 1% recurrence rate for abnormal urine SA level [95% CI] was 37 [23, 51] μmol/L. The blood nitisinone concentration that gives a 1% recurrence rate for erythrocyte PBG synthase activity [95% CI] was 70 [44, 95] μmol/L. At a blood nitisinone concentration of 30 μmol/L, the recurrence rate for abnormal urine SA level [95% CI] was 2.8% [0.5, 13.7], and the recurrence rate for abnormal erythrocyte PBG synthase activity [95% CI] was 18.6% [8.5, 38.0]. No significant relationship was observed between the age at the start of nitisinone administration and the recurrence rate for abnormal values.

A logistic regression analysis of 250 patients enrolled in the study from July 1993 to March 2000 was performed, and the results obtained were similar to the above analysis. The recurrence rates for abnormal urine SA and erythrocyte PBG synthase activity at a blood nitisinone concentration of 30 μmol/L were approximately 6%.

PMDA considers as follows:
The submitted data on the relationship between blood nitisinone concentration and therapeutic effects have suggested, albeit with limitations, that there are improvements in the clinical laboratory values with an increase in blood nitisinone concentration, as explained by the applicant. On the other hand, animal studies have suggested that administration of nitisinone increases plasma tyrosine concentrations and may cause eye disorders. PMDA will further discuss this issue as well as the safety of nitisinone administration in the clinical section [see "4.(iii).B.(2).2) Safety"].

4.(iii) Summary of clinical efficacy and safety

4.(iii).A Summary of the submitted data

The submitted reference data included the following data: the results of a phase I single dose study (Study CCT/96/001) in foreign healthy male adults and investigator-initiated NTBC study[65] (phase II/III study [main analysis], phase III study [complementary analysis]); Safety Addendum, which contains data on patients who received nitisinone without being enrolled in the NTBC study; periodic safety update reports (PSURs), which contain data on patients who received nitisinone after the NTBC study; and published literature. The results from the main studies and details of published literature are described below.

[65] The NTBC study was an investigator-initiated, worldwide study conducted by a team from Sahlgrenska University Hospital (Sweden). The NTBC study was not undertaken in accordance with the Good Clinical Practice Guidelines (GCP). Upon request, nitisinone was distributed to hospitals all over the world from Sahlgrenska University Hospital on a compassionate use basis. The investigators were to send blood and urine samples for laboratory tests, and local laboratory test results and clinical information to the Sahlgrenska University Hospital on a regular basis in accordance with the protocol designed by the research team of Sahlgrenska University Hospital, and efficacy and safety data of patients enrolled in the study were collected. In the second half of 1994, the distribution of nitisinone was transferred from Sahlgrenska University Hospital to Swedish Orphan AB (current Swedish Orphan BiovitrumAB; SOBI).

4.(iii).A.(1) Clinical pharmacology studies

For the outlines and results of the major studies in the foreign phase I single-dose study (Study CCT/96/001), see "4.(i) Summary of biopharmaceutic studies and associated analytical methods".

4.(iii).A.(2) NTBC study

NTBC study consisted of the following two parts and corresponding two reports were prepared: phase II/III study (the main NTBC analysis [5.3.5.2-1]) was conducted from February 1991 to August 1997, and 207 patients received nitisinone; phase III study (the complementary analysis [5.3.5.2-2]) was conducted from July 1993 to March 2000, and 250 patients received nitisinone. The complementary analysis was conducted to supplement the main analysis by evaluating the patients treated after the initial dose of 1 mg/kg/day was recommended and enrolled during the period from after the main analysis until the latest possible time immediately before the data compilation for marketing application in Europe. The main and complementary analyses shared some patients,[66] and the total number of patients enrolled in the NTBC study was 291 including 1 Japanese patient.

4.(iii).A.(2).1) Phase II/III study (Main analysis) (5.3.5.2-1 [from February 1991 to August 1997]; reference data)

An open-label, uncontrolled study was conducted to investigate the efficacy and safety of nitisinone in Japanese and foreign patients[67] with HT-1 who were on tyrosine and phenylalanine-restricted diets.

With regard to the dosage and administration, patients were asked to take the specified oral daily dose in 2 divided doses, and the dose interval and timing were not specified. The first patient enrolled was initially given oral doses of 0.1 mg/kg/day of nitisinone, and later the dose was increased to 0.2 mg/kg/day, and then to 0.4 mg/kg/day in the first 2 months of treatment. Four patients enrolled in March or April 1991 were initially given oral doses of 0.2 mg/kg/day, and 3 of them were later given increased doses up to 0.6 mg/kg/day.[68] An initial dose of 0.6 mg/kg/day, from December 1991 on, and then 1 mg/kg/day, from 1993 on, were recommended. The dose was adjusted individually according to the trends of erythrocyte PBG synthase activity, urine SA, plasma SA, urine 5-ALA, urine succinylacetoacetate (SAA), and blood nitisinone concentrations.

All the 207 patients treated were included in the efficacy and safety analysis sets. Of them, treatment was discontinued for the following reasons in 38 patients: death due to hepatic failure, liver transplant due to hepatic failure, liver transplant due to suspected hepatocellular carcinoma with subsequent confirmation, and elective liver transplant in 7 patients each; liver transplant due to suspected hepatocellular carcinoma without subsequent confirmation in 6 patients; death due to hepatocellular

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66 A total of 41 patients from February 1991 to July 1993; 166 patients from July 1993 to August 1997; and 84 patients from August 1997 to March 2000

67 Patients with HT-1 diagnosed based on the urine or plasma SA were included, and patients who had undergone liver transplant were excluded in the study.

68 Lindstedt S et al., *Lancet* 1992: 340; 813-7 (5.4-16)

carcinoma in 2 patients; death due to multi-organ failure, and discontinuation upon request by guardian in 1 patient each.

The median exposure duration to nitisinone in the 207 patients during the treatment period (minimum to maximum) was 22.2 (0.1 to 78) months. The treatment periods for patients who started treatment at the ages of 0 to 6 months (80 patients), 0 to 24 months (142 patients), and >24 months (65 patients) were 18.0 (0.1 to 78) months, 20.4 (0.1 to 78) months, and 27.7 (0.8 to 76) months, respectively.

As for efficacy, table 8 shows the survival during the nitisinone treatment period.

Table 8. Survival during the nitisinone treatment period

	Number of patients after treatment			Survival[a)]% [95% CI]		
	1 year later	2 years later	4 years later	1 year later	2 years later	4 years later
Analysis set (207 patients)	149	95	35	96 [93, 99]	96 [92, 100]	93 [87, 99]
Treatment start age, 0-2 months old (16 patients)	12	7	3	88 [70, 100]	88 [65, 100]	88 [52, 100]
Treatment start age, 0-6 months old (80 patients)	55	30	11	94 [88, 100]	94 [85, 100]	94 [80, 100]
Treatment start age, > 6 months old (127 patients)	94	65	24	97 [94, 100]	97 [94, 100]	93 [85, 100]

a) Estimated using the Kaplan-Meier method

Death due to hepatic failure (7 patients) and liver transplant due to hepatic failure (7 patients) during the nitisinone treatment period were observed only in patients who started treatment at the age of 0 to 24 months. The cumulative incidence of death and liver transplant due to hepatic failure after 1, 2, and 4 years of nitisinone treatment estimated by the Kaplan-Meier method [95% CI] was 9% [3, 14], 10% [4, 16], and 13% [3, 22], respectively.

During the nitisinone treatment, 9 patients[69] were found histopathologically to have hepatocellular carcinoma, and of these patients, 8 patients started treatment at the age of >24 months. The cumulative incidence of hepatocellular carcinoma after 1, 2, and 4 years of nitisinone treatment estimated by the Kaplan-Meier method [95% CI] was 3% [0, 5], 4% [0, 8], and 8% [2, 15], respectively. For patients who started treatment at the age of 0 to 24 months, the cumulative incidence of hepatocellular carcinoma after 1, 2, and 4 years of nitisinone treatment [95% CI] was 1% [0, 3], 1% [0, 3], and 1% [0, 5], respectively. In contrast, for patients who started treatment at the age of >24 months, the cumulative incidence of hepatocellular carcinoma after 1, 2, and 4 years of nitisinone treatment [95% CI] was 6% [0, 12], 10% [1, 19], and 20% [5, 35], respectively.[70]

Table 9 shows the time to reference range of clinical laboratory values after the start of nitisinone treatment.

Table 9. Time to reference range of clinical laboratory values after the start of nitisinone treatment

Clinical laboratory value[a)]	Number of patients	Time to normalization (months)	Number of patients with value that was not normalized by Month 2 visit	Number of patients with normal value at pretreatment
		Median (minimum, maximum)		
Urine SA	186	0.3 (0.2, 20.8)	0	3
Plasma SA	172	3.9 (0.2, 27.0)	22	0
Erythrocyte PBG synthase activity	180	0.3 (0.2, 7.5)	0	2
Urine 5-ALA	163	0.2 (0.2, 20.7)	2	27

a) Reference range: urine SA, <1 mmol/mol creatinine; plasma SA, <0.1 μmol/L; erythrocyte PBG synthase activity, >0.58 nkat/g hemoglobin; urine 5-ALA, <23 mmol/L, defined as the 95th percentile of all values in the study after at least 6 months of the nitisinone treatment

69 The treatment was discontinued in 6 patients who underwent liver transplant due to hepatocellular carcinoma, and in 2 patients who died due to hepatocellular carcinoma; the remaining 1 patient who underwent anticancer treatment because of suspected pulmonary metastasis from hepatocellular carcinoma and subsequent partial hepatectomy, was confirmed to be on continuous treatment with nitisinone as of August 1997. Seven patients with suspected hepatocellular carcinoma (subsequently confirmed) underwent liver transplant and nitisinone treatment was discontinued. One patient had hepatocellular carcinoma diagnosed at the start of treatment and underwent liver transplant after 2 weeks of treatment, and the data for the patient was excluded from the analysis of the incidence of hepatocellular carcinoma.

70 When 2 patients who had markedly elevated serum α-fetoprotein concentrations are included in the analysis as 2 suspected cases of hepatocellular carcinoma, the cumulative incidence of hepatocellular carcinoma after 1, 2, and 4 years of treatment [95% CI] were 3% [0, 6], 5% [1, 8], and 11% [4, 19], respectively. Among these, for patients who started the treatment at the age of 0 to 24 months, the cumulative incidence of hepatocellular carcinoma after 1, 2, and 4 years of treatment [95% CI] were 1% [0, 3], 1% [0, 3], and 1% [0, 5], respectively; for patients who started the treatment at the age of >24 months, the cumulative incidence of hepatocellular carcinoma after 1, 2, and 4 years of treatment [95% CI] were 8% [0, 15], 12% [2, 21], and 27% [11, 42], respectively.

Table 10 shows a comparison of clinical laboratory values before the start of treatment with nitisinone and at Year 1 visit.

