Clinical Study Report

A non-interventional Post Authorization Safety Study (PASS) to evaluate long-term safety of Orfadin treatment in hypertyrosinemia type 1 (HT-1) patients in standard clinical care

PASS information

Title	A non-interventional Post Authorization Safety Study (PASS) to evaluate long-term safety of Orfadin treatment in hypertyrosinemia type 1 (HT-1) patients in standard clinical care
Version identifier of the final study report	1.0
Date of last version of the final study report	March 27, 2020
EU PAS register number	EUPAS3636
Active substance	ATC code: A16A X04, other alimentary tract and metabolism products Nitisinone, 2-[2-nitro-4-(trifluoromethyl)benzoyl] cyclohexane-1,3-dione
Medicinal product	Orfadin capsules, 2 mg, 5 mg, 10 mg, 20 mg and Orfadin oral suspension 4 mg/ml
Product reference	EU/1/04/303/001-005
Procedure number	EMEA/H/C/000555
Marketing authorization holder	Swedish Orphan Biovitrum International AB, SE-112 76 Stockholm, Sweden
Joint PASS	No
Research question and objectives	The primary objective was to assess long-term safety of Orfadin used in standard clinical practice to treat patients with HT-1
Countries of study	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, the Netherlands, Norway, Poland, Portugal, Spain, Sweden, United Kingdom
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Marketing authorization holder

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Table of contents

Table of tables

Table of figures

1 Abstract

Title: A non-interventional Post Authorization Safety Study (PASS) to evaluate long-term safety of Orfadin treatment in hypertyrosinemia type 1 (HT-1) patients in standard clinical care.

Keywords: hereditary tyrosinemia, HT-1, nitisinone, Orfadin, non-interventional study, long-term safety

Rationale and background: Orfadin® (nitisinone) was approved by the European Medicines Agency (EMA) in 2005 for the treatment of HT-1. As required by the Committee for Medicinal Products for Human Use (CHMP), a post-marketing surveillance program (named Orfadin Active Surveillance Program, OAS) to monitor hepatic, renal, hematological, neurological and ophthalmic status in all patients treated with Orfadin was initiated in 2005. At the annual re-assessment in 2009, the CHMP concluded that the benefit-risk balance was positive. However, the Marketing authorization holder was requested to continue collecting and reporting safety data on an annual basis. In 2013, OAS was transformed into a PASS.

Research question and objective: The primary objective was to assess long-term safety of Orfadin used in standard clinical practice to treat patients with HT-1.

Study design: A long-term, non-interventional, non-comparative, multicenter PASS in HT-1 patients to collect retrospective and prospective longitudinal data. Data were collected from patients receiving Orfadin either at the time of market authorization in the EU (February 21, 2005) or starting treatment after this date. The patients were followed as long as they were treated or to the end of the study (September 30, 2019).

Setting: 77 sites in 17 European countries enrolled patients.

Subjects and study size, including dropouts: All HT-1 patients on Orfadin treatment in standard clinical care at the time of study start and patients diagnosed and starting Orfadin treatment during the time of the study were included. No specific exclusion criteria were applied.

Data from 315 patients were retrieved from February 2005 until end of the study. Additionally, data from 24 patients who had liver transplants or died during OAS before the PASS was initiated were retrieved.

Variables and data sources: Primary endpoints: Occurrence of adverse events (AE) related to hepatic, renal, ophthalmic, hematological or cognitive/developmental function, respectively. Secondary endpoints: Occurrence of liver transplantation or death, occurrence of hepatic malignancies, other malignancies or other AEs, discontinuation of Orfadin treatment, incorrect administration of the oral suspension formulation of Orfadin, laboratory investigations, compliance with Orfadin treatment and diet, extent of exposure to Orfadin, and overall clinical condition.

After initiation of the PASS, prospective and retrospective information was reported using an electronic case report from (eCRF). Retrospective information reported in paper-based CRF as part of OAS for patients consenting to participate in the PASS (excluding AEs reported by the investigators) were migrated to the eCRF for review by the investigator.

Results: The incidences of hepatic, renal, ophthalmic, hematological, or cognitive/ developmental function AEs were low for all AE categories. For hepatic function and

hematological status AEs, there was a trend of increased incidence of these events with higher age at start of Orfadin treatment.

36 patients (10.6 %) in the extended mortality/liver transplantation set either died or were liver transplanted and the occurrence of death or liver transplantation was more frequent the later the treatment was initiated.

4 patients (1.3 %) had reports of hepatic malignancy. No other malignancies were reported.

There were no reported events of incorrect administration of oral suspension of Orfadin.

AEs other than those related to the primary endpoint were reported with low frequency.

11 patients (3.5%) discontinued Orfadin treatment and 8 of these were due to liver transplantations, mostly in patients with late (≥6 months of age) treatment start.

The results from the laboratory investigations confirmed increased values of plasma phenylalanine and tyrosine over time, which seemed to correlate with data indicating a declined diet compliance. Even though mean plasma tyrosine was >500 µmol/L from Treatment Year 4 and onwards, less than 5 % (15 patients) experienced ophthalmic AEs. At most treatment years there were no patients with clinically relevant abnormal succinylacetone values, as judged by the Investigator.

88.6 % of the patients were assessed as having either “very good” or “good” treatment compliance throughout the study. All patients were on a diet low in phenylalanine and tyrosine at study start and the proportion remained high during the study. The compliance with Orfadin treatment and diet seemed to decline as patients grew older.

The cumulative exposure to Orfadin during the study was 3172.7 patient years. The mean daily weight-based dose of Orfadin decreased over treatment time and was highest in the infants and lowest in the adults.

Most patients were reported to have “good” (highest level of available categories for reporting) overall clinical condition throughout the study indicating a sustained effect of Orfadin during long-term exposure up to 15 years.

Discussion: These long-term results confirm the safety profile of Orfadin without any new safety findings. Thus, up to 15 years’ treatment with Orfadin in HT-1 patients was well tolerated, with no overall increase in occurrence of AEs over time. These data have not changed the benefit-risk assessment and, given the severity of HT-1 and the safety profile of Orfadin, the benefit/risk ratio remains positive.

Marketing authorization holder: Swedish Orphan Biovitrum International AB, SE-112 76 Stockholm, Sweden

Name and affiliation of International Coordinating investigator: Prof. Ute Spiekerkötter, MD, University Medical Center Freiburg, Department of Pediatric and Adolescent Medicine, Mathildenstr. 1, DE-79106 Freiburg, Germany.

2 List of abbreviations and definitions of terms

Abbreviation	Term
AE	Adverse event
AFP	Alpha-fetoprotein
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CRF	Case report form
CRO	Contract research organization
eCRF	Electronic case report form
DBL	Database lock
DBS	Dried blood spot
EEA	European Economic Area
EMA	European Medicines Agency
EU	European Union
FDA	US Food and Drug Administration
HLGT	High level group term (MedDRA)
HLT	High level term (MedDRA)
HT-1	Hereditary tyrosinemia type 1
ICF	Informed consent form
IQR	Interquartile range
MAH	Marketing authorization holder
MedDRA	Medical dictionary for regulatory activities
NDA	New Drug Application
NEC	Not elsewhere classified
OAS	Orfadin Active Surveillance Program
p-AFP	Plasma alpha-fetoprotein
PASS	Post authorization safety study
PMC	Post-marketing committment
PMS	Post-marketing surveillance
p-Phe	Plasma phenylalanine
PRAC	Pharmacovigilance Risk Assessment Committee
PSUR	Periodic safety update report
PT	Preferred term
p-Tyr	Plasma tyrosine
RMP§	Risk management plan
SA	Succinylacetone
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation

SmPC	Summary of product characteristics
SMQ	Standardized MedDRA Query
SOC	System organ class
Sobi	Swedish Orphan Biovitrum
UK	United Kingdom
USPI	United States Prescribing Information

3 Investigators

A list of names and affiliations of participating investigators are available on request as a stand-alone document (see Annex 1).

4 Other responsible parties

A list of Sobi study personnel and a list of responsible parties and names and affiliations of contractors involved in the conduct of the study are available on request as stand-alone documents (see Annex 1).

5 Milestones

Milestone	Planned date	Actual date	Comments
Start of data collection	February 2005	February 2005	Start date for OAS, and therefore also start date for the prospective collection of data used in the analysis of this study. During 2013, the original OAS was transformed into a PASS.
End of data collection	September 2017	September 2019	The study was prolonged to allow for inclusion of patients from countries where the study start was delayed due to administrative reasons. Also, prolongation would add valuable information on long-term Orfadin treatment of HT-1 patients, who are ageing. Lastly, the prolongation allowed for inclusion of additional patients on Orfadin oral suspension.
Registration in the EU PASS register	---	June 28, 2013	There was no planned date in the study protocol for the registration.
Study progress report in PSUR no. 11	April 2014	April 2014	Data cut-off for the PSUR was in line with submission of the yearly PSUR in April.
PSUR 12	April 2015	April 2015	Ditto
PSUR 13	April 2016	April 2016	Ditto
PSUR 14	April 2017	April 2017	Ditto
PSUR 15	April 2018	April 2018	Ditto
PSUR 16	April 2019	April 2019	Ditto
PSUR 17	April 2020	April 2020	Ditto
Final report of study results	April 2020	March 2020	

Abbreviations: EMA, European Medicines Agency; HT-1, Hereditary tyrosinemia type 1; OAS, Orfadin Active Surveillance Program; PSUR, Periodic Safety Update Report.

6 Rationale and background

HT-1 is an orphan disease caused by a defect in fumarylacetoacetate hydrolase, the final enzyme in the pathway of the degradation of tyrosine. As a result, toxic metabolites are formed, primarily in the liver and kidneys. One of the metabolites is SA which can be readily detected in plasma or urine of affected (untreated) patients. This is a pathognomic test for the diagnosis of HT-1. Tyrosine levels in plasma might be raised by 0 to 30 %. The liver is often the most severely affected organ. If the disease is untreated, patients will develop cirrhosis and liver nodules, potentially resulting in hepatocellular carcinoma. AFP is a marker of liver regeneration and is often elevated in newly diagnosed patients due to ongoing liver damage by formation of the toxic tyrosine metabolites. It could also signal development of malignancy. Patients can present with acute, subacute or chronic forms of HT-1. Patients with the acute form generally present with liver failure before the age of 6 months. The subacute form is somewhat less severe but will usually result in liver disease before one year of age, whereas the chronic form mainly results in liver cirrhosis and/or kidney disease. Newborn screening using SA has been implemented in some regions but is still lacking in many parts of Europe. Newborn screening using tyrosine is not specific and may at the same time miss to detect HT-1 patients because tyrosine can be normal in undiagnosed HT-1 patients.

Before 1991, treatment of HT-1 was based on diet to lower tyrosine intake, and eventually liver transplantation. However, the prognosis was generally very poor (1, 2). In 1991, an open-label study with nitisinone was initiated (3).

Nitisinone has been developed for the treatment of HT-1 and is an enzymatic competitive inhibitor of 4-hydroxyphenylpyruvate dioxygenase, an enzyme of the tyrosine catabolic pathway. By inhibiting this enzyme, nitisinone prevents the accumulation of the toxic intermediates maleylacetoacetate and fumarylacetoacetate as well as SA. Successful treatment leads to rapid decrease in plasma and urine SA to levels below the limit of quantitation. Following this, other liver parameters such as transaminases, AFP and bilirubin will normalize. The liver echogram will usually also improve. However, late initiation of treatment cannot rectify all liver damage and the risk of developing liver malignancy remains elevated.

Orfadin® (nitisinone) was approved by the EMA on February 21, 2005 under exceptional circumstances, and has become, in combination with dietary restrictions of tyrosine and phenylalanine, standard of care for HT-1 (4). At the time of approval, the MAH was required by the CHMP to conduct a PMS program (named “Orfadin Active Surveillance Program”; OAS). The purpose of OAS was to stimulate reporting of AEs and to capture additional safety data in HT-1 patients treated with Orfadin, particularly focusing on hepatic, renal, hematological, neurological and ophthalmic status. Thus, a questionnaire collecting safety data and additional data on demographics and dosage was completed for each patient by the treating physician. OAS involved 30 countries (Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the UK).

For patients treated with Orfadin in the registration studies, the following common adverse reactions were reported; thrombocytopenia, leucopenia, granulocytopenia, conjunctivitis, corneal

opacity, keratitis, photophobia and eye pain. Elevated plasma levels of tyrosine have been associated with the eye-related AEs because tyrosine at high concentrations can crystalize in the eye. It is therefore important to maintain a low tyrosine and phenylalanine diet to control plasma tyrosine levels. It has also been hypothesized that increased tyrosine levels have negative effects on neurocognitive function and behavior (5), however clear evidence of this association is still missing. In addition to the possibility of increased tyrosine levels other potential causes hypothesized in literature are the disease itself or its treatment (6), the expression of fumarylacetoacetate hydrolase in the brain but not 4-hydroxyphenylpyruvate dioxygenase (7) and low phenylalanine levels during development (8).

During the annual re-assessment in 2009, CHMP concluded the risk-benefit balance remained positive, and considered that the PMS had met its purpose. In conclusion, the obligation was considered fulfilled and the exceptional circumstances were lifted. However, the MAH was requested to continue to collect data and to report the results in annual PSURs.

Transformation of OAS into a PASS

In 2013, the MAH decided to develop a study protocol for the conduct of OAS as a PASS and the ongoing OAS was transformed into a non-interventional voluntary PASS named OPAL (study code Sobi.NTBC-005). The scope of the PASS was to collect prospective and retrospective safety data of Orfadin since the EU approval. The protocol of the PASS was developed in accordance with the EMA "Guidance for the format and content of the protocol of non-interventional post-authorization safety studies" (9). The data items agreed with the EMA for OAS, were generally not changed in the study protocol for the PASS to maintain consistency with data already collected. However, laboratory assessments for tyrosine, phenylalanine, nitisinone plasma concentration and AFP were added, as well as minor adjustments to classification of dietary and treatment compliance. By the time of the finalization of the first version of the PASS study protocol, data in OAS had been collected from approximately 400 patients. The transition was gradual, starting in 2013 in countries with the largest number of HT-1 patients and in total 17 countries (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, the Netherlands, Norway, Poland, Portugal, Spain, Sweden, and the UK) in EU and EEA were included in the PASS.

In a line extension application for an Orfadin oral suspension formulation, submitted to EMA in 2013 (EMEA/H/C/000555/X/41), the PASS was added as a pharmacovigilance activity in the updated RMP and the study protocol was submitted for the first time as an annex to the RMP. During the review of the oral suspension formulation, approved in 2015, the PRAC suggested additional items to be included in the PASS. The study protocol was therefore amended to include e.g., "incorrect administration of the oral suspension formulation of Orfadin" as a secondary endpoint and further evaluations as described in Section 8.

When Orfadin was approved in 2005, the Orfadin SmPC recommended twice daily dosage. As knowledge of once daily posology was gained through clinical research (study Sobi.NTBC-003), this posology was also incorporated into the Orfadin SmPC, approved by EMA in January 2017 (EMEA/H/C/000555/II/0057) and in the USPI, approved by the FDA September 2017 (NDA 021232/S-20 and NDA 206356/S-03). Following a PMC from the FDA to the MAH in 2017 to investigate the use of Orfadin when used on a once daily basis, analyses regarding patients who received Orfadin treatment once daily during the study were added in the SAP.

7 Research question and objectives

The primary objective of the present PASS was to assess long-term safety of Orfadin used in standard clinical practice to treat patients with HT-1, by evaluation of occurrence of AEs related to hepatic, renal, ophthalmic, hematological functions, or cognitive/development, respectively.

The study had a number of secondary endpoints, to further assess the long-term safety, including: occurrence of liver transplantation or death, occurrence of liver transplantation, occurrence of death, occurrence of hepatic malignancies, other malignancies, other AEs, discontinuation of Orfadin treatment and incorrect administration of the oral suspension formulation of Orfadin, laboratory investigations, treatment and diet compliance, extent of exposure to Orfadin, and overall clinical condition, as rated by the investigator.

8 Amendments and updates

There was one substantial amendment to the study protocol dated September 22, 2015, originating from the PRAC review of the line extension application for the Orfadin oral suspension formulation (Table 1).

Table 1 Substantial study protocol amendment

Number	Date	Section of study protocol	Amendment or update	Reason
Substantial Amendment 1.0	2015-09-22	Section 4 (Abstract) and Section 9.3.2	Secondary objectives updated with clarification on how to handle hepatic malignancies, other malignancies and incorrect administration of the oral suspension formulation of Orfadin	Amendments made to reflect comments received from the EMA PRAC (procedure EMEA/H/C/000555/X/41) partly based on a PDCO Opinion (EMA/PDCO/389530/2013)
		Section 10.5	De-identification of patient data was updated	Local data handling requirements regarding not enter patient's date of birth
		Section 11.2	Eliciting and recording AE was updated: the investigator will in addition ask for the occurrence of any significant deterioration in clinical status of hepatic malignancies, other malignancies and incorrect administration of the oral suspension formulation of Orfadin.	Amendments made to reflect comments received from the EMA PRAC (procedure EMEA/H/C/000555/X/41)

Abbreviations: AE, Adverse event; EMA, European Medicines Agency; PDCO, Paediatric Committee; PRAC, Pharmacovigilance Risk Assessment Committee.

9 Research methods

9.1 Study design

This study was designed as a long-term non-interventional, non-comparative, multicenter PASS to collect retrospective and prospective longitudinal data in the normal clinical setting of patients with HT-1. Data were collected from patients receiving Orfadin at the time of market authorization (i.e., February 21, 2005; referred to as the index date) as well as patients starting treatment after this date (see also Section 6). No outcome data were collected for the period prior to the index date. Patients were to be followed as long as they were treated, or to the end of the study (September 30, 2019; last study data collection date).

The primary endpoints were the occurrence of AEs related to hepatic, renal, ophthalmic, hematological or cognitive/developmental function, respectively.

The design of this PASS, including the primary endpoints, was endorsed by the PRAC and approved by the EU Commission.

9.2 Setting

This was a European multicenter study in 17 countries (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Spain, Sweden, and UK). A total of 88 study sites were initiated and, of those, 77 sites enrolled at least one patient with start of data collection on February 21, 2005, ending on September 30, 2019.

To support investigators' interest in patient recruitment and entry of data, the Sponsor regularly distributed newsletters to the study sites and Sponsor personnel regularly contacted or visited the study sites and organized Investigators' Meetings.

9.3 Subjects

All HT-1 patients on Orfadin treatment in standard clinical care at study initiation as well as patients diagnosed and starting Orfadin treatment during the time of the study were included. No specific exclusion criteria were applied. Hence, the study population was highly representative of the source population.

Investigators were asked to follow-up on their patients at least annually during the study period.

Written informed consent was required for all patients prior to study entry. For patients who previously participated in OAS, retrospective data available from OAS were migrated into the eCRF for the PASS. For these patients, written informed consent was required before any new data were entered in the eCRF by the site staff.

9.4 Variables

9.4.1 Primary endpoints

The primary endpoints were occurrence of AEs related to hepatic, renal, ophthalmic, hematological, or cognitive/developmental function, respectively.

At each routine patient visit and at least once every year, the investigator was requested to record the occurrence of any significant deterioration in clinical status of the above functions occurring since the previous assessment.

The definitions of AEs related to the respective area above used for the evaluation of the primary variable were based on MedDRA SOC, HLGT, HLT, and PT. Occurrence of an AE is defined as at least one AE in any of the PTs or HLGTs listed within a category.

Hepatic AE

SOC: Hepatobiliary disorders:

- HLGT: Hepatic and hepatobiliary disorders.
- HLGT: Hepatobiliary neoplasms.

SOC: Neoplasms benign, malignant and unspecified (including cysts and polyps) (NEOPL):

- HLGT: Hepatobiliary neoplasms malignant and unspecified.

SOC: Investigations:

- HLGT: Hepatobiliary investigations.
- PT: AFP increased.
- PT: AFP abnormal.
- PT: AFP decreased.

SOC: Surgical and medical procedures:

- HLT: Hepatic therapeutic procedures (excluding PT liver transplant).

Renal AE

SOC: Renal and urinary disorders:

- HLGT: Nephropathies.
- HLGT: Renal disorders (excluding nephropathies).
- HLT: Renal lithiasis.

SOC: Investigations:

- PT: Renal function test abnormal.
- SOC: Metabolism and nutrition disorders.
- PT: Rickets.

SOC: Musculoskeletal and connective tissue:

- PT: Hypophosphataemic osteomalacia.

Ophthalmic AESOC: Eye disordersSOC: Investigations:

- PT: Ophthalmological examination abnormal.

Hematological AE

SOC: Blood and lymphatic system disorders

SOC: Investigation:

- HLGT: Hematology investigations (including blood groups).
- PT: Blood test abnormal.

Cognitive/developmental function AE

SOC: General disorders and administration site conditions:

- PT: Developmental delay.

SOC: Psychiatric disorders:

- HLGT: Cognitive and attention disorders and disturbances.
- HLGT: Developmental disorders NEC.

SOC: Nervous system disorders:

- HLT: Developmental disorders cognitive.
- HLGT: Neurological disorders NEC.
- HLGT: Mental impairment disorders.

Except for the collection of these cognitive/developmental function AEs, no other assessments of cognitive function were performed in the study.

9.4.2 Secondary endpoints

9.4.2.1 Occurrence of liver transplantation or death

Prospective information on occurrence of liver transplantation or death was recorded in the study database. Retrospective information on Orfadin treated patients with liver transplantation or death during OAS prior to initiation of this PASS, was collected from the Sobi Drug Safety Database.

9.4.2.2 Occurrence of death

Prospective information on occurrence of death was recorded in the study database. Retrospective information on Orfadin treated patients deceased during OAS prior to initiation of this PASS, was collected from the Sobi Drug Safety Database.

9.4.2.3 Occurrence of liver transplantation

Prospective information on occurrence of liver transplantation was recorded in the study database. Retrospective information on patients with liver transplantation during Orfadin treatment during OAS, but no longer on treatment at the time of initiation of this study, was collected from the Sobi Drug Safety Database.

9.4.2.4 Occurrence of hepatic malignancies

Hepatic malignancies were reported after solicited questions to the investigator for the occurrence of any significant deterioration in clinical status.

The definition of hepatic malignancies was based on MedDRA SOC term and HLT.

SOC Neoplasms benign, malignant and unspecified (including cysts and polyps) (NEOPL):

- HLT: Hepatic neoplasms malignant.
- HLT: Hepatobiliary neoplasms malignancy unspecified.
- HLT: Hepatobiliary neoplasms NEC.
- HLT: Hepatoblastomas.

9.4.2.5 Occurrence of other malignancies

Other malignancies were reported after solicited questions to the investigator for the occurrence of any significant deterioration in clinical status.

The definition of other malignancies was based on MedDRA SOC terms, HLT and HLLGT.

SOC Neoplasms benign, malignant and unspecified (including cysts and polyps):

- Excluding HLT: Hepatic neoplasms malignant.
- Excluding HLT: Hepatobiliary neoplasms malignancy unspecified.
- Excluding HLT: Hepatobiliary neoplasms NEC.
- Excluding HLT: Hepatoblastomas.
- Excluding all HLGT terms containing “benign”.

9.4.2.6 Occurrence of incorrect administration of the oral suspension formulation of Orfadin

The investigator was elicited at each patient’s study visit (after oral suspension became available) to record the occurrence of any incorrect administration of the oral suspension formulation since the previous assessment.

Potential types of medication errors that might have been reported as an AE were:

- The suspension was not properly re-suspended before use.
- The wrong oral dispenser was used.
- The incorrect dose was withdrawn into the dispenser.
- The dose was not given immediately after being withdrawn into the dispenser.
- The oral dispenser was not adequately rinsed after use.

Incorrect administrations of the oral suspension formulation were captured as medication errors in the AE reports, based on SMQ Medication error.

9.4.2.7 Occurrence of other adverse events

Occurrence of AEs other than those related to hepatic, renal, ophthalmic, hematological or cognitive/developmental functions, or not previously identified as a primary or secondary endpoint were reported.

9.4.2.8 Discontinuation of Orfadin treatment

Treatment interruption or treatment discontinuation including reason(s) for discontinuation specified as death, liver transplantation or other reason were reported.

9.4.2.9 Laboratory investigations

Plasma, serum, or DBS and/or urine concentrations of SA, p-Tyr, p-Phe, and p-AFP were recorded. Investigators were instructed that values judged as clinically significant (rated by the investigator as abnormal, clinically relevant in the CRF) should be reported as AEs.

9.4.2.10 Treatment and diet compliance

The investigators assessed treatment compliance and compliance with diet restrictions (very good, good, poor, very poor, or not applicable).

9.4.2.11 Extent of exposure

Exposure to Orfadin was reported in terms of daily dose, plasma, serum or DBS concentration of nitisinone and duration of treatment.

9.4.2.12 Overall clinical condition

The investigators assessed the patient's overall clinical condition (good, poor, or very poor).

9.4.3 Demographic variables

Demographic variables collected included:

- Age at diagnosis.
- Age at Orfadin treatment start.
- Diagnosed by newborn screening.

Age at Orfadin treatment start was categorized as:

- Newborn (<28 days old).
- ≥28 days to <6 months old.
- ≥6 to <12 months old.
- ≥12 months old.

9.4.4 Medicinal product: dose and route of administration

Commercially available Orfadin capsules, 2, 5, 10 or 20 mg were prescribed according to a weight-based (mg/kg) posology. Originally at study start in 2005, the approved Orfadin SmPC recommended twice daily dosage.

Orfadin oral suspension 4 mg/mL was approved in EU on June 19, 2015, as a suitable option for patients who have difficulties swallowing capsules.

As knowledge of once daily posology was gained through clinical research, this was also incorporated into the Orfadin SmPC, approved in January 2017. The current EU SmPC (2019) recommends once daily dosage from 20 kg body weight, and dose escalation up to a maximum of 2 mg/kg body weight per day based on monitoring of urine SA.

9.5 Data sources and measurement

In this study, prospective and retrospective post authorization safety data since the EU approval of Orfadin on February 21, 2005 were collected. Baseline data were only available for patients diagnosed and prescribed Orfadin treatment during the study period (from February 2005 and later).

All data reported in study should be available in the medical records, i.e., the source for the data is the medical records.

Prospective information during the PASS, including occurrence of AEs, was reported using the eCRF designed for the study and through the standard AE reporting process. The investigators had the final responsibility for the accuracy and authenticity of all patient data entered into the eCRFs. The AE information in the eCRF was transferred into the Sobi Drug Safety Database where the information was verified, and the AEs were assigned MedDRA codes. The information in the eCRF was reconciled with the information in the Sobi Drug Safety Database.

Retrospective information (excluding AEs reported by the investigators) reported in paper-based CRF as part of the preceding OAS were migrated to the eCRF for review by the investigator. AEs reported by investigators in the paper-based CRF were entered into the Sobi Drug Safety Database, where the information was verified and MedDRA codes were assigned.

Retrospective information on Orfadin treated patients deceased before initiation of the PASS, or patients with liver transplantation during Orfadin treatment but no longer on treatment at time of initiation of the PASS, were collected from the Sobi Drug Safety Database.

The Sobi Drug Safety Database is the data source of all investigator-reported AEs presented in this clinical study report.

9.6 Bias

Analyses of the occurrence of death and liver transplantation could underestimate the proportion of HT-1 patients treated with Orfadin with death and/or liver transplantation, because only patients that have survived until the start of data collection in the PASS (starting in 2013) are included in the study analysis sets. Patients who died/were transplanted and stopped treatment during OAS, prior to start of the PASS, would not consent to transfer and are therefore not included in the standard study analysis sets (Section 9.8.3.2).

Therefore, for sensitivity analyses of the occurrence of liver transplantations and death, three additional analysis sets were applied, and these are referred to as the extended analysis sets. Patients who died/were transplanted prior to the index date are included in the extended analysis sets (for further details of the extended analysis sets, see Section 9.8.3.2).

In contrast, analyses based on the extended analysis sets will clearly overestimate the proportion of patients with death and/or liver transplantation as only these cases were added to the standard sets but patients who withdrew for other reasons were not added. Although analyses based on the extended sets will provide an overestimation of death and liver transplantation these are included to provide an alternative worst-case analysis.

9.7 Study size

The sample size was not based on a formal calculation. All HT-1 patients meeting the inclusion criteria were eligible for entry. Approximately 300 to 500 patients were estimated to be included in the study.

The precision of the incidence estimates for the events defined in the primary endpoints for various sample sizes and incidence rates are displayed in Table 2.

Table 2 Precision of the incidence estimates for events defined in the primary endpoints is shown below for various sample sizes and incidence rates

Assumed incidence	Number of patients	Width of two-sided 95% confidence interval for incidence (using normal approximation)
20 %	500	7.0
	400	7.8
	300	9.0
10 %	500	5.2
	400	5.8
	300	6.8
5 %	500	3.8
	400	4.2
	300	5.0
2%	500	2.4
	400	2.8
	300	3.2

9.8 Data transformation

9.8.1 Data management

An eCRF was used in this study. The eCRF allowed the investigator to enter data to the eCRF designed for the study. Each person involved in data entry at each study site had an individual username and password to allow record traceability. Individual access applications were handled by an eCRF vendor.

Data were captured in the eCRF to be completed for each patient at least once yearly. The eCRF included logical checks to prevent data entry errors. Data inconsistencies outside the logical checks were managed by queries directly to the study sites.

Retrospective data collected during OAS (excluding AEs reported by the investigators) were migrated to the eCRF for review of accuracy by the investigators. AEs reported by investigators during OAS were entered into the Sobi Drug Safety Database.

Downloaded data and programs used for analysis were stored and archived in Sobi's Clinical Data Repository. Due to the partially retrospective data set-up, some data queries could not be resolved before DBL and remained unresolved in the database.

Patient data were pseudonymized and patients were assigned a study identification number. Data were entered by site staff only into eCRFs. The pseudonymized data, as entered into the eCRF, was visible to the eCRF vendor, CRO and Sponsor, but only site staff could trace a case ID back to a patient identity. Due to restrictions in the permission to register the date of birth, this was imputed for all patients. In cases where the date of birth was previously collected and kept in the study database to enable the identification of patients by the investigators, the date of birth was excluded from the analysis database.

Prior to DBL, all tasks or criteria defined in the data management plan were completed and documented. The DBL was approved by relevant study personnel and subsequently all edit accesses were removed. Final DBL occurred on October 31, 2019.

9.8.2 Data conversions

9.8.2.1 Conversions of verbal descriptors collected during OAS to PASS terminology

The phrasing of assessments changed for some variables over the years from the initial OAS using paper-based CRFs. The CRF wordings changed during tAS (after the index date while still using paper-based CRFs), to the verbal descriptors used in the eCRF from the start of the PASS. When older data were imported, the data captured in OAS were mapped to the questions and response categories used in the PASS.

Overall clinical condition was in the eCRF rated by the investigator as Good, Poor, Very Poor or Not Assessed. Where older paper-based assessment had been used these were converted to the PASS ordinal scale:

- Well was transformed to Good
- Moderately ill was transformed to Poor
- Severely ill was transformed to Very poor

Orfadin treatment compliance and **Compliance with diet restrictions** were in the electronic data-entry tool rated by the investigator as Very Good, Good, Poor, Very Poor or Unknown. Where older paper-based assessment had been used these were transformed accordingly:

- The older paper-based rating Yes was transformed to Good
- The older paper-based rating No was transformed to Poor

9.8.2.2 Conversions of laboratory units

Laboratory results given in different units according to local laboratory standards, were converted to standard units (Table 3).

Table 3 Standard units employed for laboratory results (converted from local hospital units)

Assay	Standard unit
Nitisinone	µmol/L
Plasma tyrosine	µmol/L
Plasma phenylalanine	µmol/L
Plasma, serum, or dried blood spot succinylacetone	µmol/L
Urinary succinylacetone	mmol/mol creatinine
Plasma alpha-fetoprotein	ng/mL

Conversion factors used for conversion from original laboratory units to standard units are presented in Annex 2.

Nitisinone concentrations measured in DBS were converted to plasma using a conversion factor of 2.4 (10). Two different conversion factors were identified in the literature but Prieto et al (10) was considered most reliable and scientifically solid since it is based on samples from actual patients.

In samples where the SA concentration was reported as “0” this was classified as “Not detectable”. Samples where the SA concentration was reported as “999999999” was handled as “TRACE” and classified as “Detectable”.

9.8.3 Data analysis

9.8.3.1 General

All analyses were based on the combined prospectively and retrospectively collected data as described in Section 9.8.1.

All enrolled patients were included in the statistical analyses and descriptive analyses were conducted to summarize the data. In general, no imputation of missing data was performed, apart from imputation of missing dates and laboratory units, as explained in Section 9.9.3.

9.8.3.2 Analysis sets

There were 2 main sets of data analyzed:

The complete set, including all patients receiving Orfadin at the index date (February 21, 2005) or starting thereafter. For this set, the analyses of primary and secondary endpoints reflected events occurring and assessments made on or after the index date. Duration of exposure prior to the index date was ignored when estimating event rates.

The index set, including the subset of patients having their first dose of Orfadin on the index date or later. For the index set, the entire duration of treatment was used, and all events on treatment was used when estimating event rates.

The complete set thus includes more patients and patients with a longer treatment duration and is therefore a more relevant analysis set for analyzing long-term safety effects. The index set, on the other hand, informs more reliably on the safety profile after the first initiation of Orfadin treatment (i.e., label use). Since the primary objective of the present PASS was to assess long-term safety of Orfadin, the results presented in this clinical study report will generally focus on the data obtained from the complete set except for overall clinical condition. The variable overall clinical condition makes comparisons with the condition at the first treatment year and, therefore, it is deemed more relevant to use the index set for this variable.

For the analysis of the occurrence of liver transplantations and death, 3 additional analysis sets were used:

The extended mortality/liver transplantation set, including all patients receiving Orfadin prior to or on the index date or starting thereafter plus the patients withdrawn from OAS due to death or liver transplantation on or after the index date.