Table 10. Comparison of clinical laboratory values before the start of treatment and at Year 1 visit

Clinical laboratory value	Number of patients	Before treatment	Year 1 visit	Change	*P*-value[a)]
Urine SA (mmol/mol creatinine)	104	120	<1.00	-119	<0.001
Plasma SA (μmol/L)	108	13.4	<0.1	-13.3	<0.001
Erythrocyte PBG synthase activity (nkat/g haemoglobin)	100	0.02	0.98	0.92	<0.001
Urine 5-ALA (mmol/mol creatinine)	109	76.0	6.0	-66.0	<0.001
Serum α-fetoprotein	104	471[b)]	3.10[b)]	-392[b)]	<0.001
Serum ALT (μkat/L)	54	0.56	0.73	0.15	0.007
Serum AST (μkat/L)	50	0.90	0.77	-0.05	0.064
Serum γ-GTP (μkat/L)	41	1.07	0.42	-0.38	<0.001
Serum bilirubin (μmol/L)	49	17.0	6.8	-10.0	<0.001
Serum albumin (g/L)	47	37.0	42.0	4.0	<0.001
Urine α_1-microglobulin (g/mol creatinine)	100	4.3	2.6	-1.5	<0.001
Serum alkaline phosphatase (μkat/L)	51	15.1	7.4	-7.7	<0.001
Serum phosphate (mmol/L)	45	1.47	1.70	0.23	<0.001
Serum creatinine (μmol/L)	49	32.0	39.0	5.0	0.025
Urine amino acids (mmol/mol creatinine)[c)]	13	7535	1372	-4192	<0.001
Haemoglobin (g/L)	51	114	118	5	0.019
Red blood cell count ($\times 10^{12}$/L)	40	4.07	4.31	0.32	0.011
Platelet count ($\times 10^9$/L)	46	120	227	56	<0.001
Neutrophil count ($\times 10^9$/L)	41	3.00	2.98	-0.10	0.750
Urine phenolic acids (mmol/mol creatinine)	96	458	1730	663	<0.001
Plasma tyrosine (μmol/L)	110	146	387	225	<0.001
Plasma phenylalanine (μmol/L)	110	65.0	56.0	-5.0	0.021
Plasma methionine (μmol/L)	110	38.0	22.0	-12.0	<0.001
Plasma glycine (μmol/L)	109	418	376	-43	0.009
Plasma valine (μmol/L)	110	153	204	41	<0.001
Plasma alanine (μmol/L)	110	553	496	-55	0.039

Median values

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, gamma-glutamyl transpeptidase

a) Wilcoxon signed-rank test

b) Median value for the ratio of α-fetoprotein concentration to reference value

c) Followed up until falling within reference range

As for safety, the incidence of adverse events was as shown in Table 11.

Table 11. Incidence of adverse events

System organ class	Preferred term	Number of patients (incidence [%])
Body as a whole-general disorders	Death (death due to multi-organ failure)	1 (0.5)
Cardiovascular disorders	Cyanosis	1 (0.5)
Central & peripheral nervous system disorders	Convulsion	3 (1)
	Headache	1 (0.5)
	Hyperkinesia	1 (0.5)
Gastro-intestinal disorders	Diarrhoea	1 (0.5)
	Enanthema	1 (0.5)
	Gastritis	1 (0.5)
	Gastroenteritis	1 (0.5)
	Gastrointestinal haemorrhage	1 (0.5)
	Tooth discolouration	1 (0.5)
Liver and biliary disorders	Hepatic failure	14 (7)
	Porphyria	1 (0.5)
Metabolic and nutritional disorders	Dehydration	1 (0.5)
	Hypoglycaemia	1 (0.5)
	Thirst	1 (0.5)
Musculo-skeletal system disorders	Pathological fracture	1 (0.5)
Neoplasms (tumors)	Malignant liver tumor	10 (5)
	Liver tumor	6 (3)
	Benign brain tumor	1 (0.5)
Platelet, bleeding & clotting disorders	Thrombocytopenia	6 (3)
	Epistaxis	2 (1)
Psychiatric disorders	Nervousness	1 (0.5)
	Somnolence	1 (0.5)
Reproductive disorders, female	Amenorrhoea	1 (0.5)
Resistance mechanism disorders	Otitis	1 (0.5)
	Infection	1 (0.5)
Respiratory system disorders	Bronchitis	1 (0.5)
Skin and appendages disorders	Pruritus	3 (1)
	Dermatitis exfoliative	2 (1)
	Alopecia	1 (0.5)
	Maculopapular rash	1 (0.5)
	Xeroderma	1 (0.5)
White blood cell and RES disorders	Leukopenia	4 (2)
	Granulocytopenia	2 (1)
Vision disorders	Keratitis	5 (2)
	Photophobia	4 (2)
	Conjunctivitis	4 (2)
	Corneal opacity	4 (2)
	Eye pain	3 (1)
	Blepharitis	2 (1)
	Cataract	1 (0.5)
Other	Other (elective liver transplant)	7 (3)

The WHO Adverse Reactions Terminology (WHO-ART)

There were 10 deaths (7 due to hepatic failure[71]; 2 due to hepatocellular carcinoma[72]; and 1 due to multi-organ failure), none of which were determined to be adverse reactions of nitisinone. Thirteen serious adverse events were reported in 11 patients: 4 events of convulsion; 3 events of

71 Seven of 14 patients with hepatic failure listed in Table 11

72 Two of 10 patients with malignant liver tumor listed in Table 11

thrombocytopenia; 1 event each of gastrointestinal haemorrhage, porphyria, malignant tumor (hepatocellular carcinoma), benign brain tumor (craniopharyngioma), cyanosis, and hypoglycaemia. Of these, 3 events (3 patients) of thrombocytopenia were determined to be adverse reactions.

Ophthalmological examinations were performed during the study period to investigate eye symptoms. Eye disorders were found in 14 patients[73] (cataract, corneal opacity, keratitis, conjunctivitis, blepharitis, photophobia, eye pain, etc.). Although these symptoms were transient, some patients experienced repeated episodes. All but 1 case of cataract were determined to be adverse reactions. Eye symptoms were observed in 6 of 37 patients who had a plasma tyrosine concentration of >800 μmol/L at least once. This incidence (16.2%, 6 of 37 patients) was significantly higher than the incidence in patients (4.7%, 8 of 170 patients) who did not have a plasma tyrosine concentration of >800 μmol/L (Fisher's exact test, $P = 0.02$). Eye symptoms were observed in 1 of 12 patients who had plasma tyrosine concentrations of >1000 μmol/L, while no eye symptoms were reported in 4 patients who had plasma tyrosine concentrations of >1200 μmol/L.

4.(iii).A.(2).2) Phase III study (Complementary analysis) (5.3.5.2-2 [from July 1993 to March 2000]; reference data)

An open-label, uncontrolled study was conducted to investigate the efficacy and safety of nitisinone in Japanese and foreign patients[67] with HT-1 who were on tyrosine and phenylalanine-restricted diets.

The recommended initial dose was 1 mg/kg/day divided in 2 doses per day, with dose adjustment based on the trends in markers including erythrocyte PBG synthase activity; and concentrations of urine and plasma SA, urine 5-ALA and SAA, and blood nitisinone. The dose interval and timing were not specified. There were 3 groups as follows: the low,[74] recommended,[75] and high dose[76] groups. Subgroup analyses were also performed to evaluate efficacy in the low and recommended dose level groups. Of a total of 250 patients treated in the study, 192 patients were aged 0 to 24 months at the start of treatment, and 58 were aged ≥24 months. The recommended, low, and high dose levels included 198, 41, and 6 patients, respectively, at the start of the treatment, and 5 patients had unknown initial dose levels.

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73 This number includes patients who had multiple events listed in Table 11

74 The low dose group consisted of patients who received ≤0.70 mg/kg/day of nitisinone for ≥6 months in the first year of nitisinone treatment, or patients who received ≤0.70 mg/kg/day over the entire treatment period if their treatment period was <6 months. When patients received >0.70 mg/kg/day later on over the subsequent ≥6 months, the patients were included in the low dose group up to the date >0.70 mg/kg/day was administered for the first time; and when patients received ≤0.70 mg/kg/day further later on for ≥6 months, the patients were included in the low dose group when and after they first received it.

75 The recommended dose group consisted of patients who were not included either in the low dose group or in high dose group and whose doses were known.

76 The high dose group consisted of patients who received ≥1.50 mg/kg/day for 6 months or longer in the first year of nitisinone treatment, or patients who received ≥1.50 mg/kg/day over the entire treatment period if the treatment period was less than 6 months. When patients received <1.50 mg/kg/day later on over the subsequent 6 months or longer, the patients were included in the high dose group up to the date <1.50 mg/kg/day was administered for the first time; and when patients received ≥1.50 mg/kg/day further later on for 6 months or longer, the patients were included in the high dose group when and after they first received it.

All the 250 patients treated were included in the efficacy and safety analysis sets. Of the 250 patients, treatment was discontinued for the following reasons in 50 patients: elective transplant in 10 patinets; death due to hepatic failure, liver transplant due to hepatic failure, liver transplant due to suspected hepatocellular carcinoma (verified later), and liver transplant due to suspected hepatocellular carcinoma (unverified) in 8 patients each; death due to hepatocellular carcinoma and death due to multi-organ failure in 2 patients each; and death from gastrointestinal haemorrhage, death due to complications of premature labour, death due to unknown cause, and request by the guardian in 1 patient each.

The patients' exposure to nitisinone ranged from 0.1 to 80.5 months in terms of the total treatment period for the 250 patients.

As for efficacy, table 12 shows the survival during the nitisinone treatment period.

Table 12. Survival during the nitisinone treatment period

Patient groups by treatment start age/dose level	Number of patients after treatment			Survival[a]% [95% CI]		
	2 years later	4 years later	6 years later	2 years later	4 years later	6 years later
Analysis set (250 patients)	158	88	16	94 [91, 98]	94 [89, 98]	94 [84, 100]
Treatment start age, 0-2 months old (60 patients)	32	16	2	93 [85, 100]	93 [82, 100]	93 [60, 100]
Treatment start age, 0-6 months old (128 patients)	75	38	6	93 [87, 98]	93 [85, 100]	93 [74, 100]
Treatment start age, > 6 months old (122 patients)	83	50	10	96 [86, 100]	95 [89, 100]	95 [82, 100]
Low dose level group (41 patients)	14	10	2	97 [89, 100]	97 [88, 100]	97 [75, 100]
Recommended dose level group (198 patients)	111	48	5	95 [91, 98]	95 [89, 100]	95 [78, 100]

a) Estimated by the Kaplan-Meier method

Table 13 shows the percentage of patients free of the following events during the nitisinone treatment period, estimated by the Kaplan-Meier method: death, liver transplant, death due to hepatic failure, liver transplant due to hepatic failure, and hepatocellular carcinoma. During the nitisinone treatment period, 15 patients died and 34 patients underwent liver transplant. During the treatment period, 8 patients died due to hepatic failure and 8 patients underwent liver transplant due to hepatic failure, all but 1 of which occurred in patients who started treatment at the age of ≤24 months. During the treatment, 10 patients[77] were found histopathologically to have hepatocellular carcinoma, and of these patients, 9 patients started treatment at the age of >24 months.