The extended mortality set, including all patients receiving Orfadin prior to or on the index date or starting thereafter plus the patients withdrawn from OAS due to death on or after the index date.

The extended liver transplantation set, including all patients receiving Orfadin prior to or on the index date or starting thereafter plus the patients withdrawn from OAS due to liver transplantations on or after the index date.

Data evaluated for the extended analysis sets were restricted to the minimum needed for the evaluation of the occurrence of death and liver transplantation: date of death/liver transplantation, date of start of Orfadin treatment and date of birth (the latter needed for the evaluation of death and liver transplantation in relation to age at start of treatment, which was considered important to evaluate in relation to outcome).

9.8.3.3 Subgroups

Patients who received treatment once daily at any time during the study were divided into two subgroups for analysis:

Treatment-naïve: Previous treatment-naïve patients who started their Orfadin treatment with a once daily dosing regimen (confirmed by the first dose recorded for the patient).

Treatment-experienced: Previous treatment-experienced patients who had switched to once daily dosing following a twice daily dosing regimen (confirmed by twice daily regimen reported before the once daily dosing).

If a patient did not have either a confirmed once daily dosing regimen as first dose or a confirmed twice daily dosing regimen before the once daily dosing, the patient was not assigned to either of the two subgroups. Both subgroup analyses were performed for the index set, however, only the treatment-experienced analysis was performed for the complete set.

9.9 Statistical methods

9.9.1 Main summary measures

9.9.1.1 General measures

The statistical methods are described in detail in the SAP, which was finalized prior to DBL.

Continuous data were summarized in the format of descriptive statistics: n, mean, SD, median, minimum and maximum, unless otherwise indicated. Categorical data were summarized with counts and percentages. The denominator for all percentages was the number of patients within the population of interest, unless otherwise indicated.

Data presented graphically were visualized by boxplots or scatter plots.

Boxplots display the median and the mean in a box where the lower and upper boundaries represent the lower and upper quartiles. Whiskers are extended from the box to the minimum and the maximum values, or to the lower and upper fence defined as 1.5 times the IQR from the upper and lower boundaries of the box, respectively. Values outside the lower and upper fence are included as data points.

The key results are presented in the report body (Section 10). Supportive results are presented in Section 15. Results of all analyses described in the SAP including those not presented in Sections 10 or 15 are included in a stand-alone document (see Annex 1) which is available on request.

9.9.1.2 Adverse events

AEs were coded using MedDRA version 19.1 or later and were tabulated by SOC and PT.

9.9.2 Main statistical methods

Statistical analyses were performed using SAS software Version 9.4 (SAS Institute Inc, Cary, North Carolina, United States).

Results were presented descriptively, and with estimates and corresponding two-sided 95% CIs, when relevant.

9.9.2.1 Primary endpoint

For each of the AE categories (Section 9.4.1), the proportion of patients who experienced respective AE and the associated two-sided 95% CI was calculated, for the complete set and the index set, respectively.

Further, for each of the AE categories, the number of patients as well as the number of events and the incidence rate expressed as frequency of events per number of 100 patient years of exposure and the associated two-sided 95% CI was calculated, both for the complete set and the index set. The number of patient years of exposure was calculated for each patient as the time from treatment start to the study end or withdrawal from the study. The total number of patient years of exposure was obtained from summing over patients. For the complete set, only events

occurring on or after the index date were included and Orfadin treatment prior to the index date was excluded in the calculation of exposure time.

Additionally, the combination of the primary endpoints, i.e., the proportion of patients who experienced at least one of the events related to hepatic, renal, ophthalmic, hematological or cognitive/developmental function, was calculated together with the associated two-sided 95% CI, for the complete set and the index set, respectively.

9.9.2.2 Secondary endpoints

9.9.2.2.1 Occurrence of liver transplantation and/or death

Time to liver transplantation or death from start of Orfadin treatment was analyzed applying Kaplan-Meier methodology. Observations were censored at the last date of Orfadin treatment (or withdrawal date for patients who stopped treatment), if the reason for stopping medication was something else than liver transplantation or death. For patients continuing treatment, the last study data collection date (September 30, 2019) was used for censoring. The analysis was conducted on the complete set and on the extended mortality/liver transplantation set. For patients with treatment start prior to the index date the time on treatment was considered to be left truncated at the time point corresponding to the index date, i.e., they were treated as having entered the study at the index date with an observed time on treatment at study entry being larger than zero. These patients were included in the analysis as "late-entry" patients, i.e., they were part of the number of patients at risk in the Kaplan-Meier analyses after treatment times corresponding to time points after the index date.

Additional presentations of the "occurrence of liver transplantation or death" were stratified by age at start of Orfadin treatment.

The secondary endpoints of "occurrence of liver transplantation" and "occurrence of death" were analyzed similarly.

9.9.2.2.2 Occurrence of hepatic malignancies and other malignancies and other adverse events

The number and percentage of patients with hepatic malignancies and other malignancies were described both for the complete set and the index set.

9.9.2.2.3 Incorrect administration of the oral suspension

In the subgroup of patients using the oral suspension formulation of Orfadin, the occurrence of incorrect administration of this formulation was evaluated. The summary included the number and percentage of patients with at least one incorrect administration of the oral suspension formulation of Orfadin in the subgroup of patients with at least one dose of oral suspension of Orfadin.

Incorrect administration of the oral suspension formulation was captured as a medication error in the AE reports, based on SMQ Medication error, and was evaluated based on the complete set in the subgroup of patients with at least one oral suspension administration of Orfadin after June 19, 2015.

9.9.2.2.4 Occurrence of other adverse events

Other AEs were summarized by MedDRA SOC and PT. It included all other AEs not previously identified as primary or secondary endpoints. Displays by PT included both the number and percentage of patients with at least one AE recorded as well as the incidence rate per 100 patient years on Orfadin treatment.

Presentations were performed both for the complete set and the index set.

9.9.2.2.5 Discontinuation of Orfadin treatment

The number and percentage of patients that discontinued Orfadin treatment during the study were presented, both for the complete set and the index set, together with reason for discontinuation.

9.9.2.2.6 Laboratory investigations

Laboratory results were presented by year since start of Orfadin treatment. For the index set, duration of treatment may range from 1 to 15 years (corresponding to treatment start during 2005 and continuing through 2019).

In the complete set, some patients started their treatment prior to the index date, however only laboratory investigations recorded after the index date were included in the analyses. Consequently, in the complete set, some patients will not contribute to the summary by treatment year for years prior to the index date but will contribute to the summary by treatment year for years after the index date. For example, a patient in the complete analysis set may contribute laboratory data in displays from year 4 onwards, but not for year 1 to 3.

Laboratory results were summarized in the format of descriptive statistics. Additional displays used boxplots for years with data from at least 5 patients.

Clinically significant results, as judged by the investigator, from laboratory investigations of p-Tyr, p-Phe, plasma/serum/DBS concentrations of SA, urinary SA, and p-AFP were also captured as AEs (see also Section 9.4.2.9). The proportion of patients with a clinically significant result at any time after the index date were presented for the index set and the complete set, respectively.

9.9.2.2.6.1 Plasma tyrosine

Results for p-Tyr were summarized in tabular form and with boxplots, by year on Orfadin treatment, for the complete set and the index set. For patients with more than one assay of p-Tyr during a year, the maximum value was used. P-Tyr was additionally presented descriptively (tabulations and boxplots) by diet and diet compliance (not on diet, on diet and very good compliance, on diet and good compliance, on diet and poor compliance, on diet and very poor compliance).

The relation between p-Tyr and extent of exposure to Orfadin was explored graphically by use of scatter plots.

9.9.2.2.6.2 Plasma phenylalanine

Results for p-Phe were summarized in tabular form and in boxplots, by year on Orfadin treatment, for the complete set and the index set. For patients with more than 1 assay of p-Phe during a year, the minimum value was used.

9.9.2.2.6.3 Plasma/serum/dried blood spot or urinary succinylacetone

Results for detectable SA concentrations in plasma/serum/DBS or urine were summarized by year on Orfadin treatment, for the complete set and the index set. For patients with more than one assay of SA (plasma/serum/DBS or urine) during a year, the maximum value was used.

The proportion of patients having at least one detectable SA concentration in any assay were summarized by treatment year. Percentages were based on the number of patients participating in the study during the corresponding treatment year, for the respective population. It was noted that very low SA levels that are also present in a healthy population (11-14) could be detected. Therefore, in addition, the proportion of patients having at least one abnormal value classified as clinically relevant or unknown was presented.

The proportion of patients having at least one detectable SA level was also summarized by treatment year after initiation of once daily dosage for the two subgroups.

The association between a detectable level of SA in plasma, serum, blood spot or urine and the plasma/serum/DBS nitisinone concentration was explored graphically in boxplots for each year of Orfadin treatment. Similar boxplots were presented for the two subgroups for each year of once daily Orfadin treatment.

The association between a detectable level of SA in plasma, serum, DBS or urine and mean daily dose of Orfadin (mg/kg) was similarly explored for each year of Orfadin treatment, and for each year of once daily Orfadin treatment, in the two subgroups.

9.9.2.2.6.4 Plasma alpha-fetoprotein

For patients with more than one assay of p-AFP during a year, the maximum value was used. Results were summarized in tabular form and in boxplots by year on Orfadin treatment, for the complete set and the index set.

The number of patients with p-AFP levels >6.6 ng/mL was calculated. The p-AFP cut-off level of 6.6 ng/mL was defined by the Sponsor as the threshold for identifying abnormal values.

9.9.2.2.7 Orfadin treatment compliance

The investigator's rating of patient's compliance (very good, good, poor, very poor and not applicable) was presented descriptively by year on Orfadin treatment, for the complete set and the index set. This was also presented for each year on once daily Orfadin treatment for the two subgroups (complete and index set).

Percentages were based on the number of patients for which at least one visit was recorded during the year, for respective population. For patients with more than one visit during a year, the worst case of compliance was used in the presentations.

Treatment compliance was also summarized over time, presenting number and percentage of patients with at least one rating of poor or very poor treatment compliance. This was done for the index set and the complete set, in both cases only evaluating measurements after the index date.

The association between treatment compliance and age was investigated graphically with boxplots by year on Orfadin treatment.

The number and percentage of patients with any break in the Orfadin treatment were presented descriptively by year on Orfadin treatment, for the complete set and the index set. Percentages were based on the number of patients for which at least one visit was recorded during the year, for respective population.

9.9.2.2.8 Diet compliance

The number and percentage of patients on diet low in phenylalanine and tyrosine were presented descriptively for each year on Orfadin treatment, for the complete set and the index set. Percentages were based on the number of patients for which at least one visit was recorded during the year, for respective population. For patients with more than one visit during a year, they were categorized as being on diet if at least one of the responses during the year was “yes”.

For patients on diet low in phenylalanine and tyrosine, compliance to diet restrictions (very good, good, poor, very poor and unknown) was summarized. The summary was presented by year on Orfadin treatment, for the complete set and the index set. Percentages were based on the number of patients on diet in the respective year, for the complete set and the index set. Only measurements after the index date were analyzed. For patients on diet with more than one visit during a year, the worst case of diet compliance was used in the presentations.

For patients on diet low in phenylalanine and tyrosine the association between diet compliance and age was investigated graphically with boxplots by year on Orfadin treatment.

9.9.2.2.9 Exposure to Orfadin

Exposure to Orfadin was captured both in terms of daily dose of Orfadin, and plasma/serum/DBS concentration of nitisinone.

The number of patient-years of exposure was calculated for each patient as the time from treatment start to the study end, end of treatment or withdrawal from the study. For the complete set, Orfadin treatment prior to the index date was excluded in the calculation of duration of exposure for event rates.

An assessment was considered to correspond to year 1, 2, 3 etc. if the visit date occurred within one/two/three etc. years from the first start of Orfadin treatment. Therefore, many patients in the complete set had their first results in the study displayed after more than 1 year of Orfadin treatment. For example, if a patient was on treatment for 2 years before the index date, this patient’s assessments made 1 year after the index date were part of the analysis of “year 3” evaluations but were not part of year 1 and 2 evaluations.

The number of days since start of Orfadin treatment was summarized in the format of descriptive statistics. Subsequent years were summarized with descriptive statistics in terms of number of

years. The corresponding methods were applied for assessments by year since start of once daily Orfadin treatment.

In cases where dose had been recorded several times during a year, the mean dose (mg/kg) was used. The calculation of mean daily dose accounted for the duration of dose (based on start and stop dates for treatment dose) whenever data were available, as well as once or twice daily posology. In cases where a new dose was recorded without a new recording of weight, the exposure in terms of mg/kg was carried forward. For a new recording of weight, the exposure was calculated as the last dose divided by the weight from that date and onwards until the next record of weight.

The nitisinone concentration was summarized in the format of descriptive statistics by year on Orfadin treatment. In cases where a patient had several recordings of nitisinone concentration during a year the mean was used. The relation between nitisinone concentration and daily dose of Orfadin (mg/kg) was explored graphically by use of scatter plots.

9.9.2.2.10 Overall clinical condition

The investigators' rating of patient's overall clinical condition was summarized descriptively for each year on Orfadin treatment, for the complete set and the index set, and for once daily Orfadin treatment for the two subgroups.

The change in overall clinical condition from first year of Orfadin treatment as compared to each subsequent year was illustrated by cross-tabulation in a shift table based on the index analysis set.

Overall clinical condition, by year on Orfadin treatment, was further described in relation to treatment compliance, by cross-tabulation. For patients with more than one visit during a year, the worst rating of clinical condition was used in the analyses.

The number of days since start of Orfadin treatment until assessment of overall clinical condition was summarized by year with descriptive statistics. For patients with more than one visit during a year, the date of the visit with the worst rating of clinical condition was used.

9.9.3 Missing values

The analyses were based on available data, i.e., no imputation of missing assessments was performed. In the case of incomplete information on dates, e.g., for date of birth, date of start of treatment and date of event/assessment for the primary and secondary endpoints, these were imputed if enough information was available. Details regarding imputations are provided in the SAP.

Due to restrictions in the permission to register the date of birth this was imputed for all patients. In cases where the date of birth was previously collected and kept in the study database to enable the identification of patients by investigators, the date of birth was excluded from the analysis database.

Missing units for laboratory values were imputed with the site-specific unit in the case where only one unit had been used for all other laboratory values in that site. AFP values reported in IU

were mapped to either IU/L or IU/mL based on the site-specific reporting unit as confirmed by the sites.

Due to the retrospective part of the study, missing data were sometimes difficult to retrieve and had to be accepted in the final database.

9.9.4 Sensitivity analyses

Analyses of the occurrence of death and liver transplantation were subjected to sensitivity analyses as described in Section 9.6 above.

9.9.5 Amendments to the statistical analysis plan

There were no amendments to the SAP.

9.10 Quality control

The eCRF included logical checks to prevent data entry errors. Data inconsistencies outside the logical checks were managed by queries, as described above (see Section 9.8.1).

The investigators had the final responsibility for the accuracy and completeness of all patient data entered into the eCRFs and were requested to verify the data in the eCRF by signing an eCRF signature pages. For potential issues related to this process, please see Section 11.2.2.

All data were validated on an ongoing basis, and at the time for data extraction for the yearly PSUR, a specific validation process was applied.

Reconciliation between AE data (both AEs and SAEs) reported to the Sobi Drug Safety Database and the AEs in the study database was performed on a quarterly basis, and prior to database closure, to ensure completeness and consistency.

10 Results

10.1 Participants

The number of patients included in each of the 5 analyses sets and the subgroups of treatment-naïve- and treatment-experienced patients are presented in Table 4. The index set constitute approximately 2/3 of the complete set.

Table 4 Number of patients in the study

	All	Once daily subgroups	
Analysis set	Patients n	Treatment-naive patients n	Treatment-experienced patients n
Complete set	315	12	27
Index set	203	11	21
Extended mortality set	318	12	27
Extended liver transplantation set	336	12	27
Extended mortality/ liver transplantation set	339	12	27

Abbreviations: n, Number of patients in each group.
Note: Complete set includes all patients receiving Orfadin prior to or on the index date (February 21, 2005) or starting thereafter. Index set includes the subset of patients that started their Orfadin treatment on the index date or later. Extended sets consist of complete set plus patients withdrawn from the Post Marketing Surveillance program due to death or liver transplantation, respectively. Treatment-naïve patients started their Orfadin treatment with a once daily dosing regimen. Treatment-experienced patients have switched to once daily dosing following a twice daily dosing regimen.

The number of patients per participating country is presented in Table 40 in Section 15. The countries which enrolled most patients were Spain (19.7 % of the complete set), France (15.9 %), UK (12.7 %), Germany (11.4 %), and Italy (10.5 %).

In total, 11 patients (3.5 %) in the complete set withdrew from the study (Table 5). There were 7 patients who were reported to withdraw due to liver transplantation and 4 patients who withdrew due to reason "other". These reasons included 2 patients who transferred to adult care clinics which were not part of the study, 1 patient who changed to another hospital not part of the study and 1 patient who withdrew after successful liver transplantation. The latter patient should accordingly not belong to the "other" reason group but to the 7 patients with withdrawal reason "liver transplantation", however, this was never corrected in the database by the investigator. As from now, the number of patients in the complete set who withdrew from the study following liver tranplantation in the present report will be referred to as 8 patients.