Table 13. Percentage of patients free of the following events during the nitisinone treatment period: death and liver transplant; death and liver transplant due to hepatic failure; or hepatocellular carcinoma

		Analysis set (250 patients)	Low dose group (41 patients)	Recommended dose group (198 patients)
Percentage of patients free of death and liver transplant[a] (% [95% CI])	2 years later	84 [79, 89]	88 [74, 100]	84 [78, 89]
	4 years later	79 [73, 85]	88 [72, 100]	81 [74, 88]
	6 years later	75 [64, 85]	70 [25, 100]	81 [59, 100]
Percentage of patients free of death and liver transplant due to hepatic failure[a] (% [95% CI])	2 years later	94 [90, 97]	97 [91, 100]	95 [91, 98]
	4 years later	94 [89, 98]	97 [91, 100]	95 [59, 100]
	6 years later	92 [84, 100]	97 [87, 100]	95 [79, 100]
Percentage of patients free of hepatocellular carcinoma[a] (% [95% CI])	2 years later	98 [95, 100]	94 [85, 100]	98 [96, 100]
	4 years later	94 [90, 98]	94 [85, 100]	97 [93, 100]
	6 years later	91 [81, 100]	86 [67, 100]	97 [83, 100]

a) Estimated using the Kaplan-Meier method

Table 14 shows the percentages of patients with clinical laboratory values in reference ranges in selected periods shown in the table during the course of nitisinone treatment. In the majority of patients, urine SA, erythrocyte PBG synthase activity, and urine 5-ALA levels were within the reference ranges from the first week to the first month of treatment, and plasma SA and serum α-fetoprotein levels were within the reference ranges from the 6th month to the second year of treatment.

77 Treatment was discontinued in the following patients: 8 patients who underwent liver transplant due to hepatocellular carcinoma; and 2 patients who died due to hepatocellular carcinoma.

Table 14. The percentages of patients with normal clinical laboratory values in selected periods during the course of nitisinone treatment

Clinical laboratory value[a)]	Selected periods during the course of treatment	Percentage of patients with clinical laboratory values in reference range (%)	
		Low dose group	Recommended dose group
Urine SA[b)]	1-7 days	87.5 (21/24)	91.8 (67/73)
	31-45 days	100.0 (21/21)	97.2 (69/71)
	153-182 days	100.0 (13/13)	100.0 (88/88)
	305-365 days	100.0 (20/20)	100.0 (96/96)
Plasma SA[c)]	1-7 days	0.0 (0/22)	0.0 (0/79)
	31-45 days	0.0 (0/20)	1.3 (1/76)
	46-61 days	75.0 (6/8)	17.4 (12/69)
	62-91 days	50.0 (9/18)	30.4 (28/92)
	92-122 days	93.3 (14/15)	71.9 (64/89)
	123-152 days	81.3 (13/16)	82.1 (46/56)
	153-182 days	92.9 (13/14)	88.8 (79/89)
	305-365 days	95.0 (19/20)	100.0 (99/99)
Erythrocyte PBG synthase activity[d)]	1-7 days	73.9 (17/23)	64.0 (48/75)
	8-14 days	75.0 (6/8)	69.8 (44/63)
	15-30 days	100.0 (9/9)	92.8 (64/69)
	153-182 days	100.0 (12/12)	98.7 (78/79)
	305-365 days	100.0 (17/17)	98.9 (88/89)
Urine 5-ALA [e)]	1-7 days	92.9 (13/14)	77.8 (14/18)
	31-45 days	100.0 (12/12)	85.7 (6/7)
	153-182 days	100.0 (6/6)	100.0 (18/18)
	305-365 days	100.0 (11/11)	100.0 (20/20)
Serum α-fetoprotein	1-30 days	14.3 (4/28)	12.3 (18/146)
	31-61 days	17.4 (4/23)	15.3 (18/118)
	92-122 days	36.4 (4/11)	34.1 (28/82)
	153-182 days	44.4 (4/9)	47.1 (40/85)
	305-365 days	45.0 (9/20)	58.9 (56/95)
	427-487 days	66.7 (6/9)	77.8 (63/81)
	549-609 days	75.0 (3/4)	82.2 (37/45)
	671-730 days	100 (5/5)	87.8 (43/49)

a) Reference ranges: urine SA, <1 mmol/mol creatinine; plasma SA, <0.1μmol/L; erythrocyte PBG synthase activity, >0.58 nkat/g hemoglobin; urine 5-ALA, ≤12.9 to ≤6.6 mmol/mol creatinine depending on children's ages of 24 to 168 months, and ≤6.6 mmol/mol creatinine for children aged >168 months; serum α-fetoprotein, 90 percentile of the reference range for each age group

b) The percentage of patients with out-of-range urine-SA values before treatment was 92.7% and 86.9% for the low dose and recommended dose groups, respectively; the percentage of patients without values before treatment was 7.3% and 12.1%, respectively; 1.0% of patients in the recommended group had values that were within the reference range.

c) The percentage of patients with out-of-range plasma-SA values before treatment was 92.7% and 90.4% for the low dose and recommended dose groups, respectively; the percentage of patients without values before treatment was 7.3% and 9.6%, respectively.

d) The percentage of patients with out-of-range values of erythrocyte PBG synthase activity was 87.8% and 84.3% for the low dose and recommended dose groups, respectively; the percentage of patients without values before treatment was 12.2% and 14.6%, respectively; 1.0% of patients in the recommended dose group had values that were within the reference range.

e) The percentage of patients (patients with treatment start age ≥24 months) with out-of-range urine 5-ALAvalues before treatment was 70.8% and 82.4% for the low dose and recommended dose groups, respectively; the percentage of patients without values before treatment was 8.3% and 11.8%, respectively; the percentage of patients who had values within the reference range was 20.8% and 5.9%, respectively.

AS for safety, the incidence of adverse events was as shown in Table 15. The following adverse events were determined to be adverse reactions of nitisinone: keratitis (3.2%, 8 of 250), corneal opacity (2.4%, 6 of 250), photophobia (2.4%, 6 of 250), conjunctivitis (2.0%, 5 of 250), thrombocytopenia (2.0%, 5 of 250), eye pain (1.6%, 4 of 250), dermatitis exfoliative (1.2%, 3 of 250), pruritus (1.2%, 3 of 250), leukopenia (1.2%, 3 of 250), granulocytopenia (0.8%, 2 of 250), blepharitis (0.4%, 1 of 250),

maculopapular rash (0.4%, 1 of 250), and xeroderma (0.4%, 1 of 250).

Table 15. Incidence of adverse events

System organ class	Preferred term	Number of patients (% incidence)	Number of cases
Vision disorders	Keratitis	8 (3.2)	9
	Corneal opacity	6 (2.4)	16
	Photophobia	6 (2.4)	8
	Conjunctivitis	5 (2.0)	5
	Eye pain	4 (1.6)	18
	Cataract	2 (0.8)	2
	Blepharitis	1 (0.4)	1
	Retinal disorder	1 (0.4)	1
Liver and biliary system disorders	Hepatic failure	16 (6.4)	16
	Hepatic cirrhosis	2 (0.8)	4
	Hepatic enzyme increased	2 (0.8)	2
	Porphyria	2 (0.8)	2
	Hepatic function abnormal	1 (0.4)	1
	Hepatomegaly	1 (0.4)	1
Neoplasms	Malignant liver tumor	10 (4.0)	10
	Liver tumor	8 (3.2)	8
	Benign brain tumor	1 (0.4)	1
	Malignant lymphoma	1 (0.4)	1
Body as a whole, general disorders	Elective transplant	10 (4.0)	10
	Death	4 (1.6)	4
Gastro-Intestinal system disorders	Gastrointestinal haemorrhage	2 (0.8)	2
	Gastroenteritis	2 (0.8)	2
	Abdominal pain	1 (0.4)	1
	Constipation	1 (0.4)	1
	Enanthema	1 (0.4)	1
	Melena	1 (0.4)	1
	Tooth discolouration	1 (0.4)	1
Skin and appendages disorders	Dermatitis exfoliative	3 (1.2)	6
	Pruritus	3 (1.2)	3
	Alopecia	2 (0.8)	2
	Maculopapular rash	1 (0.4)	1
	Xeroderma	1 (0.4)	1
Platelet, bleeding and clotting disorders	Thrombocytopenia	5 (2.0)	6
	Epistaxis	1 (0.4)	1
Central and peripheral nervous system disorders	Convulsion	2 (0.8)	3
	Hyperkinesia	2 (0.8)	2
	Headache	1 (0.4)	2
	Hypokinesia	1 (0.4)	1
White blood cell and RES disorders	Leukopenia	3 (1.2)	3
	Granulocytopenia	2 (0.8)	2
Resistance mechanism disorders	Infection	3 (1.2)	4
	Otitis media	1 (0.4)	1
Metabolic and nutritional disorders	Dehydration	1 (0.4)	1
	Hypoglycaemia	1 (0.4)	1
Psychiatric disorders	Nervousness	2 (0.8)	2
Cardiovascular disorders, general	Cyanosis	1 (0.4)	1
Red blood cell disorders	Anaemia	1 (0.4)	1
Reproductive disorders, female	Amenorrhoea	1 (0.4)	1
Urinary system disorders	Haematuria	1 (0.4)	1

The WHO Adverse Reactions Terminology (WHO-ART)

There were 15 deaths (8 due to hepatic failure,[78] 2 due to hepatocellular carcinoma,[79] 2 due to multi-organ failure, 1 due to gastrointestinal haemorrhage,[80] 1 due to complications of premature labour, and 1 due to unknown cause), and none of these were determined to be adverse reactions of nitisinone. Following 19 serious adverse events were reported in 15 patients: 4 events of hepatic cirrhosis; 3 events of convulsion; 2 events each of thrombocytopenia and porphyria; 1 event each of gastrointestinal haemorrhage, malignant lymphoma, malignant tumor (hepatocellular carcinoma), benign brain tumor (craniopharyngioma), cyanosis, hypoglycaemia, infection, and retinal disorder. Of these, the 2 events of thrombocytopenia in 2 patients were determined to be adverse reactions.

4.(iii).A.(3) Overseas use studies (5.3.5.4-1, 5.3.5.4-2, 5.3.5.4-3; reference data)

Table 16 shows the incidence of serious adverse events reported in the Safety Addendum, which includes data on patients who received nitisinone without being enrolled in the NTBC study, and in the PSURs which include data on patients who received nitisinone after the NTBC study and before the approval in the US. No serious adverse reactions were reported.

Table 16. Incidence of serious adverse events reported in the Safety Addendum and PSURs

System organ class	Preferred term	Safety Addendum (March 1995 to August 1997)	PSUR 1 (January 2000 to April 2001)	PSUR 2 (May 2001 to January 2002)
		24 patients [13.0 patient-years]	318 patients [383 patient-years]	358 patients [241 patient-years]
Body as a whole-general disorders	Anaemia	-	1 (0.3)	-
	Death	1 (4.0)	-	-
	Haemolytic anaemia	-	-	1 (0.3)
Central & peripheral nervous system disorders	Encephalopathy	1 (4.0)[a)]	-	-
Liver and biliary disorders	Hepatic cirrhosis	-	-	1 (0.3)[b)]
	Hepatic failure	-	7 (2.2)[a), b)]	2 (0.6)[a)]
Neoplasms (tumors)	Liver tumor	1 (4.0)	1 (0.3)[b)]	3 (0.8)[b)]
	Malignant liver tumor	-	4 (1.3)[a), b)]	3 (0.8)[b)]
	Malignant lymphoma	-	1 (0.3)[a)]	-
Respiratory system disorders	Respiratory failure	1 (4.0)[a)]	-	-
Other	Elective liver transplant	1 (4.0)	1 (0.3)	6 (1.7)
	Liver transplant	-	1 (0.3)	-

Number of patients (% incidence); -, no occurrence

a) Death due to serious adverse events (1 of 7 patients with hepatic failure in PSUR 1, and 1 of 4 patients with malignant liver tumor)

b) Liver transplant due to serious adverse events (6 of 7 patients with hepatic failure, and 1 of 4 patients with malignant liver tumor in PSUR 1; 1 of 3 patients with liver tumor in PSUR 2)

4.(iii).A.(4) Published studies (reference data)

No clinical studies have been conducted in Japanese patients because the number of patients is extremely limited in Japan. Therefore, published papers were submitted in addition to the results of the NTBC study. Table 17 outlines the papers reporting the clinical results of nitisinone administration,

78 Eight of 16 patients with hepatic failure listed in Table 15

79 Two of 10 patients with malignant liver tumor listed in Table 15

80 One of 2 patients with gastrointestinal haemorrhage listed in Table 15

published in and out of Japan.

Table 17. Outlines of publications reporting the clinical results of nitisinone treatments in and out of Japan

Literature	Objectives	Study design	Results
5.4-14 Larochelle J, et al., *Mol Genet Metab*, 2012;107:49-54	A retrospective examination of the clinical results in patients who had not received nitisinone was performed and was compared with the clinical results of patients who received nitisinone after the start of the NTBC study. The study used the data on 78 patients with HT-1 who were born in Quebec between 1984 and 2004 to compare clinical results. Details are as follows: 28 patients in the untreated group; 24 patients with start age ≤30 days after birth in the early-treated group; 26 patients with start age ≥31 days in the late-treated group.	In the NTBC study, patients in the nitisinone treatment group received 0.6 mg/kg/day or 1.0 mg/kg/day, and analysis was performed on the clinical course which was recorded until death, liver transplant, or the completion of the observation period.	No hospitalization occurred during the nitisinone treatment period (5731 patient-months), in contrast to 184 hospitalizations during the period without treatment (1312 patient-months). Liver transplant was performed in 20 of 28 patients in the untreated group, none of the 24 patients in the early treatment group, and 7 of 26 patients in the late treatment group. Two of 26 patients died in the late treatment group, presumably from complications of transplant, while 10 of 28 patients died in the untreated group. One patient in the late treatment group developed corneal crystals with photophobia, for which a causal relationship with nitisinone administration was suspected.
5.4-16 Lindstedt S, et al., *Lancet*, 1992;340:813-7	A pilot study in patients treated with nitisinone at the beginning stage of the NTBC study. The tolerability and efficacy of nitisinone were studied.	Nitisinone was administered to 5 patients with HT-1 at an initial dose of 0.1 or 0.2 mg/kg/day and then the dose was increased to the highest dose of 0.6 mg/kg/day. The patients were monitored individually for 7-9 months.	HT-1 specific biochemical parameters (urine SA, plasma SA, erythrocyte PBG synthase activity, and urine 5-ALA) improved immediately after the start of nitisinone treatment, and improvements in liver functions were observed in all patients. The serum α-fetoprotein decreased in all patients, although 1 of the patients experienced a relapse of elevated blood α-fetoprotein levels up to 1500 μg/L at the end of the observation period.
5.4-26 Ito M, et al., *Bulletin on Special Formula: Dietary Management of Inborn Errors of Metabolism*, 2005;41:27-30	[redacted]	[redacted]	One week after administration, erythrocyte PBG synthase activity was within the reference range, and urine SA also decreased to below the detection limit. Values for blood clotting were also normalized. Two months after administration, γ-GTP, plasma SA, and total bile acids levels were normalized. Although urine 5-ALA decreased to 8.5 mmol/mol creatinine 1 week after administration, it was still higher than the reference range. One month after administration, the spleen was no longer palpable. The liver surface became smooth 8 months later. One year after administration, the lower edge of liver was approximately 2 cm below the right costal arch. Thereafter, gradual amelioration continued, and hepatomegaly was no longer observed.
5.4-27 Ueta A, et al., *Bulletin on Special Formula: Dietary Management of Inborn Errors of Metabolism*, 2005;41:23-6	A case report of nitisinone administration to a patient[a)] with HT-1 aged 2 months	Nitisinone was administered to a patient with HT-1 for approximately 18 months until liver transplant was performed (dose level not known).	After treatment comprising dietary restriction of tyrosine and phenylalanine, nitisinone treatment was started. Several days after the start of administration, skin rash, anemia, hypoproteinemia, liver functions, and other blood test values improved. Improvements were also observed in hepatomegaly and activity of the child with good body weight increase. Computerized tomography (CT) of the liver did not show any signs of abnormalities. The blood α-fetoprotein levels decreased from 12663 ng/mL measured at the time of hospitalization to 297.2 ng/mL at the time of discharge from the hospital. Nitisinone was continuously administered until liver transplant, which was requested by the family.

Literature	Objectives	Study design	Results
5.4-28 Hata I, et al., *Journal of Japanese Society for Mass-screening*, 2005;15(3):27-31	A case report of nitisinone administration to a Japanese patient with HT-1 aged 4 months	Nitisinone was administered to a patient with HT-1 at a dose of 1 mg/kg/day for approximately 2 weeks until a liver transplant was performed.	A tyrosine/phenylalanine free formula was given to the patient, and 5 days after hospitalization, administration of nitisinone was started at 1 mg/kg/day. Urine SA levels dropped immediately to below the detection limit; however, blood clotting ability did not improve. Hyperbilirubinemia and hyperammonemia worsened 1 week after the start of treatment. With progression of hepatic failure, nitisinone administration was not considered to be effective, and the patient was transferred to a different hospital to undergo a liver transplant at 5 months of age.
5.4-29 Nakabayashi H, et al., *Bulletin on Special Formula: Dietary Management of Inborn Errors of Metabolism*, 2004;40:30-5	A clinical report of nitisinone administration to a Japanese patient with citrin deficiency, which has similar clinical conditions and laboratory findings as those of HT-1. The infant underwent liver transplant due to hepatic failure.	Nitisinone was administered to a patient with citrin deficiency at 10 mg/day (approximately 0.9 mg/kg/day) for approximately 3 weeks until a liver transplant was performed.	An infant was given a tyrosine/phenylalanine restricted diet due to suspected HT-1, and administration of nitisinone was started. Serum tyrosine and related metabolites (4-hydroxyphenylacetic acid, 4-hydroxyphenyllactic acid, and 4-hydroxyphenylpyruvic acid) increased in the patient; however, blood α-fetoprotein, blood clotting ability, and liver functions were not improved. Consequently, the patient underwent liver transplant at 12 months of age (after treated with nitisinone for approximately 3 weeks). The analysis of genomic DNA extracted from the removed liver tissue detected mutations in the SLC25A13 (citrin gene), which led to the diagnosis of citrin deficiency.

a) The patient's father and mother are a Japanese Brazilian and a Brazilian, respectively.

4.(iii).B Outline of the review by PMDA

4.(iii).B.(1) Clinical positioning

The applicant explained as follows:

Conventional treatment options for HT-1 consist of dietary restriction of tyrosine and phenylalanine and liver transplant.[6] Dietary therapy alone provides HT-1 patients with suppression of hepatic and renal injuries, but does not prevent a fatal outcome.[5] Given the concerns arising from the difficulty in finding a compatible donor liver, the risk of perioperative morbidity and mortality, and the necessity for long-term immunosuppressive therapy, a liver transplant is difficult to perform, in particular in pediatric patients with severe hepatic dysfunction in the acute phase of HT-1.

Nitisinone is an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme in the second step of the tyrosine catabolic pathway. Nitisinone is expected to improve the patient's condition by inhibiting the production and accumulation of maleylacetoacetate (MAA), fumarylacetoacetate (FAA), and other toxic intermediate metabolites in the pathway, which is caused by a genetically determined deficiency in fumarylacetoacetate hydrolase (FAH) activity in patients with HT-1. Based on the results of studies including the NTBC study, nitisinone is currently used as first-line treatment[8] in many countries in combination with dietary restriction of tyrosine and phenylalanine; therefore, nitisinone is an essential drug for HT-1.

PMDA considers as follows:

Nitisinone can be expected to become the standard treatment in Japan because the HT-1 is a serious

disorder and no other drugs have been approved in Japan for this indication, and because nitisinone is already being used as the standard treatment of HT-1 in other countries.

4.(iii).B.(2) Efficacy and safety

PMDA evaluated the efficacy and safety of nitisinone based on the data from the NTBC study, in which 1 Japanese patient participated, and other data from foreign use for the following reasons: although the submitted clinical data package does not provide sufficient results to justify the extrapolation of data from foreign patients, pharmacokinetic studies in healthy Japanese adults were not considered feasible because of the possible risk of eye disorders; furthermore, the number of patients is very limited, and there is only 1 patient in Japan as of July 2014.

4.(iii).B.(2).1) Efficacy

4.(iii).B.(2).1).(a) Survival

The applicant explained as follows:

The NTBC study was conducted as an uncontrolled study for ethical reasons. Therefore, the estimated survival [5] of 108 patients with HT-1 who were treated only with dietary restriction of tyrosine and phenylalanine in the international survey cohort reported by van Spronsen et al., was compared with the survival in the complementary analysis of the NTBC study. Figure 2 shows the Kaplan-Meier plots for the NTBC study (complementary analysis) and the international survey cohort reported by van Spronsen et al. In the analysis of NTBC study, the survival after the start of treatment was estimated, whereas in the international survey cohort reported by van Spronsen et al., the survival after the first onset of symptoms was estimated. Patients who underwent liver transplant were censored at the time of transplant. The number of censored patients was 35 and 26 for the NTBC study and the international survey cohort, respectively.