Table 5 Study withdrawal

	Complete set	Index set
Reason for withdrawal	Patients (N= 315) n (%)	Patients (N= 203) n (%)
Total	11 (3.5)	5 (2.5)
Liver transplantation	7 (2.2)[a]	4 (2.0)
Other	4 (1.3)[a]	1 (0.5)

Abbreviations: N, Total number of patients; n, Number of patients in each group.
[a]One patient in the complete set was erroneuously reported as being withdrawn from the study due to other reason even though the patient underwent a liver transplantation.

In total, 76 patients in the complete set were reported to have been treated with Orfadin in a once daily dosing frequency at least once during the study (Table 6). One treatment-naïve patient in the complete set reported once daily use of Orfadin already in 2003-2004 but after those years there is no information about the dosing regimen in this patient. There were about twice as many patients who switched from twice daily to once daily dosing frequency than who started on once daily dosing regimen directly. For 37 patients who were treated with a once daily regimen, it was not possible to determine from the data reported by the investigator if they were treatment-naïve or switched from twice daily dosing regimen or how long they were treated with once daily and they could therefore not be included in any of the two subgroups.

Table 6 Proportion of patients on confirmed once daily treatment regimens

	Complete set	Index set
OD (at any time)	**(N= 315) n (%)**	**(N= 203) n (%)**
Treatment-naïve (started on OD)	12 (3.8)	11 (5.4)
Treatment-experienced (switched from BID to OD)	27 (8.6)	21 (10.3)
No confirmed first treatment regimen	37 (11.7)	16 (7.9)

Abbreviations: BID, Twice daily dosing regimen; N, Total number of patients; n, Number of patients on a certain dosing regimen; OD, Once daily dosing regimen.

10.2 Descriptive data

Demographic and baseline characteristics for the complete and index sets are presented in Table 7.

In the complete set, there was an equal distribution of male and female participants. The mean age at diagnosis was 9.5 months (median 3.1 months) in the complete set. 20.0 % of the patients in the complete set were diagnosed with HT-1 by newborn screening, and 43.8 % by clinical symptoms. For 36.2 % of the patients, it was unknown whether the diagnosis was made after screening or due to clinical symptoms. The mean age at start of Orfadin treatment was 22.4 months and about 55 % of the patients were <6 months at the start of Orfadin treatment and about 45 % were ≥6 months old.

The demographics and baseline characteristics in the index set were comparable with the complete set.

Table 7 Demographics and baseline characteristics

	Complete set	Index set
	Patients (N= 315)	**Patients (N= 203)**
Sex, n (%)		
Female	158 (50.2)	102 (50.2)
Male	157 (49.8)	101 (49.8)
Age at diagnosis (months)		

	Complete set	Index set
	Patients (N= 315)	**Patients (N= 203)**
N	279	195
Mean	9.5	8.7
SD	19.14	15.00
Median	3.1	2.9
Range	0.0 - 172.2	0.0 - 141.7
Diagnosed by newborn screening, n (%)		
Yes	63 (20.0)	52 (25.6)
No	138 (43.8)	89 (43.8)
Unknown	114 (36.2)	62 (30.5)
Age at first visit (years)		
n	315	203
Mean	5.4	3.6
SD	5.57	4.94
Median	3.3	1.9
Range	0.0 - 28.0	0.0 - 28.0
Age category at first visit, n (%)		
Newborn (<28 days)	16 (5.1)	15 (7.4)
Infant (28 days - <2 years)	96 (30.5)	87 (42.9)
Child (2 years - <12 years)	162 (51.4)	85 (41.9)
Adolescent (12 years - <18 years)	30 (9.5)	10 (4.9)
Adult (>= 18 years)	11 (3.5)	6 (3.0)
Age at Orfadin treatment start (months)		
n	315	203
Mean	22.4	28.4
SD	48.18	55.92
Median	4.9	5.9
Range	0.0 - 336.6	0.0 - 336.6
Age category at Orfadin treatment start, n (%)		
Newborn (<28 days)	70 (22.2)	48 (23.6)
>=28 days - <6 months	101 (32.1)	54 (26.6)
>=6 months -<12 months	46 (14.6)	26 (12.8)
>=12 months	98 (31.1)	75 (36.9)

Abbreviations: N, Total number of patients; n, Number of patients in each group; SD, Standard deviation.

Demographics and baseline characteristics of treatment-naïve patients on once daily dosage and treatment-experienced patients after initiation of once daily dosage for the index set are presented in Table 41.

The distribution of patients' age groups at start of Orfadin treatment in the 3 extended analysis sets are presented in Table 8. As in the complete and index sets (Table 7), approximately one third of the patients in the 3 extended analysis sets were either ≥28 days - <6 months old or ≥12 months old respectively at the time of Orfadin treatment initiation.

Table 8 Age group at start of Orfadin treatment in the extended analysis sets

	Extended mortality/liver transplantation set	Extended mortality set	Extended liver transplantation set
	Patients (N= 339) n (%)	**Patients (N= 318) n (%)**	**Patients (N= 336) n (%)**
Age category at Orfadin treatment start			
<28 days	70 (20.6)	70 (22.0)	70 (20.8)
≥28 days - <6 months	103 (30.4)	101 (31.8)	103 (30.7)
≥6 months - <12 months	52 (15.3)	46 (14.5)	52 (15.5)
≥12 months	113 (33.3)	101 (31.8)	110 (32.7)
Unknown	1 (0.3)	0	1 (0.3)

Abbreviations: N, Total number of patients; n, Number of patients in each group.
Note: Extended mortality/liver transplantation set consists of complete set plus patients withdrawn from OAS due to death or liver transplantation. Extended mortality set consists of complete set plus patients withdrawn from the Post Marketing Surveillance program due to death. Extended liver transplantation set consists of complete set plus patients withdrawn from the Post Marketing Surveillance program due to liver transplantation.

10.3 Outcome data

Not applicable.

10.4 Main results

10.4.1 Primary endpoint: hepatic, renal, ophthalmic, hematological, cognitive/developmental function AEs

The incidences and incidence rates per 100 patient years for patients observed to have hepatic, renal, ophthalmic, hematological, or cognitive/developmental function AEs in the complete set were low for all AE categories (Table 9).

Similar results for the primary endpoint were obtained in the index set (Table 42).

Table 9 Number of patients with hepatic, renal, ophthalmic, hematological, or cognitive/development function adverse events with event counts and incidence rates per 100 patient years (complete set)

	Patients (N=315) n	Percentage (95% CI)	Events f	Incidence rate per 100 patient years (95% CI)
Hepatic function	18	5.7 (3.4-8.9)	18	0.6 (0.4-0.9)
Renal function	1	0.3 (0.0-1.8)	1	0.0 (0.0-0.2)
Ophthalmic function	15	4.8 (2.7-7.7)	19	0.6 (0.4-0.9)
Hematological status	7	2.2 (0.9-4.5)	9	0.3 (0.1-0.5)
Cognitive/developmental function	8	2.5 (1.1-4.9)	9	0.3 (0.1-0.5)
Total (any of the above)	43	13.7 (10.1-17.9)	56	1.8 (1.4-2.3)

Abbreviations: N, Total number of patients; n, Number of patients with at least one AE in the pre-defined group; f, Total number of AEs in the pre-defined group; CI, Confidence interval; AE, Adverse event.
Note: Patient years of exposure = 3172.7.

There was no apparent relation between the age at start of Orfadin treatment and the total incidences for patients observed to have any of the hepatic, renal, ophthalmic, hematological, and cognitive/developmental function AEs in the complete set (Table 10). However, for hepatic function and hematological status AEs, there was a trend of increased incidence of these events with higher age at start of Orfadin treatment. For renal function AEs, the only event was reported for a patient in the ≥12 months age at start of Orfadin treatment group.

Similar results were obtained in the index set (Table 43).

Table 10 Number of patients with hepatic, renal, ophthalmic, hematological, cognitive/development function adverse events with event counts and incidence rates per 100 patient years by age at start of Orfadin treatment (complete set)

Adverse Event category/ Age at start of Orfadin treatment	N	Patients with events n	Percentage (95% CI)	Events f	Incidence rate per 100 patient years (95% CI)
Hepatic function	315	18	5.7 (3.4-8.9)	18	0.6 (0.4-0.9)
<28 days	70	3	4.3 (0.9-12.0)	3	0.4 (0.1-1.3)
>=28 days - <6 months	101	5	5.0 (1.6-11.2)	5	0.4 (0.2-1.0)
>=6 months - <12 months	46	4	8.7 (2.4-20.8)	4	0.8 (0.3-2.2)
>=12 months	98	6	6.1 (2.3-12.9)	6	0.7 (0.3-1.7)
Renal function	315	1	0.3 (0.0-1.8)	1	0.0 (0.0-0.2)
<28 days	70	0	-	0	-
>=28 days - <6 months	101	0	-	0	-
>=6 months - <12 months	46	0	-	0	-
>=12 months	98	1	1.0 (0.0-5.6)	1	0.1 (0.0-0.9)
Ophthalmic function	315	15	4.8 (2.7-7.7)	19	0.6 (0.4-0.9)
<28 days	70	4	5.7 (1.6-14.0)	7	1.0 (0.5-2.1)
>=28 days - <6 months	101	8	7.9 (3.5-15.0)	9	0.8 (0.4-1.5)
>=6 months - <12 months	46	0	-	0	-
>=12 months	98	3	3.1 (0.6-8.7)	3	0.4 (0.1-1.2)
Hematological status	315	7	2.2 (0.9-4.5)	9	0.3 (0.1-0.5)
<28 days	70	0	-	0	-
>=28 days - <6 months	101	0	-	0	-
>=6 months - <12 months	46	5	10.9 (3.6-23.6)	6	1.2 (0.6-2.8)
>=12 months	98	2	2.0 (0.2-7.2)	3	0.4 (0.1-1.2)
Cognitive/developmental function	315	8	2.5 (1.1-4.9)	9	0.3 (0.1-0.5)
<28 days	70	2	2.9 (0.3-9.9)	3	0.4 (0.1-1.3)
>=28 days - <6 months	101	4	4.0 (1.1-9.8)	4	0.3 (0.1-0.9)
>=6 months - <12 months	46	1	2.2 (0.1-11.5)	1	0.2 (0.0-1.5)
>=12 months	98	1	1.0 (0.0-5.6)	1	0.1 (0.0-0.9)
Total	315	43	13.7 (10.1-17.9)	56	1.8 (1.4-2.3)
<28 days	70	8	11.4 (5.1-21.3)	13	1.8 (1.1-3.2)
>=28 days - <6 months	101	16	15.8 (9.3-24.4)	18	1.5 (1.0-2.4)
>=6 months - <12 months	46	7	15.2 (6.3-28.9)	11	2.3 (1.3-4.1)
>=12 months	98	12	12.2 (6.5-20.4)	14	1.7 (1.0-2.9)

Abbreviations: AE, Adverse event; CI, Confidence interval; f, Total number of AEs in the pre-defined group; N, Total number of patients; n, Number of patients with at least one AE in the pre-defined group.
Note: Patient years of exposure in total = 3172.7, and by age at start of Orfadin treatment: <28 days = 706.8, ≥28 days - <6 months = 1174.1, 6 - <12 months = 483.4, ≥12 month = 808.5.

10.4.2 Secondary endpoints

10.4.2.1 Occurrence of liver transplantation or death

In total, 12 patients (3.8 %) and 36 patients (10.6 %) in the complete and extended mortality/liver transplantation sets, respectively, either died or were liver transplanted (Table 11). As can be seen in Table 11, the occurrence of death or liver transplantation was more frequent the older the patients were at the start of Orfadin treatment.

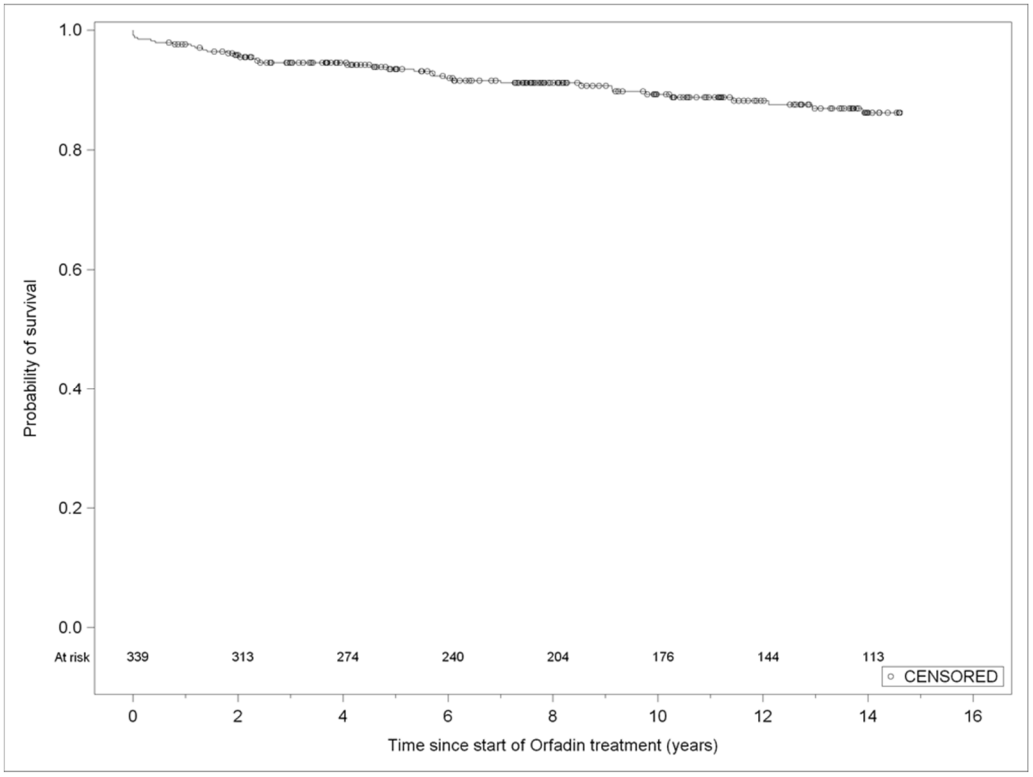
Table 11 Number of patients deceased or liver transplanted, by age at start of Orfadin treatment

	Complete set		Extended mortality/liver transplantation set	
	Patients N	Deceased or liver transplanted n (%)	Patients N	Deceased or liver transplanted n (%)
Total	315	12 (3.8)	339	36 (10.6)
Age at start of Orfadin treatment				
<28 days	70	0 (0.0)	70	0 (0.0)
>=28 days - <6 month	101	4 (4.0)	103	6 (5.8)
>=6 months - <12 months	46	3 (6.5)	52	9 (17.3)
>=12 months	98	5 (5.1)	113	20 (17.7)
Unknown	0	0	1	1 (100.0)

Abbreviations: N, Number of patients at risk; n (%), Number (and percentage) of patients deceased or liver transplanted among the patients at risk.

The time to death or liver transplantation in the extended mortality/liver transplantation set is graphically presented as Kaplan-Meier survival graph in Figure 1. The occurrence of death or liver transplantation does not seem to change over time.

Figure 1 Kaplan-Meier curve of time to liver transplantation or death (extended mortality/liver transplantation set)



10.4.2.2 Occurrence of death

No patients and 3 patients (0.9 %) in the complete and extended mortality sets, respectively, died (Table 12). All deceased patients were ≥12 months old at the start of Orfadin treatment.

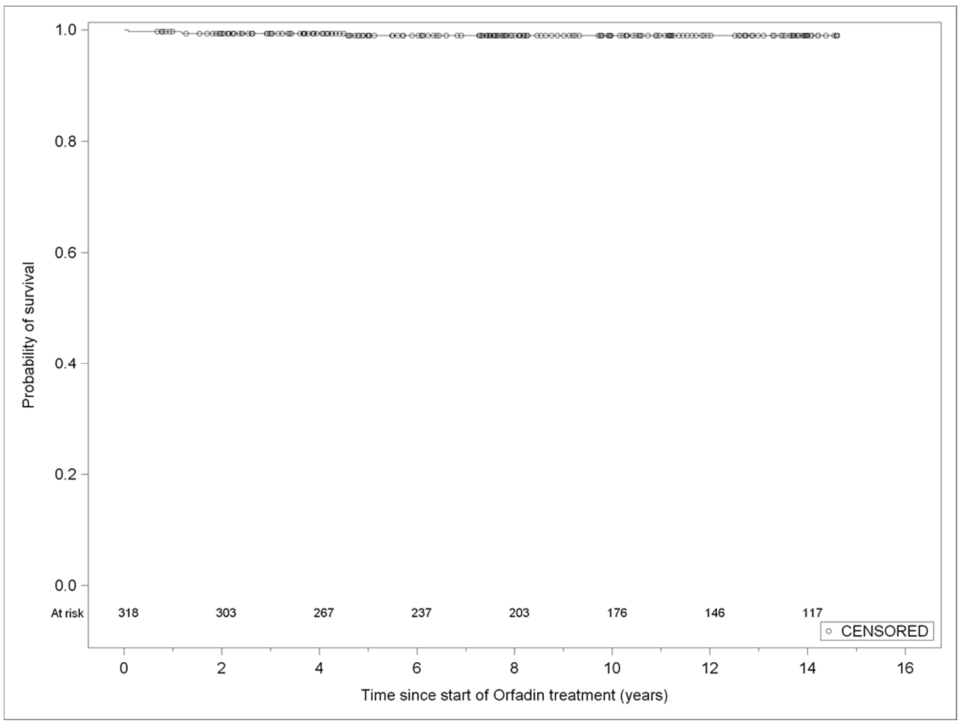
Table 12 Number of patients deceased, by age at start of Orfadin treatment

	Complete set		Extended mortality set	
	Patients N	Deceased n (%)	Patients N	Deceased n (%)
Total	315	0 (0.0)	318	3 (0.9)
Age at start of Orfadin treatment				
<28 days	70	0 (0.0)	70	0 (0.0)
>=28 days - <6 month	101	0 (0.0)	101	0 (0.0)
>=6 months - <12 months	46	0 (0.0)	46	0 (0.0)
>=12 months	98	0 (0.0)	101	3 (3.0)

Abbreviations: N, Number of patients at risk; n (%), Number (and percentage) of patients deceased or liver transplanted among the patients at risk.