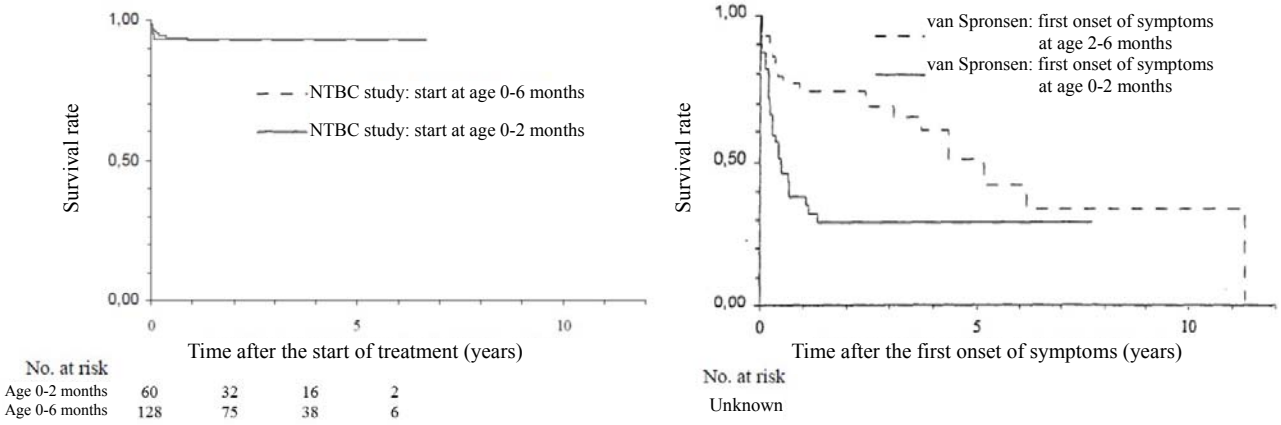

Figure 2. The survival rate estimated by the Kaplan-Meier method for the complementary analysis of the NTBC study (left) and the international survey cohort reported by van Spronsen et al. (right)

The data from the international survey cohort reported by van Spronsen et al. showed that the survival of patients with HT-1 who were treated with dietary restriction alone after the onset of symptoms

varied with the age of onset of the symptoms. Among patients treated with dietary restriction alone, the 2-year and 4-year survival were 29% for patients in whom symptoms developed at age <2 months, and 74% and 60%, respectively, for patients in whom symptoms developed at age 2 to 6 months. In contrast, the 4-year survival after the start of treatment was all 93% in patients with start age of <2 months and <6 months in the NTBC study (complementary analysis).

Although it is difficult to perform a strict comparison between the data from the international survey cohort reported by van Spronsen et al. and the NTBC study (complementary analysis) because of the lack of a precise observation period for data in the former study, there was an outstanding difference in survival between the patients who were treated with nitisinone and dietary restriction, and the patients who were treated with dietary restriction alone.

4.(iii).B.(2).1).(b) Incidence of hepatic failure and hepatocellular carcinoma, and clinical laboratory values

The applicant explained as follows:

Based on the data from the international survey cohort reported by van Spronsen et al., hepatic failure and recurring hemorrhage accounted for 67% (35 of 52 patients) of total deaths in patients who were treated with tyrosine and phenylalanine diet restriction alone. Among patients in whom signs and symptoms developed at age <6 months, 42% (35 of 83 patients) died from hepatic failure or recurring hemorrhage. Based on the data from the NTBC study (complementary analysis), 6% (8 of 128 patients) of patients who started treatment with nitisinone at the age of <6 months died or underwent liver transplant due to hepatic failure during the observation period, all of which occurred in the first year of treatment.

It is known that patients with HT-1 exhibiting hepatic cirrhosis are at higher risk of developing hepatocellular carcinoma. Weinberg et al. reported that 37% (16 of 43 patients) of patients with HT-1 aged >24 months had hepatocellular carcinoma.[81] In the international survey cohort reported by van Spronsen et al., 18% (9 of 52 patients) of the total number of patients aged ≥24 months who were treated only with dietary restriction of tyrosine and phenylalanine were diagnosed as having hepatocellular carcinoma.[5] In the NTBC study (complementary analysis), only 1 patient developed hepatocellular carcinoma among the patients who started receiving nitisinone at the age of 0 to 24 months. This patient underwent a partial hepatectomy following chemotherapy and subsequently remained healthy. The incidence of hepatocellular carcinoma after 6 years of treatment estimated by the Kaplan-Meier method was approximately 1% in patients who started nitisinone treatment at the age of 0 to 24 months, while the estimated incidence of hepatocellular carcinoma after 2, 4, and 6 years of nitisinone treatment in all patients who started treatment at the age of ≥ 24 months was 8%, 18%, and 25%, respectively. These results indicate that patients who started nitisinone treatment at the age of <24 months have a significantly reduced incidence of hepatocellular carcinoma. Starting

[81] Weinberg, et al., *J Pediatr*. 1976;88:434-8

nitisinone treatment as young as possible is expected to reduce the risk of developing hepatocellular carcinoma as well as progressive liver injury.

SA is not detected in urine or plasma under normal circumstances; therefore, in the NTBC study, the detection limits of the analysis method (1.0 mmol/mol creatinine for urine SA; 0.1 μmol/L for plasma SA) were used as the reference limits for urine SA and plasma SA to evaluate the efficacy of nitisinone. Almost all the HT-1 patients enrolled in the NTBC study showed markedly elevated urine SA and plasma SA before the start of treatment with nitisinone in both the main and complementary analyses. In approximately 90% or more patients, urine SA levels were in the reference range within 1 week after the start of treatment; however, some patients experienced reccurent increase of SA excretion in urine after normalization. Because there was a correlation between the recurrent increase of SA excretion in urine and blood nitisinone concentration [see "4.(ii).B. Relationship between blood nitisinone concentration and efficacy"], it was assumed that recurrence of elevated urine SA occurred when the blood nitisinone levels decreased. The plasma SA concentrations also decreased to reference range in most patients following nitisinone administration (Tables 9 and 14), but more slowly, which was attributable to SA bound to plasma proteins.

Alpha-fetoprotein is extensively produced along with rapid hepatocellular proliferation in the liver of normal fetuses. Serum α-fetoprotein levels are high at birth, but rapidly decrease during the first year of life in the normal population.[82] In HT-1 patients, especially in acute phase, serum α-fetoprotein levels are markedly elevated compared to the reference range. Serum α-fetoprotein levels decrease while receiving a dietary restriction treatment, but still remain higher than the reference range.[83] In the main and complementary analyses of the NTBC study, serum α-fetoprotein levels decreased in the first year of nitisinone treatment (Tables 10 and 14), and after 2 years of treatment, the levels of approximately 90% of patients were in the reference range. The decreased serum α-fetoprotein levels are considered to represent the change from a high rate of hepatocyte turnover to a normal rate of hepatocyte proliferation.

Neurological complications in patients with HT-1 are considered secondary to metabolic disorder of porphyrin. Inhibition of erythrocyte PBG synthase activity by SA leads to accumulation of 5-ALA, which is likely to be involved in neurotoxicity.[84] The main and complementary analyses of the NTBC study showed that administration of nitisinone decreased the 5-ALA levels in urine (Tables 10 and 14), and that acute porphyria only developed in 2 patients, both of whom subsequently recovered.

[redacted]

82 Lahdenne P, et al., *J Pediatr*. 1991;118:272-6.

83 Halvorsen S, *Inborn metabolic diseases.* ed. by Fernandes J, et al., Springer Verlag, Berlin, 1990;199-209

84 Gibbs TC, et al., *J Neurol Neurosurg Psychiatry*. 1993;56:1129-32, Mitchell G, et al., *N Engl J Med*. 1990;322:432-7.

[redacted] One Japanese patient who was enrolled in the NTBC study was treated with nitisinone at a daily dose in the range of the recommended dose levels. This patient's blood nitisinone concentrations were generally the same as those of all the patients enrolled in the study, and no adverse events were reported for this patient.

PMDA considers as follows:
It is difficult to fully evaluate the efficacy of nitisinone based on the results of studies including the NTBC study in which only 1 Japanese patient was enrolled, and the submitted clinical study data consisted only of reference data. However, it can be concluded that the efficacy of nitisinone has been suggested for the following reasons: the comparison of the survival provided by the NTBC study and the estimated survival of patients treated only with dietary restriction of tyrosine and phenylalanine from the data of the international survey cohort on patients with HT-1 reported by van Spronsen et al., suggests that survival is improved by nitisinone treatment, although it is difficult to strictly compare these two data; urine SA and other HT-1 specific biochemical parameters were controlled to be within reference range by nitisinone treatment, and tended to be stabilized by the treatment in combination with dietary restriction of tyrosine and phenylalanine. The above issues will be finalized, taking into account the comments made in the Expert Discussion.

4.(iii).B.(2).2) Safety

The applicant explained as follows:
The NTBC study consists of the main analysis (5.3.5.2-1) and complementary analysis (5.3.5.2-2): the former was conducted as a Phases II/III study in patients who were treated with nitisinone from February 1991 to August 1997; and the latter was conducted as a Phase III study in patients who were treated with nitisinone from July 1993 to March 2000. Because of the overlapping period, these two analyses shared a number of patients[66]; therefore, patients' data were pooled into a dataset of 291 patients who were treated during the period from February 1991 to March 2000, excluding duplicated patients, to evaluate safety information of nitisinone as follows.[85]

As for exposure to nitisinone in the overall NTBC study, the numbers of patients exposed for a treatment duration of 1 day, 1 week, 4 weeks, 3 months, 6 months, and 1 year were 291, 289, 277, 264, 252, and 229, respectively. Seventy-nine percent of the patients (229 of 291 patients) were administered nitisinone for at least 1 year. The total treatment period was 971.6 patient-years, with a median and a maximum period of 3.3 years and 9.1 years, respectively. Table 18 shows the number of patients by dose level of nitisinone in the NTBC study, indicating that most patients (224 of 291 patients, 77%) were treated with a daily nitisinone dose of 0.8 to 1.2 mg/kg/day, equal or close to the recommended starting dose of 1 mg/kg/day. Many patients started receiving nitisinone at the age of <2 years (216 of 291 patients, 74%), and only 3 patients started at the age of ≥18 years.

[85] The adverse event data in the NTBC study were first presented using the WHO Adverse Reactions Terminology (WHO-ART), and then they were converted into the ICH Medical Dictionary for Regulatory Activities (MedDRA) terminology and summarized for the pooled analysis.

Table 18. The number of patients by dose level of nitisinone in the NTBC study

Dose level (mg/kg/day)	Unknown	<0.6	≥0.6 to <0.8	≥0.8 to <1.2	≥1.2	Total
Number of patients (patient-years)	38 (54.4)	90 (124.6)	131 (130.4)	224 (542.9)	69 (119.2)	- (971.6)

Tables 19 and 20 show the adverse events that occurred in 3 or more patients, and adverse events by treatment duration in the NTBC study, respectively. The data on adverse events by treatment duration show that the majority of adverse events occurred in the first 4 years of nitisinone treatment, and particularly, patients were more likely to develop hepatic failure in the first 6 months of treatment, and undergo a liver transplant in the first year of treatment than in subsequent periods.

Table 19. Adverse events that occurred in ≥3 patients (NTBC study)

System organ class	Preferred term	291 patients (971.6 patient-years)	
		Number of patients (number of patients per 100 patient-years)	Number of events (number of events per 100 patient-years)
Any adverse event		123 (12.7)	224 (23.1)
Eye disorders	Keratitis	10 (1.0)	12 (1.2)
	Corneal opacity	7 (0.7)	17 (1.7)
	Conjunctivitis	6 (0.6)	8 (0.8)
	Photophobia	6 (0.6)	8 (0.8)
	Eye pain	4 (0.4)	18 (1.9)
	Cataract	3 (0.3)	3 (0.3)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Hepatic neoplasm malignant	15 (1.5)	15 (1.5)
	Hepatic neoplasm	12 (1.2)	12 (1.2)
Hepatobiliary disorders	Hepatic failure	23 (2.4)	23 (2.4)
General disorders and administration site conditions	Adverse event[a)]	15 (1.5)	15 (1.5)
	Death	4 (0.4)	4 (0.4)
Blood and lymphatic system disorders	Thrombocytopenia	7 (0.7)	8 (0.8)
	Leukopenia	5 (0.5)	6 (0.6)
Skin and subcutaneous tissue disorders	Dermatitis exfoliative	3 (0.3)	6 (0.6)
	Pruritus	3 (0.3)	3 (0.3)
Nervous system disorders	Convulsion	3 (0.3)	4 (0.4)
Infections and infestations	Infection	3 (0.3)	4 (0.4)

MedDRA version 15.1

a) Liver transplant

Table 20. Incidence of adverse events by treatment duration (NTBC study, adverse events that occurring in 3 or more patients)

System organ class	Preferred term	291 patients (971.6 patient-years)			
		0 to 6 months (291 patients, 133.1 patient-years)	7 months to 1 year (252 patients, 329.9 patient-years)	2 to 4 years (187 patients, 405.1 patient-years)	5 or more years (75 patients, 103.5 patient-years)
Eye disorders	Keratitis	0	4 (1.2)	5 (1.2)	1 (1.0)
	Corneal opacity	0	5 (1.5)	5 (1.2)	0
	Conjunctivitis	1 (0.8)	3 (0.9)	3 (0.7)	1 (1.0)
	Photophobia	1 (0.8)	3 (0.9)	2 (0.5)	0
	Eye pain	0	1 (0.3)	4 (1.0)	1 (1.0)
	Cataract	0	2 (0.6)	1 (0.2)	0
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Hepatic neoplasm malignant	2 (1.5)	6 (1.8)	6 (1.5)	1 (1.0)
	Hepatic neoplasm	3 (2.3)	4 (1.2)	4 (1.0)	1 (1.0)
Hepatobiliary disorders	Hepatic failure	18 (13.5)	2 (0.6)	3 (0.7)	0
General disorders and administration site conditions	Adverse event[a)]	6 (4.5)	6 (1.8)	2 (0.5)	1 (1.0)
	Death	3 (2.3)	1 (0.3)	0	0
Blood and lymphatic system disorders	Thrombocytopenia	5 (3.8)	1 (0.3)	1 (0.2)	0
	Leukopenia	4 (3.0)	1 (0.3)	1 (0.2)	0
Skin and subcutaneous tissue disorders	Dermatitis exfoliative	2 (1.5)	1 (0.3)	0	1 (1.0)
	Pruritus	2 (1.5)	1 (0.3)	0	0
Nervous system disorders	Convulsion	1 (0.8)	1 (0.3)	2 (0.5)	0
Infections and infestations	Infection	0	3 (0.9)	0	0

Number of patients (number of patients per 100 patient-years); MedDRA version 15.1

a) Liver transplant

There were 22 deaths in the entire NTBC study due to the following causes (preferred terms): 10 hepatic failures; 5 hepatic neoplasms malignant; 4 deaths (2 deaths due to multi-organ failure; complications of premature labour, unknown cause [1 each]); 2 adverse events (operational death during elective liver transplant and death due to unknown cause several months after withdrawal from nitisinone treatment because of liver transplant); 1 gastrointestinal haemorrhage. The incidence of non-fatal, serious adverse events is shown in Table 21.

Table 21. The incidence of non-fatal, serious adverse events (NTBC study)

System organ class	Preferred term	291 patients (971.6 patient-years)	
		Number of patients (number of patients per 100 patient-years)	Number of events (number of events per 100 patient-years)
Any adverse event		55 (5.7)	69 (7.1)
Infections and infestations	Infection	1 (0.1)	1 (1.0)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Hepatic neoplasm	12 (1.2)	12 (1.2)
	Hepatic neoplasm malignant	10 (1.0)	10 (1.0)
	Lymphoma	1 (0.1)	2 (0.2)
	Brain neoplasm benign	1 (0.1)	1 (0.1)
	Neoplasm malignant	1 (0.1)	1 (0.1)
Hepatobiliary disorders	Hepatic failure	12 (1.2)	12 (1.2)
	Hepatic cirrhosis	2 (0.2)	4 (0.4)
General disorders and administration site conditions	Adverse event[a)]	13 (1.3)	13 (1.3)
Nervous system disorders	Convulsion	3 (0.3)	4 (0.4)
Blood and lymphatic system disorders	Thrombocytopenia	3 (0.3)	3 (0.3)
Congenital, familial and genetic disorders	Porphyria	2 (0.2)	2 (0.2)
Cardiac disorders	Cyanosis	1 (0.1)	1 (0.1)
Eye disorders	Retinal disorder	1 (0.1)	1 (0.1)
Gastrointestinal disorders	Gastrointestinal haemorrhage	1 (0.1)	1 (0.1)
Metabolism and nutrition disorders	Hypoglycaemia	1 (0.1)	1 (0.1)

MedDRA version 15.1

a) Liver transplant

Nitisinone was approved in the US in January 2002, and subsequently in the Europe in 2005. The post-marketing experience of nitisinone is presumed as follows: the number of cumulative patients who were treated with nitisinone is estimated to be 8135 patient-years over a period of more than 10 years, from January 18, 2002 to February 20, 2013. The additional safety database[86] managed by SOBI as part of post-marketing surveillance program contains the data of 862 patients, and 932 adverse events have been reported in 492 post-marketing case reports. The most frequently reported adverse events are related to clinical laboratory values, particularly, case reports[87] on patients with elevated plasma tyrosine levels, higher than 500 μmol/L. Other common adverse events included complications due to increased plasma tyrosine levels (eye disorders, 63 events), HT-1 related complications (surgical and medical procedures, 92 events; neoplasms benign, malignant and unspecified [including cysts and polyps], 77 events; and hepatobiliary disorders, 69 events).

PMDA considers as follows:

It is difficult to fully evaluate the safety of nitisinone based on the results of studies including the NTBC study in which only 1 Japanese patient was enrolled, and the submitted clinical study data consisted only of reference data. However, safety information has been obtained from the NTBC study,

86 The database was intended to include information of patients in Europe, but actually it also contains data on patients who received nitisinone in other regions including the US.

87 Blood tyrosine concentration exceeding 500 μmol/L was considered to be an adverse event by SOBI, regardless of associated symptoms.

as well as from clinical experience (after the approvals in the US and Europe) at a certain level. Therefore, the safety of nitisinone is acceptable when nitisinone is administered in combination with dietary restriction of tyrosine and phenylalanine, as long as appropriate precautionary statements are provided. PMDA discussed important adverse events in evaluating safety as follows.

4.(iii).B.(2).2).(a) Eye disorders

The applicant explained as follows:

As expected, when patients were treated with nitisinone, plasma tyrosine levels increased significantly from the pretreatment levels. This was a direct effect of inhibition of HPPD by nitisinone, and because an increase in tyrosine concentration leads to the formation of tyrosine crystals in the eye, promoting deposition of crystals in the eye, possibly resulting in corneal opacity, tyrosinemia-related eye symptoms are considered to be an identified risk. It is also known that corneal lesions occur in patients with type II tyrosinemia, or HT-2, in whom plasma tyrosine concentrations are higher than those in HT-1 patients.[6]

Because eye disorders are identified risks resulting from nitisinone administration, eye examinations were performed during the NTBC study to monitor the patients if they have symptoms. The incidence of eye disorders in the NTBC study is shown in Table 22. The most common adverse events reported in the NTBC study were eye disorders (29 of 291 patients, 69 events), including keratitis, corneal opacity, conjunctivitis, photophobia, and eye pain, most of which were transient, but recurrence was observed in some patients. Among the eye disorders, retinal disorder was determined to be a serious adverse event. This patient started receiving nitisinone from the age of approximately 6 weeks, developed retinal disorder 340 days after starting nitisinone treatment and the outcome is unknown. Retinal disorder was not determined to be an adverse reaction. The incidence of adverse events was examined on the basis of treatment duration at the onset of the symptoms (0 to 6 months, 7 months to 1 year, 2 to 4 years, and ≥5 years), and the results showed that eye disorders tended not to develop until at least 7 months had passed after the start of treatment (Table 20).

Table 22. The incidence of eye disorders in the NTBC study

Eye disorders/Preferred term	291 patients (971.6 patient-years)	
	Number of patients (number of patients per 100 patient-years)	Number of events (number of events per 100 patient-years)
Adverse event	29 (3.0)	69 (7.1)
Adverse reaction	27 (2.8)	65 (6.7)
Serious adverse events	1 (0.1)	1 (0.1)
Keratitis	10 (1.0)	12 (1.2)
Corneal opacity	7 (0.7)	17 (1.7)
Conjunctivitis	6 (0.6)	8 (0.8)
Photophobia	6 (0.6)	8 (0.8)
Eye pain	4 (0.4)	18 (1.9)
Cataract	3 (0.3)	3 (0.3)
Blepharitis	2 (0.2)	2 (0.2)
Retinal disorder	1 (0.1)	1 (0.1)

MedDRA version 15.1

Among the 207 patients included in the main analysis of the NTBC study, 37 patients had a plasma tyrosine concentration exceeding 800 μmol/L at least once during the study period, and 6 patients developed eye disorders. The incidence of eye disorders in patients who had a plasma tyrosine concentration of >800 μmol/L at least once (16.2%, 6 of 37 patients) was significantly higher than in patients who did not have at all (4.7%, 8 of 170 patients), suggesting a higher risk in the former patients. In the 250 patients included in the complementary analysis of the NTBC study, the incidence of eye disorders was analyzed using a logistic regression model including plasma tyrosine concentration and the age at which nitisinone treatment was started. The results showed that the incidence of eye disorders tended to increase with an increase in the age at which treatment started and with an increase in the peak plasma tyrosine concentration during treatment. The estimated incidence of eye disorders in patients with a peak plasma tyrosine concentration of 850 μmol/L for the treatment start ages of 0, 1, 2, and 3 years was approximately 11%, 12%, 13%, and 15%, respectively. In patients with a peak plasma tyrosine concentration of 500 μmol/L, the estimated incidence for the treatment start age groups was 5%, 5%, 6%, and 6%, respectively.

Among 932 adverse events included in the post-marketing safety information for nitisinone, 63 events were related to eye disorders, and the majority of these were considered associated with tyrosinemia. The following 6 events were considered to be serious adverse events: 2 events of corneal opacity; and 1 event each of eye pain, photophobia, lenticular opacity, and visual acuity reduced. The remaining 57 events were determined to be non-serious.

As discussed above, tyrosine concentrations increase as a result of nitisinone administration, resulting in the development of eye disorders, which are not considered serious adverse events. However, in order to lower the risk of eye disorders caused by an elevation in tyrosine concentrations, adherence to dietary restriction to prevent a significant elevation in tyrosine concentrations is important; therefore, appropriate precautionary statements will be included in the package insert.