The time to death in the extended mortality set is graphically presented as Kaplan-Meier survival graph in Figure 2. The occurrence of death does not seem to change over time even though the graph is based on very few cases.

Figure 2 Kaplan-Meier curve of time death (extended mortality set)



10.4.2.3 Occurrence of liver transplantation

In total, 12 patients (3.8 %) and 33 patients (9.8 %) in the complete and extended liver transplantation sets, respectively, were liver transplanted (Table 13). As can be seen in Table 13, the occurrence of liver transplantation was more frequent in the 2 older age groups of start of Orfadin treatment. As presented in Section 10.1, 8 patients in the complete set were withdrawn from the study following liver transplantation, i.e., 4 patients continued in the study after being transplanted.

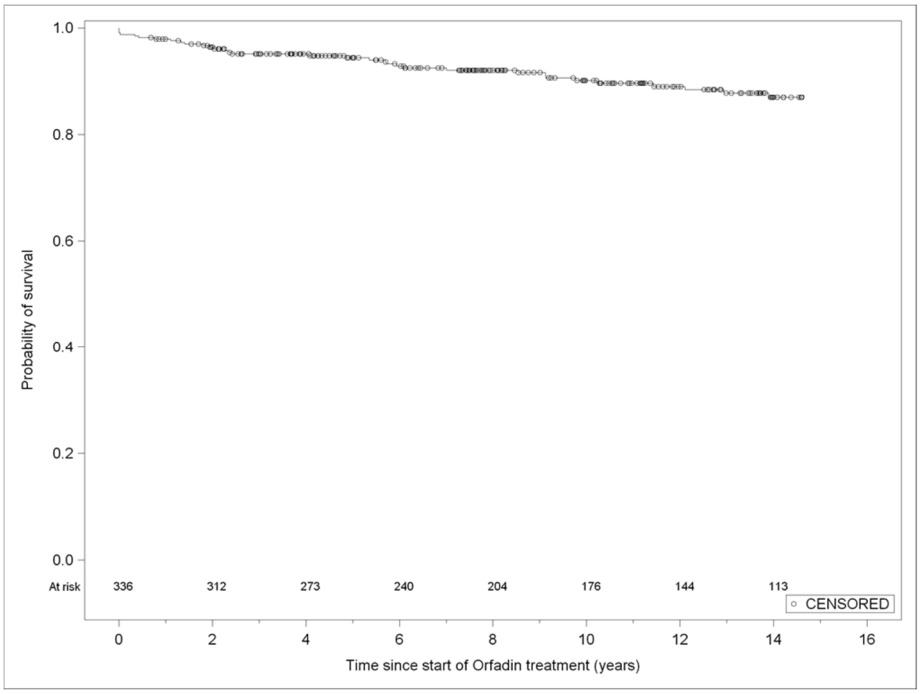
Table 13 Number of liver transplanted patients, by age at start of Orfadin treatment

	Complete set		Extended liver transplantation set	
	Patients N	Liver transplanted n (%)	Patients N	Liver transplanted n (%)
Total	315	12 (3.8)	336	33 (9.8)
Age at start of Orfadin treatment				
<28 days	70	0 (0.0)	70	0 (0.0)
>=28 days - <6 month	101	4 (4.0)	103	6 (5.8)
>=6 months - <12 months	46	3 (6.5)	52	9 (17.3)
>=12 months	98	5 (5.1)	110	17 (15.5)
Unknown	0	0	1	1 (100.0)

Abbreviations: N, Number of patients at risk; n (%), Number (and percentage) of patients deceased or liver transplanted among the patients at risk.

The time to liver transplantation in the extended liver transplantation set is graphically presented as Kaplan-Meier survival graph in Figure 3. The occurrence of liver transplantation does not seem to change over time.

Figure 3 Kaplan-Meier curve of time to liver transplantation (extended liver transplantation set)



10.4.2.4 Occurrence of hepatic malignancies

In total, 4 patients (1.3 %) in the complete set had reports of hepatic malignancy (Table 14). 2 of these patients were ≥12 months at start of Orfadin treatment.

Table 14 Occurrence of hepatic malignancy, by age at start of Orfadin treatment (complete set)

	Patients (N=313) n	Percentage (95% CI)
Total	4	1.3 (0.3-3.2)
Age at start of Orfadin treatment		
>=28 days - <6 month	1	0.3 (0.0-1.8)
>=6 months - <12 months	1	0.3 (0.0-1.8)
>=12 months	2	0.6 (0.1-2.3)

Abbreviations: CI, Confidence interval; N, Total number of patients at risk; n, Number of patients with hepatic malignancy in each age group.

In the index set, 2 patients (1.0 %) had reports of hepatic malignancy and both these patients were ≥12 months at start of Orfadin treatment (Table 44).

10.4.2.5 Occurrence of other malignancies

There were no other malignancies reported, either in the complete or index set.

10.4.2.6 Occurrence of incorrect administration of the oral suspension formulation of Orfadin

There were no reported events of incorrect administration of oral suspension of Orfadin (Table 45).

10.4.2.7 Occurrence of other adverse events

The occurrence of other AEs reported by the investigator not previously identified as primary or secondary endpoints in the complete set are displayed in Table 46.

The most commonly reported other AEs by SOCs in the complete set were Investigations (9.8 %), Gastrointestinal disorders (3.5 %), Injury, poisoning and procedural complications (2.5 %) and Nervous system disorders (2.5 %). The most commonly reported events by PTs were amino acid level (i.e., p-Tyr and p-Phe) increased (6.3 %) and drug level decreased (2.5 %) (both events within the SOC Investigations).

The occurrences of other AEs in the index set were very similar as for the complete set (data not shown).

10.4.2.8 Discontinuation of Orfadin treatment

In total, 11 patients (3.5 %) in the complete set discontinued Orfadin treatment (Table 15). 8 of the discontinuations were due to liver transplantations and 3 discontinuations were due to “other” reasons (see also information regarding study discontinuations in Section 10.1). In the index set, 5 patients (2.5 %) discontinued Orfadin treatment. 4 of the discontinuations in the index were due to liver transplantations and 1 discontinuation was due to “other” reason.

Table 15 Treatment discontinuation

	Complete set	Index set
	Patients (N=315) n (%)	**Patients (N= 203) n (%)**
Discontinued treatment	11 (3.5)	5 (2.5)
Reason for discontinuation		
Death	0 (0.0)	0 (0.0)
Withdrawal by subject	0 (0.0)	0 (0.0)
Liver transplantation	7 (2.2)[a]	4 (2.0)
Other	4 (1.3)[a]	1 (0.5)
Missing	0 (0.0)	0 (0.0)

Abbreviations: N, Total number of patients; n, Number of patients in each group.
[a]One patient in the complete set was erroneuously reported as being discontinued from treatment due to other reason even though the patient underwent a liver transplantation.

10.4.2.9 Laboratory investigations

10.4.2.9.1 Plasma tyrosine

10.4.2.9.1.1 Plasma tyrosine levels by treatment year

Because laboratory assessments for p-Tyr, p-Phe, SA, p-AFP and nitisinone plasma concentration were not assessed regularly in OAS but added to the PASS protocol, only a limited number of patients had these laboratory variables assessed during the study. For p-Tyr, 36 patients at Treatment Year 15 up to 144 patients at Treatment Year 7 in the complete set had these levels measured (Table 16). The p-Tyr levels increased rather constantly during the study, from a median (min, max) at Treatment Year 1 of 386 µmol/L (2, 949 µmol/L) to 648 µmol/L (30, 1167 µmol/L) at Treatment Year 15.

Similar results were obtained in the index set (data not shown).

Table 16 Plasma tyrosine concentration (µmol/L) by treatment year (complete set)

	n	Mean	SD	Median	Min, Max
Year 1	77	418.9	199.09	386	2, 949
Year 2	77	486.8	196.83	443	104, 1265
Year 3	78	462.8	177.94	441	23, 937
Year 4	81	503.6	213.16	454	189, 1098
Year 5	73	483.4	177.20	440	168, 1029
Year 6	82	547.4	218.40	521	63, 1031
Year 7	144	503.7	208.46	483	0, 1607
Year 8	119	536.4	213.08	537	114, 1065
Year 9	76	535.1	193.73	533	47, 1117
Year 10	87	502.8	247.18	482	52, 1394
Year 11	99	555.4	208.72	544	107, 1218
Year 12	104	586.2	205.82	575	69, 1152
Year 13	107	637.8	202.10	633	223, 1158
Year 14	86	623.3	216.29	626	123, 1092
Year 15	36	665.0	245.42	648	30, 1167

Abbreviations: n, Total number of patients with at least one assessment of plasma tyrosine during the corresponding year; SD, Standard deviation.
Note: For patients with more than one assay of plasma tyrosine during a year, the maximum value is displayed

10.4.2.9.1.2 Plasma tyrosine levels by diet compliance and treatment year

As expected, patients who had a “very good” or “good” diet compliance as assessed by the investigator, had lower p-Tyr levels throughout the study than patients who had a “poor” or “very poor” diet compliance (Table 17).

Similar results were obtained in the index set (data not shown).

Table 17 Plasma tyrosine concentration (µmol/L) by diet compliance and treatment year (complete set)

Year/ Diet compliance	n	Mean	SD	Median	Min, Max
Year 1					
On diet	67	413.0	191.55	370	2, 949
Very good	30	387.6	162.25	344	59, 760
Good	26	376.6	180.21	356	2, 847
Poor	9	514.6	207.95	576	114, 793
Very poor	1	948.8		949	949, 949
Unknown	1	671.0		671	671, 671
Missing	10	458.3	252.30	490	42, 811
Year 2					
Not on diet	2	284.0	254.56	284	104, 464

Year/ Diet compliance	n	Mean	SD	Median	Min, Max
On diet	64	484.1	191.69	431	196, 1265
Very good	24	390.7	119.82	360	196, 657
Good	29	485.6	156.09	447	274, 807
Poor	6	653.7	121.24	639	482, 854
Very poor	2	1115.4	211.28	1115	966, 1265
Unknown	3	457.3	40.45	443	426, 503
Missing	11	539.2	212.52	541	237, 868
Year 3					
Not on diet	3	430.0	143.36	501	265, 524
On diet	64	469.9	175.57	441	23, 927
Very good	23	428.2	110.12	424	237, 674
Good	31	450.0	188.94	461	23, 812
Poor	7	674.3	182.59	734	440, 927
Unknown	3	517.7	162.19	583	333, 637
Missing	11	430.7	209.10	426	192, 937
Year 4					
Not on diet	1	388.0		388	388, 388
On diet	70	483.7	188.21	452	191, 978
Very good	28	433.8	141.40	409	191, 935
Good	26	446.8	194.67	415	220, 969
Poor	12	609.0	180.50	588	334, 934
Very poor	1	978.0		978	978, 978
Unknown	3	603.3	93.82	657	495, 658
Missing	10	654.4	320.14	589	189, 1098
Year 5					
Not on diet	1	384.0		384	384, 384
On diet	65	475.3	169.21	440	168, 1029
Very good	24	397.3	122.00	395	179, 754
Good	28	472.6	149.70	448	168, 744
Poor	7	620.3	123.30	608	467, 805
Very poor	4	722.3	275.44	737	386, 1029
Unknown	2	447.0	165.46	447	330, 564
Missing	7	572.7	244.40	510	290, 875
Year 6					
Not on diet	1	342.0		342	342, 342
On diet	73	553.2	207.03	521	185, 1031
Very good	25	502.1	209.58	419	258, 1015
Good	26	492.6	188.72	473	185, 833
Poor	16	721.6	151.62	739	471, 1031

Year/ Diet compliance	n	Mean	SD	Median	Min, Max
Very poor	3	626.7	223.38	542	458, 880
Unknown	3	533.3	180.01	549	346, 705
Missing	8	520.3	322.28	542	63, 1001
Year 7					
Not on diet	6	390.2	167.30	408	158, 565
On diet	128	512.9	212.96	483	0, 1607
Very good	21	385.6	120.53	387	167, 615
Good	73	484.6	167.17	469	0, 896
Poor	27	670.5	273.44	676	258, 1607
Very poor	1	921.0		921	921, 921
Unknown	6	525.5	234.92	610	139, 751
Missing	10	454.0	149.69	509	62, 566
Year 8					
Not on diet	1	460.0		460	460, 460
On diet	100	532.4	213.81	534	114, 1065
Very good	17	384.4	123.46	401	187, 560
Good	52	494.3	183.13	447	114, 882
Poor	25	697.9	228.09	709	254, 1065
Very poor	2	787.5	21.92	788	772, 803
Unknown	4	495.0	134.01	472	379, 657
Missing	18	562.9	218.57	541	276, 997
Year 9					
Not on diet	1	277.0		277	277, 277
On diet	58	528.8	205.29	516	47, 1117
Very good	12	380.8	97.54	382	247, 526
Good	31	511.7	151.11	514	221, 848
Poor	9	768.5	213.46	659	552, 1117
Very poor	3	542.7	101.91	600	425, 603
Unknown	3	563.7	465.67	693	47, 951
Missing	17	571.9	141.60	548	304, 901
Year 10					
Not on diet	3	271.3	205.80	240	83, 491
On diet	77	510.4	251.82	486	52, 1394
Very good	27	335.9	151.25	327	52, 652
Good	29	482.6	178.23	476	52, 905
Poor	17	784.1	234.00	755	499, 1394
Very poor	3	794.7	222.19	737	607, 1040
Unknown	1	525.0		525	525, 525
Missing	7	518.4	174.87	473	325, 820

Year/ Diet compliance	n	Mean	SD	Median	Min, Max
Year 11					
Not on diet	4	724.3	180.04	771	468, 887
On diet	80	528.1	207.00	490	107, 1218
Very good	19	366.5	120.90	370	107, 590
Good	32	529.5	167.08	514	116, 942
Poor	25	626.7	228.48	651	241, 1218
Very poor	3	771.9	168.19	860	578, 878
Unknown	1	357.0		357	357, 357
Missing	15	656.2	181.43	615	353, 968
Year 12					
Not on diet	3	451.7	382.00	453	69, 833
On diet	89	586.6	199.81	570	193, 1152
Very good	14	346.3	110.85	337	193, 524
Good	28	575.5	179.44	545	282, 974
Poor	36	655.4	168.13	654	382, 1152
Very poor	7	815.3	107.92	800	647, 989
Unknown	4	486.3	109.78	511	332, 592
Missing	12	616.4	211.67	644	103, 968
Year 13					
On diet	100	639.7	197.50	635	223, 1140
Very good	19	445.8	144.23	466	223, 700
Good	29	631.4	166.52	622	314, 987
Poor	35	685.1	170.20	659	311, 1081
Very poor	15	801.5	185.69	778	494, 1140
Unknown	2	593.0	240.42	593	423, 763
Missing	7	610.3	277.59	585	279, 1158
Year 14					
Not on diet	4	777.3	110.33	810	626, 863
On diet	72	638.9	212.56	651	175, 1092
Very good	10	417.8	244.71	325	175, 1008
Good	32	583.1	146.45	588	265, 912
Poor	17	722.2	141.63	708	460, 934
Very poor	11	882.2	155.82	927	657, 1092
Unknown	2	591.0	280.01	591	393, 789
Missing	10	449.1	187.41	434	123, 816
Year 15					
Not on diet	1	821.0		821	821, 821
On diet	29	676.4	232.52	630	213, 1167
Very good	3	580.7	136.62	512	492, 738

Year/ Diet compliance	n	Mean	SD	Median	Min, Max
Good	8	481.9	132.34	513	213, 609
Poor	13	753.8	240.79	690	345, 1167
Very poor	5	844.0	165.55	951	623, 976
Missing	6	583.5	324.40	657	30, 975

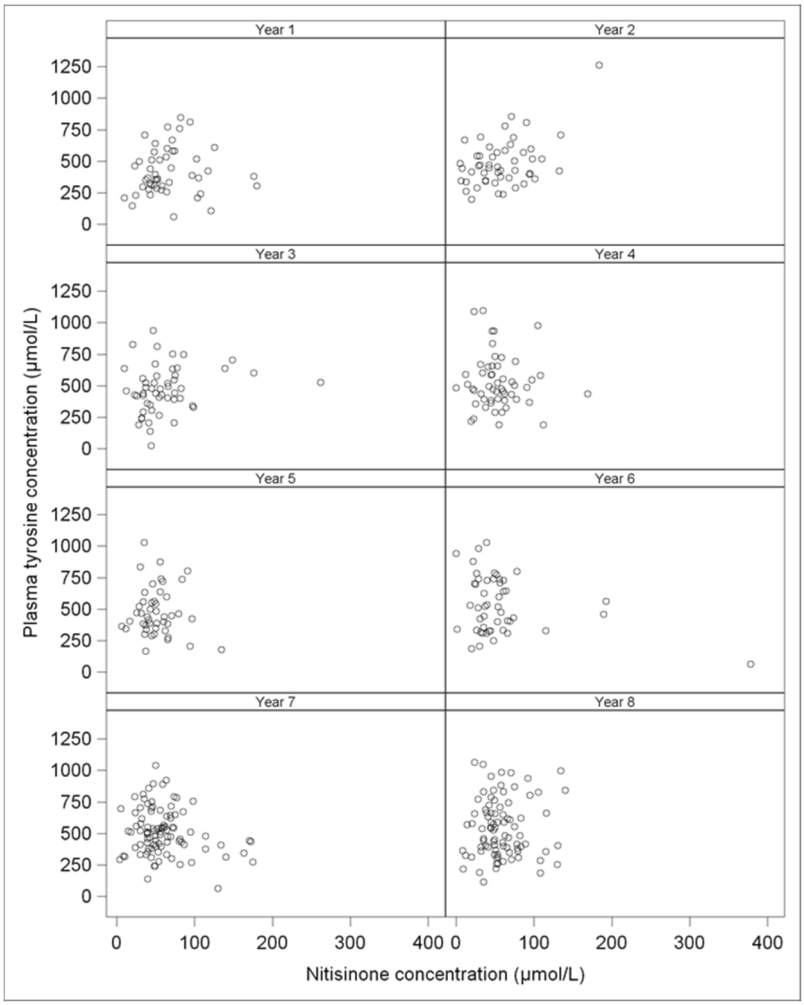
Abbreviations: n, Total number of patients with at least one assessment of plasma tyrosine during the corresponding year; SD, Standard deviation.
Note: For patients with more than one assay of plasma tyrosine during a year, the maximum value is displayed.

10.4.2.9.1.3 Plasma tyrosine levels versus nitisinone concentration by treatment year

The p-Tyr levels versus nitisinone concentration by treatment year in the complete set are presented graphically in Figure 4 (Treatment Year 1-8) and Figure 5 (Treatment Year 9-16). There was a tendency to a positive relation between the p-Tyr levels and the nitisinone concentrations even though the variability was large.

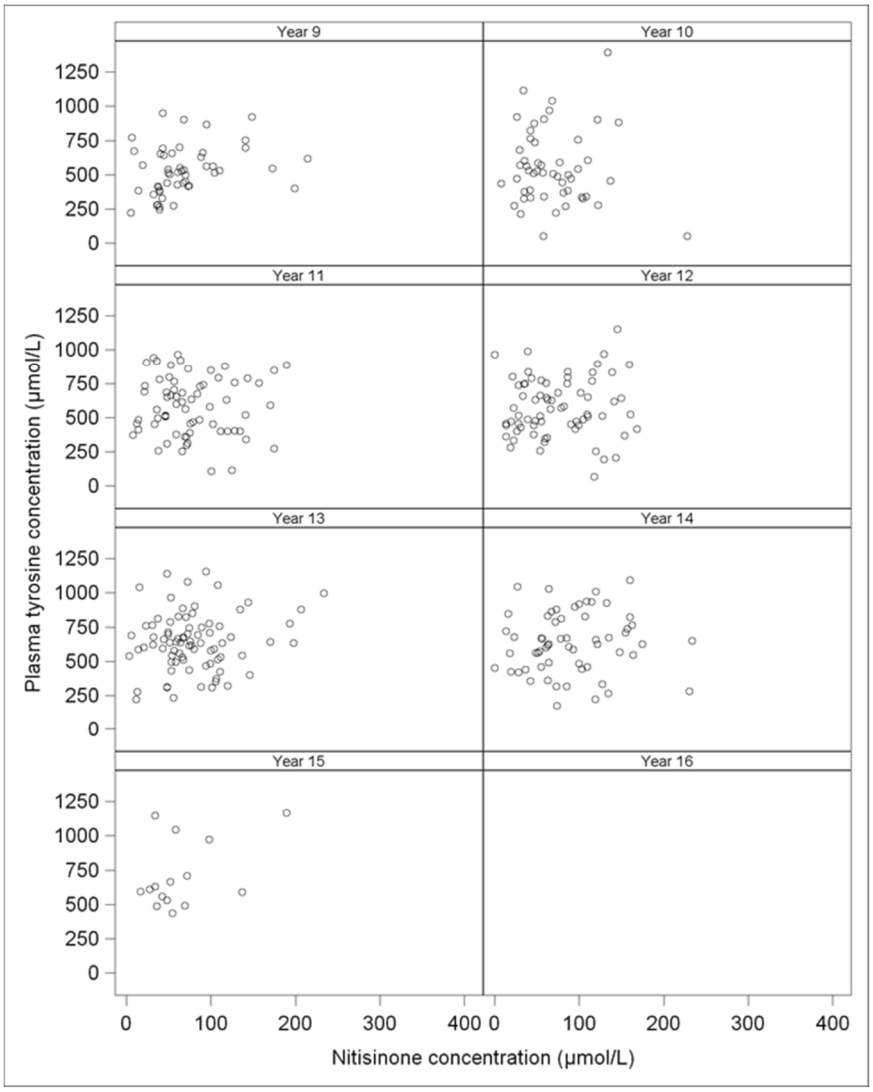
Similar results were obtained in the index set (data not shown).

Figure 4 Plasma tyrosine concentration versus nitisinone concentration by treatment year, treatment year 1-8 (complete set)



Note: For patients with more than one assay of plasma tyrosine during a year, the maximum value is displayed. For patients with more than one assay of nitisinone during a year, the mean recorded concentration was used.

Figure 5 Plasma tyrosine concentration versus nitisinone concentration by treatment year, treatment year 9-16 (complete set)



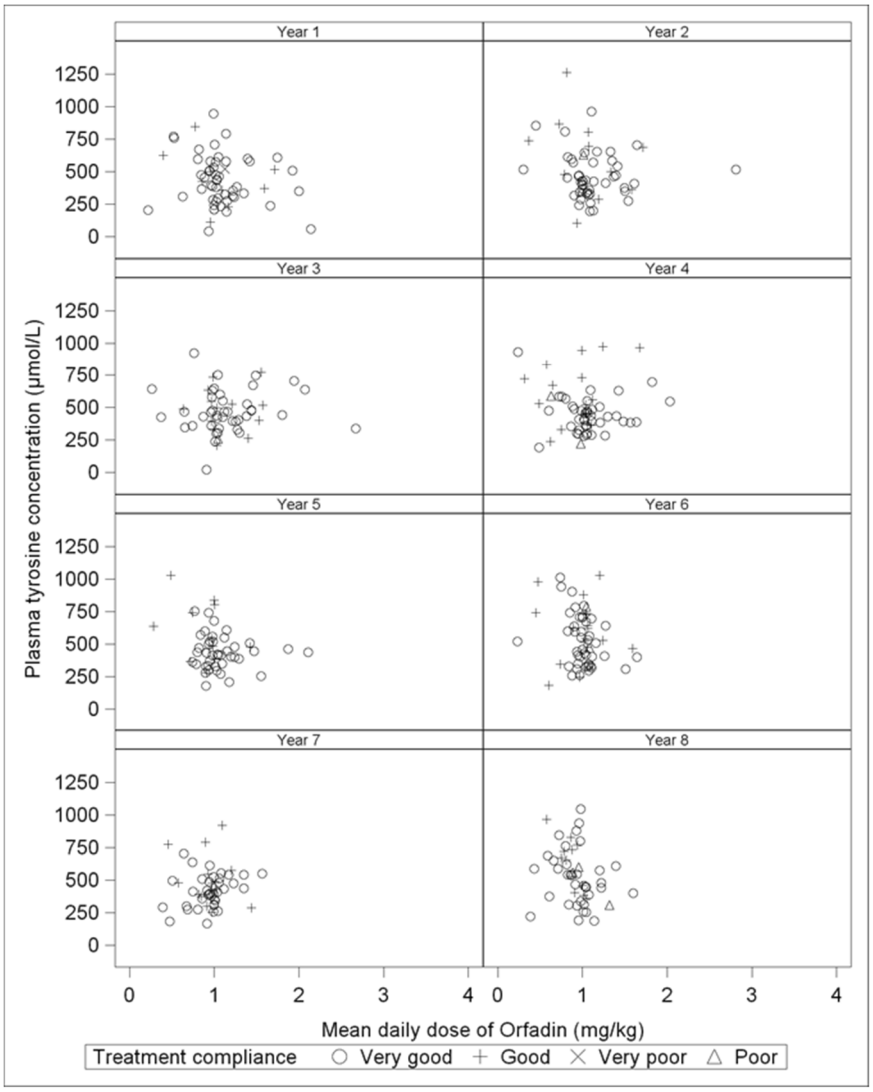
Note: For patients with more than one assay of plasma tyrosine during a year, the maximum value is displayed. For patients with more than one assay of nitisinone during a year, the mean recorded concentration was used.

10.4.2.9.1.4 Plasma tyrosine levels versus mean daily dose of Orfadin by treatment year

The p-Tyr levels versus mean daily dose of Orfadin by treatment year in the complete set are presented graphically in Figure 6 (Treatment Year 1-8) and Figure 7 (Treatment Year 9-16). The treatment compliance is also indicated in the graphs. There was no clear relation between the p-Tyr levels and the mean daily dose of Orfadin.

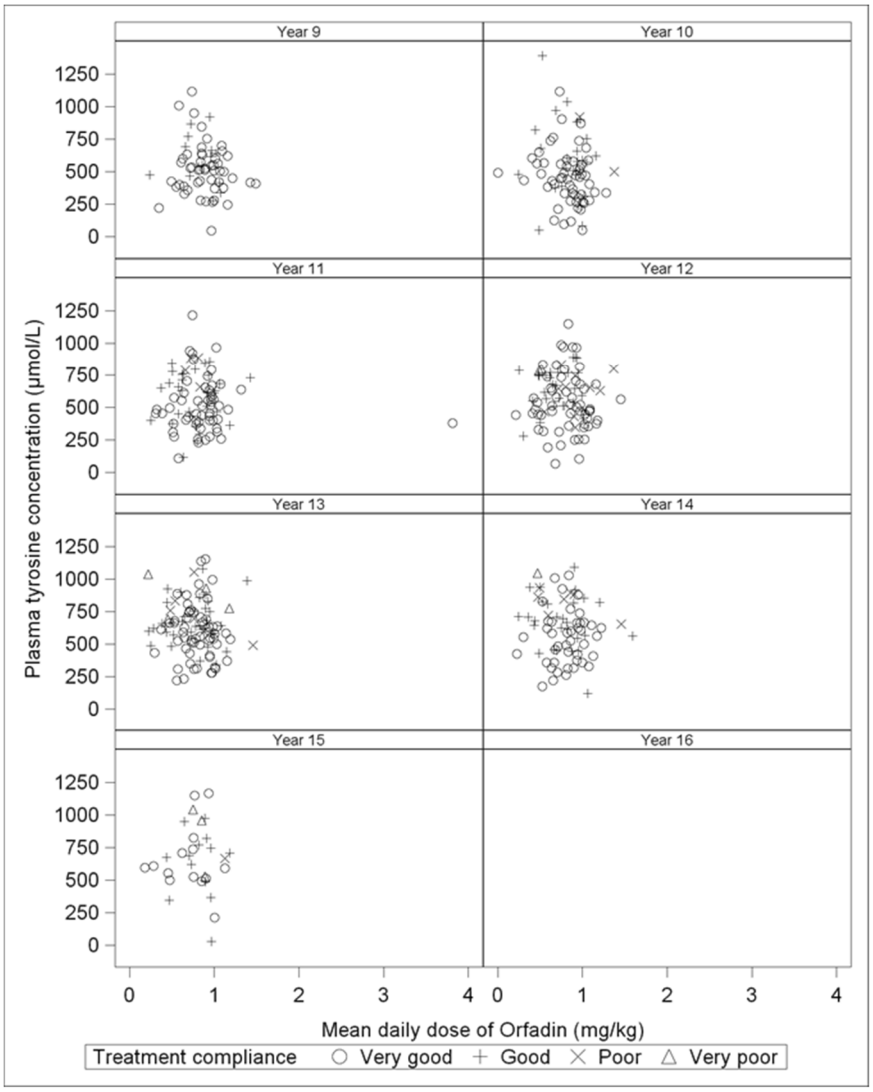
Similar results were obtained in the index set (data not shown).

Figure 6 Plasma tyrosine concentration versus mean daily dose of Orfadin (mg/kg) by treatment year, treatment year 1-8 (complete set)



Note: For patients with more than one assay of plasma tyrosine during a year, the maximum value is displayed.

Figure 7 Plasma tyrosine concentration versus mean daily dose of Orfadin (mg/kg) by treatment year, treatment year 9-16 (complete set)



Note: For patients with more than one assay of plasma tyrosine during a year, the maximum value is displayed.

10.4.2.9.2 Plasma phenylalanine

As stated above, since assessment of p-Phe levels were not measured in OAS but introduced in the PASS, only a limited number of patients had p-Phe levels measured during the study (from 34 patients at Treatment Year 15 up to 98 patients at Treatment Years 12 and 13 in the complete set) (Table 18).

Similar results were obtained in the index set (data not shown).

Table 18 Plasma phenylalanine concentration (µmol/L) by treatment year (complete set)

	n	Mean	SD	Median	Min, Max
Year 1	63	41.4	24.45	40	5, 153
Year 2	61	42.0	19.26	44	4, 90
Year 3	56	38.0	17.34	38	4, 81
Year 4	62	47.6	20.94	43	4, 108
Year 5	56	41.8	21.05	41	7, 128
Year 6	55	48.8	19.24	47	4, 106
Year 7	45	46.9	24.15	45	6, 171
Year 8	51	48.9	13.61	47	20, 87
Year 9	65	48.1	18.16	46	7, 103
Year 10	78	52.4	33.22	47	8, 299
Year 11	90	47.5	14.78	47	12, 107
Year 12	98	51.0	18.75	49	7, 112
Year 13	98	80.1	264.42	50	6, 2664
Year 14	79	48.6	17.01	48	12, 93
Year 15	34	48.7	19.00	48	13, 94

Abbreviations: n, Total number of patients with at least one assessment of phenylalanine during the corresponding year; SD, Standard deviation.
Note: For patients with more than one assay of phenylalanine during a year, the maximum value is displayed.

10.4.2.9.3 Plasma/serum/dried blood spot or urinary succinylacetone

10.4.2.9.3.1 Number of patients with detectable level of succinylacetone in plasma, serum, dried blood spot or urine

Only a limited number of patients had SA levels measured during the study (from 35 patients at Treatment Year 15 up to 102 patients at Treatment Year 7 in the complete set) (Table 19). The total proportion of patients with detectable level of SA in plasma, serum, DBS or urine, among those patients who had SA levels measured, was 62.9 % at Treatment Year 1 and 48.6 % at Treatment Year 15.