PMDA considers as follows:
The risk of eye disorders due to an increase in tyrosine concentration is represented by the serious adverse events reported in foreign post-marketing safety information. Therefore, in regard to the use of nitisinone, appropriate precautionary statements should be provided to ensure adequate management of plasma tyrosine concentration and strict adherence to dietary therapy. Data on eye disorders should be continuously collected through the post-marketing surveillance program. The above issues will be finalized, taking into account the comments made in the Expert Discussion.

4.(iii).B.(2).2).(b) Leukopenia, granulocytopenia, and thrombocytopenia

The applicant explained as follows:
Leukopenia, granulocytopenia, and thrombocytopenia are known risks during nitisinone treatment. In the NTBC study, the following adverse events were observed: 6 events (0.6 events per 100 patient-years) of leukopenia in 5 of 291 patients (0.5 patients per 100 patient-years); 2 events (0.2 events per

100 patient-years) of granulocytopenia in 2 of 291 patients (0.2 patients per 100 patient-years); and 8 events (0.8 events per 100 patient-years) of thrombocytopenia in 7 of 291 patients (0.7 patients per 100 patient-years). The incidence of adverse events was examined on the basis of treatment duration at the onset (0 to 6 months, 7 months to 1 year, 2 to 4 years, and ≥5 years), and the results showed that the incidence of thrombocytopenia and leukopenia was higher in the first 6 months of treatment than in the subsequent periods (Table 20).

In the post-marketing safety information for nitisinone, 8 events in 6 patients (3, 4, and 1 event of leukopenia, neutropenia, and pancytopenia, respectively) were reported, and 5 of these 8 events were non-serious. Of the 8 events, 3 events resolved, and the outcomes of the remaining 5 events were unresolved or unknown. One event of leukopenia was reported as a serious adverse event. The patient experienced a comorbid condition of mild leukopenia and staphylococcal infection. There are no other reports of comorbidity with infection.

Although no specific risk populations, risk factors, or mechanisms of leukopenia, granulocytopenia, and thrombocytopenia are known, appropriate precautionary statements will be included in the package insert.

PMDA considers that there are no major problems in the manner the applicant addresses these issues by providing precautionary statements concerning the risks of leukopenia, granulocytopenia, and thrombocytopenia. However, it is necessary to gather information on leukopenia, granulocytopenia, and thrombocytopenia in the post-marketing surveillance. This issue will be finalized, taking into account the comments made in the Expert Discussion.

4.(iii).B.(2).2).(c) Skin symptoms

PMDA asked the applicant to explain the development of skin symptoms associated with elevated tyrosine levels induced by nitisinone administration.

The applicant responded as follows:

Skin lesions and corneal lesions occur in patients with hereditary tyrosinemia type II, in whom plasma tyrosine concentrations are higher than those in HT-1 patients.[88] Skin symptoms are considered to be caused by formed needle-shaped tyrosine crystals, causing hyperkeratosis or erosion on the hands and soles of the feet. Because skin and eye symptoms are alleviated when tyrosine concentrations decrease in patients with hereditary tyrosinemia type II, the goal of the treatment is to lower tyrosine concentrations; consequently, dietary restriction of tyrosine and phenylalanine are introduced.

However, nitisinone inhibits HPPD, an enzyme in the second step of the tyrosine catabolic pathway;

[88] Nakamura K, Endo F. *Supplement to Japanese Journal of Clinical Medicine, New series of syndromes by clinical specialty,* No.19: Inborn Error of Metabolism. 2nd ed. Book 1, Osaka, Japan, Nippon Rinsho-sha. 2012;162-3

therefore, nitisinone treatment may cause tyrosine levels to rise. In the NTBC study, 15 adverse events that were classified as "skin and subcutaneous tissue disorders" occurred (1.5 events per 100 patient-years) in 9 of 291 patients (0.9 patients per 100 patient-years). Commonly reported adverse events (≥3 patients) were 6 events (0.6 events per 100 patient-years) of dermatitis exfoliative in 3 of 291 patients (0.3 patients per 100 patient-years), 3 events (0.3 events per 100 patient-years) of pruritus in 3 of 291 patients (0.3 patients per 100 patient-years).

In the post-marketing safety information on nitisinone, 11 adverse events in 9 patients were reported as follows: 2 events of rash; 1 event each of rash macular, pruritus, purpura, dermatitis diaper, exfoliative rash, dermatitis herpetiformis, eczema, erythema, and skin discolouration.

Because skin symptoms are observed in patients with hereditary tyrosinemia type II when tyrosine concentrations increase, skin symptoms are considered to be risks associated with nitisinone treatment; however, the risk of developing skin symptoms can also be increased in patients who have elevated tyrosine concentration due to poor adherence to dietary restriction. Therefore, elevated tyrosine levels and resulting skin symptoms can be prevented by adherence to dietary restriction of tyrosine and phenylalanine.

PMDA accepted the applicant's response, and data on skin symptoms should be gathered continuously in the post-marketing surveillance program. This issue will be finalized, taking into account the comments made in the Expert Discussion.

4.(iii).B.(3) Indications

The applicant explained as follows:

In the treatment of HT-1 patients, preventing the progression of liver disorder at an early stage is of great importance; therefore, patients should be treated at as young an age as possible with nitisinone, an HPPD inhibitor, in combination with dietary restriction of tyrosine and phenylalanine. The NTBC study is the only clinical study which evaluated the clinical efficacy of nitisinone in the treatment of patients with HT-1 and in which 1 Japanese patient was enrolled. The Japanese patient was the only one patient in Japan when the study was conducted, and still remains the only patient as of July 2014. The number of HT-1 patients is extremely limited, and no clinical studies have been conducted in Japan; however, the efficacy of nitisinone in the treatment of HT-1 is expected based on the results of the NTBC study. Also, there are no specific differences in the diagnostic criteria, pathology, or course of treatment for HT-1 between in Japan and other countries.[89]

[89] FY2011 Annual Report, Research group of Research on protocol for definitive diagnosis of neonates with signs of tyrosinemia and treatment guidelines, Research Project on Measures for Intractable Disease, Health and Labour Science Research Grants. 2012;86-9; Nakamura K, Endo F. *Supplement to Japanese Journal of Clinical Medicine. New series of syndromes by clinical specialty,* No.19: Inborn Errors of Metabolism. 2nd ed. Book 1, Osaka, Japan, Nippon Rinsho-sha. 2012;159-66; de Laet C, et al., Orphanet J Rare Dis. 2013;8(1):8; McKiernan PJ. Expert Opinion on Orphan Drug. 2013;1:491-7

Based on the above, tyrosinemia type I is an appropriate indication for nitisinone.

PMDA considers that there are no particular problems in selecting "tyrosinemia type I" as the indication for nitisinone, given that efficacy in the treatment of patients with HT-1 has been demonstrated by the NTBC and other studies. The indication will be finalized, taking into account the comments made in the Expert Discussion, including the consistency of expressions with those used for indications in foreign countries.

4.(iii).B.(4) Dosage and administration

The applicant explained as follows:

The dosage and administration were selected based on the results of the NTBC study, the only clinical study that evaluated the efficacy and safety, and no dose-finding studies were conducted. In the pilot study (5.4-16) in which nitisinone was given to patients for the first time, nitisinone was first administered at a dose of 0.1 mg/kg/day based on the results of non-clinical toxicity studies, but was increased to 0.6 mg/kg/day because of elevated 5-ALA excretion in urine. Based on the results of the study, 0.6 mg/kg/day was recommended thereafter. However, among an increasing number of patients treated with nitisinone, plasma SA did not subside sufficiently, or even increased in many cases, with signs of persistent high urine 5-ALA levels or of high urine SA levels due to the recurrence of inhibition of erythrocyte PBG synthase activity. At the daily dose of 0.6 mg/kg/day, nitisinone was not effective enough particularly in infants, and the decision was made to increase the initial daily dose to 1 mg/kg in patients with acute form. The blood nitisinone concentration to obtain the desired biochemical effects in patients with HT-1 was determined to be at least 20 to 30 μmol/L [see "4.(ii).B. Relationship between blood nitisinone concentration and efficacy"]; however, it was suggested that this concentration may not be obtained at the dose level of 0.6 mg/kg/day of nitisinone. Although no consensus has been found in the subsequent literatures on the recommended blood nitisinone concentrations, it is estimated to be in the order of 30 to 50 μmol/L,[90] which does not differ greatly from 20 to 30 μmol/L, the concentration recommended in the NTBC study. The blood nitisinone concentration normalized by the levels of doses administered in the first year of treatment for the recommended dose group (0.7-1.5 mg/kg) in the complementary analysis of the NTBC study was 28, 31, and 36 μmol/L per mg/kg for the treatment start ages of 0 to 6 months, 6 to 24 months, and >24 months, respectively; therefore, the recommended dose was determined to be 1 mg/kg/day. In fact, 207 patients received nitisinone during the period covered by the main analysis of the NTBC study, and only 5 patients received starting doses of >1.2 mg/kg/day, and many of the 5 patients received reduced doses subsequently. When the response is inadequate, dose adjustment was recommended on an individual basis. The highest dose used was 3.0 mg/kg/day. The dose may be increased up to 2 mg/kg/day for patients who do not respond to the treatment adequately, because it can be attributable to lower-than-effective blood nitisinone concentrations. On the other hand, because of insufficient

90 De Laet C, et al., *Orphanet J Rate Dis.* 2013;8:8

experience in the administration of higher doses in the NTBC study, it was determined that dose levels exceeding 2 mg/kg/day should be avoided. The Japanese patient who was enrolled in the NTBC study received a dose of approximately 1 mg/kg/day (0.86-0.94 mg/kg/day), and the blood nitisinone concentration was approximately 20 μmol/L (20.6-26.5 μmol/L) after ≥2 months of treatment.

With regard to dosage regimen, in the pilot study, nitisinone was initially administered 3 times daily. In the NTBC study, the daily dose was to be administered in 2 divided doses with no further instructions on dose interval or timing.

PMDA considers as follows:
Although no dose-finding studies have been conducted for nitisinone, there are no particular problems with respect to the initial dose of 1 mg/kg/day in 2 divided doses for the following reasons: from the treatment experience in the NTBC study and other studies, the initial dose of nitisinone was stepped up to the recommended dose of 1 mg/kg/day in 2 divided doses; the dose of 1 mg/kg/day is expected to provide a sufficient blood nitisinone concentration for the desired biochemical effect in HT-1 patients. On the other hand, the dose needs to be increased in patients whose response is unsatisfactory because their blood nitisinone concentration may not be in the effective range; however, the applicant did not provide a clear rationale for the selection of a maximum daily dose of 2 mg/kg. Therefore, it is inevitable to select a maximum daily dose of 2 mg/kg in line with the labels approved by foreign authorities, given that the maximum daily dose according to the dosage and administration approved in the US and Europe is 2 mg/kg, and that no specific problems have been reported at doses up to 2 mg/kg/day, although the maximum dose is difficult to assess accurately from clinical study results available. Because the number of Japanese patients investigated in previous studies is extremely limited, and also because of the lack of efficacy and safety details at increased dose levels, it is necessary to continue to collect information on the dosage and administration, efficacy, and safety of nitisinone through the post-marketing surveillance program. Precautionary remarks stating that patients should be treated with nitisinone in combination with dietary restriction of tyrosine and phenylalanine should be provided in the “Precautions for Dosage and Administration” section of the package insert. These issues will be finalized, taking into account the comments made in the Expert Discussion.