Table 19 Number of patients with detectable level of succinylacetone in plasma, serum, dried blood spot or urine (complete set)

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Year 1				
Total	62	39 (62.9)	0 (0.0)	7 (11.3)
Plasma/serum	23	13 (56.5)	0 (0.0)	4 (17.4)
Dried blood spot	25	18 (72.0)	0 (0.0)	0 (0.0)
Urine	22	10 (45.5)	0 (0.0)	3 (13.6)
Year 2				
Total	60	33 (55.0)	0 (0.0)	2 (3.3)
Plasma/serum	25	11 (44.0)	0 (0.0)	1 (4.0)
Dried blood spot	29	19 (65.5)	0 (0.0)	0 (0.0)
Urine	20	5 (25.0)	0 (0.0)	1 (5.0)
Year 3				
Total	58	25 (43.1)	0 (0.0)	0 (0.0)
Plasma/serum	23	6 (26.1)	0 (0.0)	0 (0.0)
Dried blood spot	25	16 (64.0)	0 (0.0)	0 (0.0)
Urine	23	6 (26.1)	0 (0.0)	0 (0.0)
Year 4				
Total	56	32 (57.1)	0 (0.0)	5 (8.9)
Plasma/serum	22	13 (59.1)	0 (0.0)	4 (18.2)
Dried blood spot	23	17 (73.9)	0 (0.0)	0 (0.0)
Urine	25	6 (24.0)	0 (0.0)	1 (4.0)
Year 5				
Total	54	20 (37.0)	1 (1.9)	6 (11.1)
Plasma/serum	27	11 (40.7)	0 (0.0)	6 (22.2)
Dried blood spot	12	8 (66.7)	1 (8.3)	0 (0.0)
Urine	25	3 (12.0)	0 (0.0)	0 (0.0)
Year 6				
Total	58	23 (39.7)	1 (1.7)	2 (3.4)
Plasma/serum	28	8 (28.6)	0 (0.0)	1 (3.6)
Dried blood spot	16	12 (75.0)	1 (6.3)	0 (0.0)
Urine	23	4 (17.4)	0 (0.0)	1 (4.3)
Year 7				
Total	102	35 (34.3)	0 (0.0)	13 (12.7)
Plasma/serum	71	21 (29.6)	0 (0.0)	11 (15.5)
Dried blood spot	14	10 (71.4)	0 (0.0)	0 (0.0)

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Urine	25	6 (24.0)	0 (0.0)	2 (8.0)
Year 8				
Total	81	48 (59.3)	1 (1.2)	26 (32.1)
Plasma/serum	32	20 (62.5)	0 (0.0)	13 (40.6)
Dried blood spot	15	10 (66.7)	0 (0.0)	0 (0.0)
Urine	46	20 (43.5)	1 (2.2)	14 (30.4)
Year 9				
Total	54	24 (44.4)	0 (0.0)	5 (9.3)
Plasma/serum	22	14 (63.6)	0 (0.0)	5 (22.7)
Dried blood spot	15	7 (46.7)	0 (0.0)	0 (0.0)
Urine	27	4 (14.8)	0 (0.0)	0 (0.0)
Year 10				
Total	63	36 (57.1)	0 (0.0)	0 (0.0)
Plasma/serum	23	18 (78.3)	0 (0.0)	0 (0.0)
Dried blood spot	20	14 (70.0)	0 (0.0)	0 (0.0)
Urine	27	5 (18.5)	0 (0.0)	0 (0.0)
Year 11				
Total	78	36 (46.2)	1 (1.3)	0 (0.0)
Plasma/serum	28	20 (71.4)	1 (3.6)	0 (0.0)
Dried blood spot	29	12 (41.4)	0 (0.0)	0 (0.0)
Urine	45	14 (31.1)	1 (2.2)	0 (0.0)
Year 12				
Total	92	50 (54.3)	3 (3.3)	0 (0.0)
Plasma/serum	27	13 (48.1)	2 (7.4)	0 (0.0)
Dried blood spot	43	34 (79.1)	1 (2.3)	0 (0.0)
Urine	38	8 (21.1)	1 (2.6)	0 (0.0)
Year 13				
Total	96	50 (52.1)	0 (0.0)	0 (0.0)
Plasma/serum	14	10 (71.4)	0 (0.0)	0 (0.0)
Dried blood spot	57	41 (71.9)	0 (0.0)	0 (0.0)
Urine	43	7 (16.3)	0 (0.0)	0 (0.0)
Year 14				
Total	71	40 (56.3)	0 (0.0)	0 (0.0)
Plasma/serum	14	11 (78.6)	0 (0.0)	0 (0.0)
Dried blood spot	38	29 (76.3)	0 (0.0)	0 (0.0)
Urine	34	6 (17.6)	0 (0.0)	0 (0.0)

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Year 15				
Total	35	17 (48.6)	0 (0.0)	0 (0.0)
Plasma/serum	11	8 (72.7)	0 (0.0)	0 (0.0)
Dried blood spot	15	8 (53.3)	0 (0.0)	0 (0.0)
Urine	16	3 (18.8)	0 (0.0)	0 (0.0)

Abbreviations: n, Number of patients.
Note: Percentage based on number of patients with at least one assay result.

To further explore the levels of SA in plasma, serum, DBS or urine, the levels were split into 5 frequency categories; 0-0.1, >0.1-0.5, >0.5-1, >1-2 and >2 µmol/L (Table 20). About 78 % and 88 % of the patients had SA levels ≤0.5 µmol/L and ≤2 µmol/L, respectively, at Treatment Year 1 and at the last assessment (Treatment Year 15), 80 % and 97 % of the patients had SA levels ≤0.5 µmol/L and ≤2 µmol/L, respectively.

Table 20 Frequency table of succinylacetone levels in plasma, serum, dried blood spot or urine (complete set)

Treatment year	**Patients with at least one assay result n**	**Succinylacetone level (µmol/L)**	**Number of patients n (%)**	**Cumulative number of patients n (%)**
Year 1	60	0-0.1	31 (51.7)	31 (51.7)
		>0.1-0.5	16 (26.7)	47 (78.3)
		>0.5-1	4 (6.7)	51 (85.0)
		>1-2	2 (3.3)	53 (88.3)
		>2	7 (11.7)	60 (100.0)
Year 2	59	0-0.1	30 (50.8)	30 (50.8)
		>0.1-0.5	22 (37.3)	52 (88.1)
		>0.5-1	6 (10.2)	58 (98.3)
		>1-2	1 (1.7)	59 (100.0)
		>2	0 (0.0)	59 (100.0)
Year 3	58	0-0.1	35 (60.3)	35 (60.3)
		>0.1-0.5	15 (25.9)	50 (86.2)
		>0.5-1	5 (8.6)	55 (94.8)
		>1-2	2 (3.4)	57 (98.3)
		>2	1 (1.7)	58 (100.0)
Year 4	56	0-0.1	30 (53.6)	30 (53.6)
		>0.1-0.5	16 (28.6)	46 (82.1)

Treatment year	Patients with at least one assay result n	Succinylacetone level (µmol/L)	Number of patients n (%)	Cumulative number of patients n (%)
		>0.5-1	3 (5.4)	49 (87.5)
		>1-2	4 (7.1)	53 (94.6)
		>2	3 (5.4)	56 (100.0)
Year 5	54	0-0.1	40 (74.1)	40 (74.1)
		>0.1-0.5	9 (16.7)	49 (90.7)
		>0.5-1	2 (3.7)	51 (94.4)
		>1-2	0 (0.0)	51 (94.4)
		>2	3 (5.6)	54 (100.0)
Year 6	58	0-0.1	40 (69.0)	40 (69.0)
		>0.1-0.5	9 (15.5)	49 (84.5)
		>0.5-1	5 (8.6)	54 (93.1)
		>1-2	1 (1.7)	55 (94.8)
		>2	3 (5.2)	58 (100.0)
Year 7	102	0-0.1	83 (81.4)	83 (81.4)
		>0.1-0.5	12 (11.8)	95 (93.1)
		>0.5-1	4 (3.9)	99 (97.1)
		>1-2	1 (1.0)	100 (98.0)
		>2	2 (2.0)	102 (100.0)
Year 8	81	0-0.1	56 (69.1)	56 (69.1)
		>0.1-0.5	8 (9.9)	64 (79.0)
		>0.5-1	7 (8.6)	71 (87.7)
		>1-2	7 (8.6)	78 (96.3)
		>2	3 (3.7)	81 (100.0)
Year 9	54	0-0.1	35 (64.8)	35 (64.8)
		>0.1-0.5	12 (22.2)	47 (87.0)
		>0.5-1	6 (11.1)	53 (98.1)
		>1-2	0 (0.0)	53 (98.1)
		>2	1 (1.9)	54 (100.0)
Year 10	63	0-0.1	38 (60.3)	38 (60.3)
		>0.1-0.5	16 (25.4)	54 (85.7)
		>0.5-1	6 (9.5)	60 (95.2)
		>1-2	3 (4.8)	63 (100.0)
		>2	0 (0.0)	63 (100.0)
Year 11	78	0-0.1	49 (62.8)	49 (62.8)
		>0.1-0.5	17 (21.8)	66 (84.6)
		>0.5-1	10 (12.8)	76 (97.4)
		>1-2	1 (1.3)	77 (98.7)

Treatment year	Patients with at least one assay result n	Succinylacetone level (µmol/L)	Number of patients n (%)	Cumulative number of patients n (%)
		>2	1 (1.3)	78 (100.0)
Year 12	92	0-0.1	47 (51.1)	47 (51.1)
		>0.1-0.5	18 (19.6)	65 (70.7)
		>0.5-1	15 (16.3)	80 (87.0)
		>1-2	8 (8.7)	88 (95.7)
		>2	4 (4.3)	92 (100.0)
Year 13	96	0-0.1	50 (52.1)	50 (52.1)
		>0.1-0.5	15 (15.6)	65 (67.7)
		>0.5-1	22 (22.9)	87 (90.6)
		>1-2	7 (7.3)	94 (97.9)
		>2	2 (2.1)	96 (100.0)
Year 14	71	0-0.1	36 (50.7)	36 (50.7)
		>0.1-0.5	17 (23.9)	53 (74.6)
		>0.5-1	8 (11.3)	61 (85.9)
		>1-2	4 (5.6)	65 (91.5)
		>2	6 (8.5)	71 (100.0)
Year 15	35	0-0.1	24 (68.6)	24 (68.6)
		>0.1-0.5	4 (11.4)	28 (80.0)
		>0.5-1	1 (2.9)	29 (82.9)
		>1-2	5 (14.3)	34 (97.1)
		>2	1 (2.9)	35 (100.0)

Abbreviations: n, Number of patients.

Note: Percentage based on number of patients with at least one assay result. For patients with more than one assay of succinylacetone in plasma, serum, dried blood spot or urine during a year, the maximum value is displayed. Only assessments with numeric values included, i.e., TRACE findings are excluded.

When the SA levels were classified as abnormal and assessed by clinical relevance, as judged by the investigator, the proportion of patients with clinically relevant SA values, among those patients who had SA levels measured, was very low during the study; e.g. at most treatment years there were no patients with clinically relevant abnormal SA values (Table 19).

Similar results were obtained in the index set (data not shown).

10.4.2.9.3.2 Succinylacetone by treatment year

Except for a few patients with high SA values at Treatment Years 1, 3, 4 and 9, the SA levels were generally low during the study with mean and median values ranging 0.18 to 0.49 µmol/L and 0.0 to 0.1 µmol/L, respectively (Table 21).

Similar results were obtained in the index set (data not shown).

Table 21 Succinylacetone (µmol/L) by treatment year (complete set)

	n	Mean	SD	Median	Min, Max
Year 1	60	8.68	45.832	0.1	0.0, 325.0
Year 2	59	0.24	0.347	0.1	0.0, 2.0
Year 3	58	1.07	6.548	0.0	0.0, 50.0
Year 4	56	2.11	13.330	0.1	0.0, 100.0
Year 5	54	0.22	0.571	0.0	0.0, 2.9
Year 6	58	0.27	0.600	0.0	0.0, 2.6
Year 7	102	0.18	0.624	0.0	0.0, 5.4
Year 8	81	0.41	0.825	0.1	0.0, 4.2
Year 9	54	18.68	136.061	0.0	0.0, 1000.0
Year 10	63	0.25	0.439	0.1	0.0, 2.0
Year 11	78	0.26	0.558	0.0	0.0, 4.0
Year 12	92	0.45	0.682	0.1	0.0, 3.2
Year 13	96	0.41	0.604	0.1	0.0, 3.4
Year 14	71	0.49	0.788	0.1	0.0, 3.1
Year 15	35	0.33	0.613	0.0	0.0, 2.5

Abbreviations: n, Total number of patients with at least one assessment of succinylacetone in plasma, serum, dried blood spot or urine during the corresponding year; SD, Standard deviation.
Note: For patients with more than one assay of succinylacetone in plasma, serum, dried blood spot or urine during a year, the maximum value is displayed. Only assessments with numeric values included, i.e., TRACE findings are excluded.
Several succinylacetone values were reported with a suspected erroneous unit. The maximum value at Year 9 indicates that at least one value was likely to be reported with mmol/L instead of µmol/L resulting in a value 1000 times higher than the actual value.

10.4.2.9.3.3 Detectable level of succinylacetone in plasma, serum, blood spot or urine versus the plasma/serum/dried blood spot nitisinone concentration

The association between a detectable level of SA in plasma, serum, blood spot or urine and the plasma/serum/DBS nitisinone concentration is presented graphically for each year of Orfadin treatment in Figure 9 (Treatment Year 1-8) and Figure 10 (Treatment Year 9-16). There was no clear relation between a detectable level of SA in plasma, serum, dried blood or urine and the plasma/serum/DBS nitisinone concentration but the plasma/serum/DBS nitisinone concentration seemed to be similar in patients with or without detectable SA levels.

Similar results were obtained in the index set (data not shown).

10.4.2.9.3.4 Succinylacetone levels versus Orfadin dose by treatment year

The association between a detectable level of SA in plasma, serum, DBS or urine and mean daily dose of Orfadin is presented graphically for each year of Orfadin treatment in Figure 11 (Treatment Year 1-8) and Figure 12 (Treatment Year 9-16). There was no clear relation between a detectable level of SA in plasma, serum, blood spot or urine and the mean daily dose of Orfadin.

Similar results were obtained in the index set (data not shown).

10.4.2.9.3.5 Succinylacetone levels in patients on once daily dosing

Throughout the study, very few patients had detectable SA level among those patients who had SA levels measured, in either of the two once daily subgroups (Table 47 and Table 48). In addition, when the SA levels were classified as abnormal and assessed by clinical relevance, there were no cases of patients with clinically relevant (or unknown) SA values except for the urine SA level in one treatment-naïve patient at Treatment Year 1 (Table 47) and the DBS SA level in one treatment-experienced patient at Treatment Year 5 (Table 48).

For further information about SA levels in patients on once daily dosing, see Table 49 and Table 50 and Figure 13 to Figure 16.

10.4.2.9.4 Plasma alpha-fetoprotein

Only a limited number of patients had p-AFP levels measured during the study (from 29 patients at Treatment Year 15 up to 100 patients at Treatment Years 12 and 13 in the complete set) (Table 22). The p-AFP levels decreased during the study (Figure 8), from a median (min, max) at Treatment Year 1 of 31 ng/mL (0, 326707 ng/mL) to 4 ng/mL (0, 70 ng/mL) at Treatment Year 15 (Table 22).

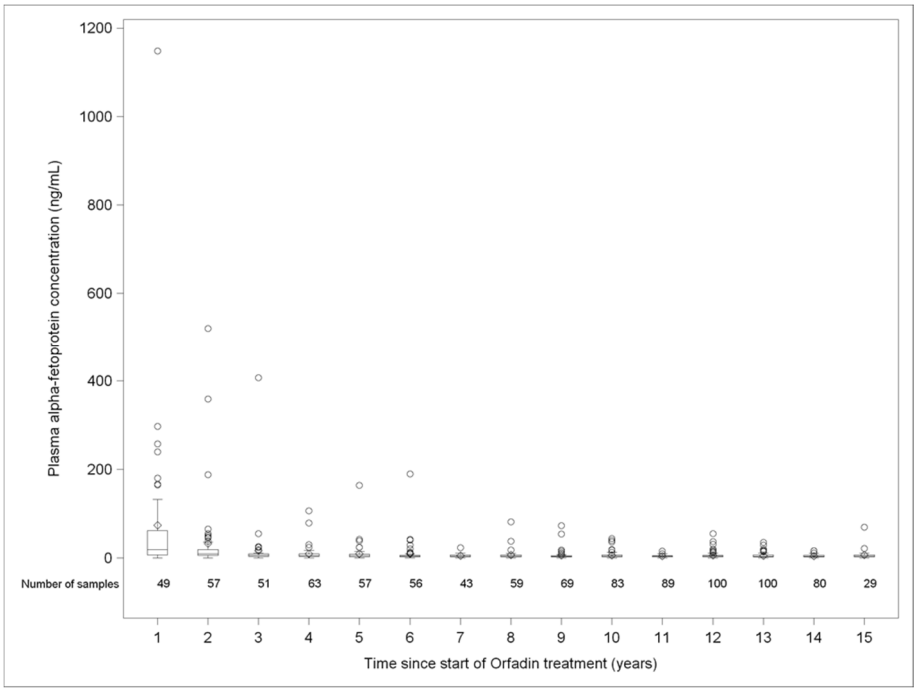
Similar results were obtained in the index set (data not shown).

Table 22 Plasma alpha-fetoprotein concentration (ng/mL) by treatment year (complete set)

	n	Mean	SD	Median	Min, Max
Year 1	59	15663.2	53891.45	31	0, 326707
Year 2	57	32.7	84.24	9	0, 520
Year 3	51	16.8	56.41	6	0, 407
Year 4	63	9.7	16.32	6	0, 107
Year 5	57	10.0	22.22	5	0, 164
Year 6	56	9.8	25.86	4	0, 190
Year 7	43	5.1	4.04	4	0, 24
Year 8	59	6.7	11.28	5	0, 82
Year 9	69	6.1	10.60	4	0, 73
Year 10	83	6.5	7.43	4	0, 44
Year 11	89	4.5	2.60	4	0, 16
Year 12	100	6.1	7.31	4	0, 55
Year 13	100	5.7	5.29	4	0, 35
Year 14	80	4.4	3.15	4	0, 16
Year 15	29	7.2	12.81	4	0, 70

Abbreviations: n, Total number of patients with at least one assessment of plasma alpha-fetoprotein during the corresponding year; SD, Standard deviation.
Note: For patients with more than one assay of plasma alpha-fetoprotein during a year, the maximum value is displayed.