4.(iii).B.(5) Special populations

Women who are or may be pregnant, or are breastfeeding

The applicant explained as follows:
Teratogenicity (skeletal abnormalities, external abnormalities including umbilical hernia and gastroschisis) and fetal toxicity were reported in reproductive and developmental toxicity studies. Corneal opacity and weight decreased have also been reported as toxic effects in newborns via breastfeeding. The effects on bone in fetuses and corneal opacity in newborns via breastfeeding represent changes for which NOAEL has not been established; in addition, species difference in the sensitivity to nitisinone between humans and other animals is unknown. Therefore, the following

precautionary statements should be provided: women who are or may be pregnant, or are breastfeeding should use nitisinone only when the expected therapeutic benefits outweigh the possible risks, and breastfeeding women should stop breastfeeding immediately when treatment with nitisinone is initiated.

PMDA considers that there are no particular problems in the precautionary statement concerning administration to women who are or may be pregnant, or are breastfeeding; however, all women of reproductive age should be informed that nitisinone was found to have teratogenicity in the non-clinical studies.

4.(iii).B.(6) Post-marketing investigations

The applicant explained as follows:

The drug use results survey will be conducted in all patients to be treated with nitisinone to evaluate the safety and efficacy of nitisinone in routine clinical use. In the drug use results survey, the following information will be collected: patients' characteristics, status of their dietary restriction, urine SA and serum α-fetoprotein levels, results of ophthalmological examinations, and clinical findings.

PMDA considers as follows:

It is appropriate to collect safety and efficacy data from all patients to be treated with nitisinone, considering the extremely limited number of patients studied in Japan; however, the specific survey methodology, duration of survey, and survey items will be finalized, taking into account the comments made in the Expert Discussion.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection and data integrity assessment were conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

No GCP on-site inspection has been conducted because data requiring GCP on-site inspection were not included in this submission.

IV. Overall Evaluation

Based on the submitted data, which have demonstrated the efficacy of nitisinone in HT-1 patients, PMDA considers that the safety of nitisinone is acceptable in light of its observed benefits. Nitisinone is expected to provide a new option in the treatment of patients with HT-1, and is therefore of clinical

significance. It is important to study the safety and efficacy of nitisinone based on the data collected from all patients treated with nitisinone via the post-marketing surveillance because the number of Japanese patients investigated in submitted studies was extremely limited, resulting in limitations on the evaluation of safety and efficacy, and because nitisinone is to be administered for a long period of time.

PMDA considers that the product may be approved if it can be concluded based on the comments from the Expert Discussion that there are no particular problems.

Review Report (2)

October 3, 2014

I. Product Submitted for Registration

[Brand name]	Orfadin Capsules 2 mg, 5 mg, and 10 mg
[Non-proprietary name]	Nitisinone
[Applicant]	Astellas Pharma Inc.
[Date of application]	December 25, 2013

II. Content of the Review

The outline of the comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) is described in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations etc., concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc., by Pharmaceuticals and Medical Devices Agency" (PMDA administrative Rule No. 8/2008 dated December 25, 2008).

(1) Efficacy

PMDA considered as follows:

It was difficult to fully evaluate the efficacy of Orfadin Capsules 2 mg, 5 mg, and 10 mg (hereinafter referred to as nitisinone) based on the results of studies including the NTBC study in which only 1 Japanese patient was enrolled, and the submitted clinical study data consisted only of reference data. However, it can be concluded that the efficacy of nitisinone has been demonstrated for the following reasons: (i) although it is difficult to perform a strict comparison, the comparison of the estimated survival rate of patients treated only with dietary restriction of tyrosine and phenylalanine from the data of the international survey cohort on patients with type-I hereditary tyrosinemia (HT-1) reported by van Spronsen et al., and the survival provided by the NTBC study suggests that survival may be improved by nitisinone treatment; (ii) urine SA and other HT-1 specific biochemical parameters were controlled to be within the reference range by nitisinone treatment, and thus tended to be stabilized by the treatment in combination with dietary restriction of tyrosine and phenylalanine.

The above conclusion by PMDA was supported by the expert advisors.

(2) Safety

PMDA considered as follows:

It was difficult to fully evaluate the safety of nitisinone, based on the results of studies including the NTBC study in which only 1 Japanese patient was enrolled, and the submitted clinical study data consisted only of reference data. However, safety information has been obtained from the NTBC study, as well as from clinical experience (after the approvals in the US and Europe) at a certain level. Therefore, the safety of nitisinone is acceptable if nitisinone is administered in combination with dietary restriction of tyrosine and phenylalanine, and if appropriate precautionary statements are

provided. Data on eye disorders, leukopenia, granulocytopenia, and thrombocytopenia, and skin symptoms should be continuously collected through the post-marketing surveillance program.

The above conclusion by PMDA was supported by the expert advisors [see "(5) Risk Management Plan (draft)" for post-marketing investigations].

(3) Indications

PMDA considers that there are no particular problems in selecting "tyrosinemia type I" as the indication for nitisinone, given that efficacy in the treatment of patients with HT-1 has been demonstrated by the NTBC study and other studies.

The above conclusion by PMDA was supported by the expert advisors.

(4) Dosage and administration

PMDA considered as follows:

Although no dose-finding studies have been conducted for nitisinone, there are no particular problems with respect to the initial dose of 1 mg/kg/day in 2 divided doses for the following reasons: from the treatment experience in the NTBC and other studies, the initial dose of nitisinone was stepped up to the recommended dose of 1 mg/kg/day in 2 divided doses; the dose of 1 mg/kg/day is expected to provide a sufficient blood nitisinone concentration for the desired biochemical effect in HT-1 patients. On the other hand, the dose needs to be increased in patients whose response to the initial dose is inadequate because their blood nitisinone concentrations may not be in the effective range; however, the applicant did not provide a clear rationale for the selection of a maximum daily dose of 2 mg/kg. The maximum dose is difficult to assess accurately from clinical study results available, but it is inevitable to select a maximum daily dose of 2 mg/kg in line with the labels approved by foreign authorities, given that the maximum daily dose according to the dosage and administration approved in the US and Europe is 2 mg/kg, and that no specific problems have been reported at doses up to 2 mg/kg/day. Because the number of Japanese patients investigated in submitted studies is extremely limited, and also because of the lack of efficacy and safety details at increased dose levels, it is necessary to continue to collect information on the dosage and administration, efficacy, and safety of nitisinone through the post-marketing surveillance program. Precautionary remarks stating that patients should be treated with nitisinone in combination with dietary restriction of tyrosine and phenylalanine should be provided in the "Precautions for Dosage and Administration" section of the package insert. These issues will be finalized, taking into account the comments made in the Expert Discussion.

The above conclusion by PMDA was supported by the expert advisors.

PMDA requested the applicant to correct the description of "Dosage and administration" as shown below and provide remarks stating that patients should be treated with nitisinone in combination with

dietary restriction of tyrosine and phenylalanine in the "Precautions for Dosage and Administration" section of the package insert.

[Dosage and administration]
The usual dosage is 1 mg/kg/day as nitisinone divided in 2 doses administered orally.
The dose may be adjusted according to the patient's condition. The maximum dose is 2 mg/kg/day.
(Underlined phrases indicate revisions)

The applicant responded as follows:
The applicant will make revisions to the dosage and administration as shown above, and provide in the "Precautions for Dosage and Administration" section of the package insert a statement to the effect that patients should be treated with nitisinone in combination with dietary restriction of tyrosine and phenylalanine. PMDA accepted the applicant's response.

(5) Risk Management Plan (draft)

Based on the discussions in the "4.(iii).B.(6) Post-marketing investigations" section of the Review Report (1) and comments of the expert advisors for the Expert Discussion, PMDA has concluded that it is appropriate to take the following measures specified in the current Risk Management Plan (draft) for nitisinone: safety specification and efficacy follow-up studies shown in Table 23; and additional pharmacovigilance activities and risk minimization activities as well as the use results survey to be conducted during the re-examination period on an all-case surveillance basis as shown in Tables 24 and 25.

Table 23. Considerations on safety and efficacy in the Risk Management Plan (draft)

Safety Specification		
Important identified risks	Important potential risks	Important missing information
• Eye disorders • Thrombocytopenia, leukopenia, and granulocytopenia	• Effects of elevated tyrosine concentrations[a)] • Developmental and cognitive disorder • Reproductive and developmental toxicity	• Effects by age
Efficacy Follow-up Studies		
• Efficacy in long-term use		

a) Effects of elevated tyrosine concentrations excluding eye disorders and developmental and cognitive disorders (including skin disorders)

Table 24. Outlines of additional pharmacovigilance activities and risk minimization activity in the Risk Management Plan (draft)

Additional pharmacovigilance activity	Additional risk minimization activity
• Early post-marketing phase vigilance • Use results survey (all-case surveillance)	• Providing information obtained from early post-marketing phase vigilance • Preparation and distribution of information leaflet to promote the proper use (information leaflets for medical professionals and patients)

Table 25. Outline (draft) of use-results survey

Objectives	To study safety and efficacy of nitisinone in patients with HT-1 in routine clinical use
Survey method	On an all-case surveillance basis
Target patients	All patients who were treated with nitisinone
Observation period	Observation period per patient is up to 7 years
Estimated number of patients	All patients who were treated with nitisinone
Major survey items	Patient characteristics, presence/absence of pregnancy, presence/absence of breastfeeding, administration status of nitisinone, concomitantly used drugs, status of tyrosine and phenylalanine dietary restriction, plasma tyrosine concentrations, results of ophthalmological examination, developmental and cognitive disorder, efficacy evaluation (urine SA, serum α-fetoprotein, clinical conditions related to liver function), safety evaluation (eye disorders, skin disorders, thrombocytopenia, leukopenia, granulocytopenia, adverse events, and the like)

III. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved after modifying the indication and dosage and administration as shown below, with the following conditions. As it is a drug with a new active ingredient, the re-examination period is 8 years. The drug substance and the drug product are both classified as powerful drugs, and the product is not classified as a biological product or a specified biological product.

[Indication] Tyrosinemia type I

[Dosage and administration] The usual dosage is 1 mg/kg/day as nitisinone divided in 2 doses administered orally. The dose may be adjusted according to the patient's condition. The maximum dose is 2 mg/kg/day.

[Conditions for approval] The applicant is required to:

- Prepare a Risk Management Plan, and implement it appropriately.
- Conduct a post-marketing drug use-results survey covering all patients treated with the product during the re-examination period because clinical experiences in Japan are extremely limited. Based on the survey data, identify characteristics of patients treated with the product and compile safety and efficacy data for the product in the early post-marketing period, thereby taking necessary measures to ensure proper use of the product.