Figure 8 Plasma alpha-fetoprotein concentration (ng/mL) by treatment year (complete set)



Note: 10 outliers with alpha-fetoprotein values ranging between 4458 and 326707 were excluded from the plot for readability.

The number of patients with p-AFP levels >6.6 ng/mL also decreased during the study from 81.4 % at Treatment Year 1 to 27.6 % at Treatment Year 15 (Table 23).

Table 23 Number of patients with plasma alpha-fetoprotein concentration >6.6 ng/mL (complete set)

Treatment year	Patients with at least one assay result n	Patients with sample >6.6 ng/mL n (%)
Year 1	59	48 (81.4)
Year 2	57	40 (70.2)
Year 3	51	23 (45.1)
Year 4	63	24 (38.1)
Year 5	57	19 (33.3)
Year 6	56	13 (23.2)
Year 7	43	10 (23.3)
Year 8	59	16 (27.1)
Year 9	69	11 (15.9)
Year 10	83	23 (27.7)
Year 11	89	17 (19.1)
Year 12	100	22 (22.0)
Year 13	100	25 (25.0)
Year 14	80	11 (13.8)
Year 15	29	8 (27.6)

Abbreviations: n, Number of patients.
Note: Percentage based on number of patients with at least one assay result.

10.4.2.10 Compliance with Orfadin treatment and diet

10.4.2.10.1 Summary of treatment compliance

Most of the patients (88.6 %) in the complete set were assessed as having either “very good” or “good” treatment compliance, as assessed by the investigator, throughout the study (Table 24).

Table 24 Treatment compliance summarized over time (complete set)

	Patients (N=315) n (%)
Very good	154 (48.9)
Good	125 (39.7)
Poor	21 (6.7)
Very poor	9 (2.9)
Missing	6 (1.9)

Abbreviations: N, Total number of patients; n, Number of patients in each group.
Note: For each patient the worst treatment compliance over time is presented. Only assessments on or after the index date (February 21, 2005) are included.

The treatment compliance seemed to decline as the patient's aged, e.g. the proportion of patients who were assessed as having "very good" compliance decreased from 82.7 % at Treatment Year 1 to 43.2 % at Treatment Year 15 while the proportion of patients who were assessed as having "very poor" compliance increased from 1.3 % at Treatment Year 1 to 8.1 % at Treatment Year 15 (Table 25). This decline in treatment compliance can also be seen in Figure 17 and Figure 18.

Similar results were obtained in the index set (data not shown).

Table 25 Treatment compliance by treatment year (complete set)

Treatment year	Very good n (%)	Good n (%)	Poor n (%)	Very poor n (%)	Total n
Year 1	62 (82.7)	12 (16.0)	0 (0.0)	1 (1.3)	75
Year 2	52 (77.6)	13 (19.4)	2 (3.0)	0 (0.0)	67
Year 3	50 (75.8)	15 (22.7)	1 (1.5)	0 (0.0)	66
Year 4	47 (71.2)	16 (24.2)	3 (4.5)	0 (0.0)	66
Year 5	50 (76.9)	15 (23.1)	0 (0.0)	0 (0.0)	65
Year 6	51 (72.9)	17 (24.3)	2 (2.9)	0 (0.0)	70
Year 7	41 (70.7)	16 (27.6)	1 (1.7)	0 (0.0)	58
Year 8	41 (68.3)	15 (25.0)	4 (6.7)	0 (0.0)	60
Year 9	61 (73.5)	20 (24.1)	1 (1.2)	1 (1.2)	83
Year 10	64 (68.8)	27 (29.0)	2 (2.2)	0 (0.0)	93
Year 11	62 (62.0)	33 (33.0)	5 (5.0)	0 (0.0)	100
Year 12	61 (58.7)	31 (29.8)	10 (9.6)	2 (1.9)	104
Year 13	61 (55.5)	38 (34.5)	6 (5.5)	5 (4.5)	110
Year 14	47 (56.6)	28 (33.7)	7 (8.4)	1 (1.2)	83
Year 15	16 (43.2)	16 (43.2)	2 (5.4)	3 (8.1)	37

Abbreviations: n, Number of patients in each group.
Note: The percentage is based on the total number of patients with compliance assessments at each year.

10.4.2.10.2 Treatment compliance in patients on once daily dosing

All (100 %) treatment-naïve patients on once daily dosing and 85.7 % (9.5 % of these patients had missing data) of the treatment-experienced patients after initiation of once daily dosage for the index set were assessed as having either "very good" or "good" treatment compliance during the study (Table 51).

10.4.2.10.3 Diet compliance

At Treatment Year 1, all (100 %) patients in the complete set were on a diet low in phenylalanine and tyrosine (Table 26). The proportion of patients on a diet remained high and the lowest proportion was seen at Treatment Year 14 when 93.7 % of the patients were on such diet.

The diet compliance was relatively high in the beginning of the treatment but declined over time; e.g. the proportion of patients who were assessed as having "very good" or "good" diet

compliance decreased from 70.6 % at Treatment Year 1 to 43.8 % at Treatment Year 15 while the proportion of patients who were assessed as having "very poor" or "poor" compliance increased from 11.0 % at Treatment Year 1 to 56.2 % at Treatment Year 15 (Table 26). This decline in treatment compliance can also be seen in Figure 19 and Figure 20.

Similar results were obtained in the index set (data not shown).

Table 26 Diet information by treatment year (complete set)

	On diet low in phenylalanine and tyrosine				If yes, diet compliance				
	Yes n (%)	No n (%)	Missing n (%)	Total n	Very good n (%)	Good n (%)	Poor n (%)	Very poor n (%)	Unknown n (%)
Year 1	109 (100.0)	0 (0.0)	0	109	37 (33.9)	40 (36.7)	11 (10.1)	1 (0.9)	20 (18.3)
Year 2	95 (97.9)	2 (2.1)	0	97	25 (26.3)	49 (51.6)	7 (7.4)	2 (2.1)	12 (12.6)
Year 3	144 (96.6)	5 (3.4)	0	149	26 (18.1)	47 (32.6)	9 (6.3)	0 (0.0)	62 (43.1)
Year 4	154 (98.7)	2 (1.3)	0	156	28 (18.2)	91 (59.1)	24 (15.6)	2 (1.3)	9 (5.8)
Year 5	145 (98.0)	3 (2.0)	0	148	27 (18.6)	86 (59.3)	23 (15.9)	4 (2.8)	5 (3.4)
Year 6	126 (99.2)	1 (0.8)	0	127	27 (21.4)	62 (49.2)	23 (18.3)	3 (2.4)	11 (8.7)
Year 7	146 (96.1)	6 (3.9)	0	152	22 (15.1)	88 (60.3)	29 (19.9)	1 (0.7)	6 (4.1)
Year 8	121 (97.6)	3 (2.4)	0	124	18 (14.9)	64 (52.9)	33 (27.3)	2 (1.7)	4 (3.3)
Year 9	75 (97.4)	2 (2.6)	0	77	17 (22.7)	41 (54.7)	11 (14.7)	3 (4.0)	3 (4.0)
Year 10	87 (95.6)	4 (4.4)	0	91	30 (34.5)	34 (39.1)	19 (21.8)	3 (3.4)	1 (1.1)
Year 11	91 (95.8)	4 (4.2)	0	95	24 (26.4)	36 (39.6)	27 (29.7)	3 (3.3)	1 (1.1)
Year 12	96 (97.0)	3 (3.0)	0	99	15 (15.6)	32 (33.3)	38 (39.6)	7 (7.3)	4 (4.2)
Year 13	103 (99.0)	1 (1.0)	0	104	19 (18.4)	32 (31.1)	35 (34.0)	15 (14.6)	2 (1.9)
Year 14	74 (93.7)	5 (6.3)	0	79	11 (14.9)	33 (44.6)	17 (23.0)	11 (14.9)	2 (2.7)
Year 15	32 (94.1)	2 (5.9)	0	34	4 (12.5)	10 (31.3)	13 (40.6)	5 (15.6)	0 (0.0)

Abbreviations: n, Number of patients in each group for a given year.
Note: The percentage is based on the total number of patients at each year.

10.4.2.11 Extent of exposure

10.4.2.11.1 Cumulative exposure

The cumulative exposure to Orfadin during the study was 3172.7 patient years in the complete set (Table 9) and 1558.6 patient years in the index set (Table 42).

10.4.2.11.2 Daily dose of Orfadin

The mean daily weight-based dose of Orfadin decreased over time, from 1.13 mg/kg/day at Treatment Year 1 to 0.77 mg/kg/day at Treatment Year 15 in the complete set (Table 27). The mean daily weight-based dose was highest in the infants (1.20 mg/kg) and lowest in the adults (0.79 mg/kg) (Table 28).

Similar results were obtained in the index set (data not shown).

Table 27 Orfadin mean daily dose (mg/kg) by treatment year (complete set)

	n	Mean	SD	Median	Min, Max
Year 1	136	1.13	0.443	1.0	0.1, 4.3
Year 2	156	1.11	0.444	1.0	0.1, 4.3
Year 3	218	1.06	0.359	1.0	0.1, 2.7
Year 4	238	1.01	0.325	1.0	0.1, 2.0
Year 5	238	0.98	0.316	1.0	0.1, 2.1
Year 6	228	0.94	0.281	1.0	0.0, 2.1
Year 7	226	0.91	0.263	0.9	0.0, 1.7
Year 8	218	0.89	0.384	0.9	0.0, 5.2
Year 9	194	0.89	0.414	0.9	0.0, 5.6
Year 10	183	0.88	0.427	0.9	0.0, 5.6
Year 11	172	0.85	0.335	0.9	0.2, 3.8
Year 12	158	0.81	0.241	0.8	0.2, 1.7
Year 13	144	0.79	0.250	0.8	0.0, 1.5
Year 14	135	0.78	0.244	0.8	0.0, 1.6
Year 15	118	0.77	0.275	0.7	0.0, 1.8

Abbreviations: n, Total number of patients with dosing information for a given year; SD, Standard deviation.

Table 28 Development of Orfadin mean daily weight-based dose (mg/kg) with increasing age over time (complete set)

Mean daily dose	n	Mean	SD	Median	Min, Max
Newborn (<28 days)	10	1.08	0.212	1.1	0.5, 1.2
Infant (28 days - <2 years)	99	1.20	0.479	1.1	0.2, 4.3
Child (2 years - <12 years)	261	1.00	0.332	1.0	0.2, 3.3
Adolescent (12 years - <18 years)	161	0.81	0.228	0.8	0.2, 1.7
Adult (>=18 years)	92	0.79	0.255	0.8	0.2, 1.5

Abbreviations: n, Total number of patients with dosing information in each group; SD, Standard deviation.
Note: Every patient only contributes once to each age category, the mean is used if several values for a patient are available.

10.4.2.11.3 Plasma, serum or dried blood spot concentration of nitisinone

Only a limited number of patients had plasma, serum or DBS concentration of nitisinone measured during the study (from 16 patients at Treatment Year 15 up to 99 patients at Treatment Year 7 in the complete set) (Table 29). The mean plasma, serum or DBS concentration of nitisinone tended to increase over treatment time, especially during the latter part of the study; e.g. it increased from 63.03 µmol/L at Treatment Year 1 to 90.69 µmol/L at Treatment Year 14 in the complete set. At Treatment Year 15, the nitisinone concentration was decreased again but at this timepoint, only a limited number of nitisinone values were available.

The plasma, serum or DBS concentration of nitisinone versus mean daily dose of Orfadin during the study is presented graphically for each year of Orfadin treatment in Figure 21 (Treatment

Year 1-8) and Figure 22 (Treatment Year 9-16). The treatment compliance is also indicated in the graphs. As expected, there was a positive relation between the concentration of nitisinone in plasma, serum or blood spot and the mean daily dose of Orfadin.

Similar results were obtained in the index set (data not shown).

Table 29 Plasma/serum/dried blood spot nitisinone concentration (µmol/L) by treatment year (complete set)

	n	Mean	SD	Median	Min, Max
Year 1	56	63.03	35.054	52.1	9.7, 180.0
Year 2	54	60.19	37.436	54.1	5.0, 183.8
Year 3	55	60.69	42.035	50.0	9.8, 261.6
Year 4	54	54.28	29.196	49.9	0.0, 169.2
Year 5	49	50.15	23.543	48.0	6.5, 134.4
Year 6	59	54.11	54.183	42.3	0.0, 378.0
Year 7	99	60.35	36.259	51.0	4.0, 174.7
Year 8	95	57.06	28.419	51.0	6.0, 139.9
Year 9	52	66.61	45.805	55.0	5.3, 214.3
Year 10	58	65.33	39.679	56.0	0.2, 228.1
Year 11	74	75.95	42.768	67.5	8.0, 189.6
Year 12	73	75.50	43.904	63.0	0.2, 168.5
Year 13	82	79.47	45.076	73.2	3.1, 233.5
Year 14	60	90.69	51.312	81.8	0.1, 233.9
Year 15	16	61.16	46.440	49.8	10.0, 189.6

Abbreviations: n, Total number of patients with at least one assessment of nitisinone during the corresponding year; SD, Standard deviation.
Note: For patients with more than one assay of nitisinone during a year, the mean recorded concentration was used.

The plasma, serum or DBS concentration of nitisinone versus mean daily dose of Orfadin by treatment year (Treatment Year 1-8) in treatment-naïve patients on once daily dosing and treatment-experienced patients after initiation of once daily dosage for the index set are presented graphically in Figure 23 and Figure 24, respectively. No nitisinone concentrations were assessed after Treatment Year 7 in the treatment-experienced patients and after Treatment Year 11 in the treatment-naïve patients. There were few nitisinone concentration values in these subgroups and therefore it is difficult to draw any conclusions from these data.

10.4.2.12 Overall clinical condition

10.4.2.12.1 Summary of overall clinical condition

For assessment of overall clinical condition, only assessments on or after the index date (February 21, 2005) were included. The number of days since start of Orfadin treatment until assessment of overall clinical condition in the index set is summarized by treatment year in Table 52.

Most of the patients (86.7 %) in the index set were assessed as having "good" overall clinical condition during the study (Table 30).

Table 30 Overall clinical condition summarized over time on Orfadin treatment (index set)

	Patients (N=203) n (%)
Good	176 (86.7)
Poor	13 (6.4)
Very poor	13 (6.4)
Not assessed	0 (0.0)
Missing	1 (0.5)

Abbreviations: N, Total number of patients; n, Number of patients in each group.
Note: For each patient the worst overall clinical condition over time is presented. Only assessments on or after the index date (February 21, 2005) are included.

The overall clinical condition improved from Treatment Year 1 to Treatment Year 2 and thereafter there was a consistently high proportion of patients with "good" overall condition throughout the study (Table 31).

Table 31 Overall clinical condition by treatment year (index set)

Treatment year	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Total n
Year 1	96 (84.2)	6 (5.3)	11 (9.6)	1 (0.9)	114
Year 2	98 (95.1)	4 (3.9)	1 (1.0)	0 (0.0)	103
Year 3	102 (98.1)	1 (1.0)	1 (1.0)	0 (0.0)	104
Year 4	93 (96.9)	2 (2.1)	0 (0.0)	1 (1.0)	96
Year 5	84 (98.8)	1 (1.2)	0 (0.0)	0 (0.0)	85
Year 6	91 (98.9)	1 (1.1)	0 (0.0)	0 (0.0)	92
Year 7	76 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	76
Year 8	59 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	59
Year 9	51 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	51
Year 10	48 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	48
Year 11	41 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	41
Year 12	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	30
Year 13	22 (95.7)	0 (0.0)	0 (0.0)	1 (4.3)	23
Year 14	9 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	9
Year 15	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

Abbreviations: n, Number of patients in each group.
Note: For patients with more than one visit for a given year the worst case of overall clinical condition is used.

Similar results were obtained in the complete set (data not shown).

10.4.2.12.2 Change in overall clinical condition

The change in overall clinical condition from first year of Orfadin treatment as compared to each subsequent year is illustrated in Table 32. The overall clinical condition for the patients with overall condition rated as "good" at Treatment Year 1, remained generally "good" for the rest of the study. Furthermore, for the few patients who had "poor" or "very poor" overall clinical condition at Treatment Year 1, the clinical condition at Treatment Year 2 and all subsequent treatment years, was assessed as "good".

Table 32 Shift table for overall clinical condition versus treatment year 1 (index set)

Year 1	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Missing n (%)
Year 2					
Good	61 (98.4)	4 (100.0)	6 (100.0)	0 (0.0)	27 (87.1)
Poor	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (9.7)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 3					
Good	50 (100.0)	3 (100.0)	7 (100.0)	0 (0.0)	42 (95.5)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 4					
Good	39 (97.5)	4 (100.0)	8 (100.0)	0 (0.0)	42 (95.5)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.5)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 5					
Good	33 (100.0)	4 (100.0)	5 (100.0)	0 (0.0)	42 (97.7)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 6					
Good	33 (97.1)	3 (100.0)	7 (100.0)	1 (100.0)	47 (100.0)
Poor	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 7					
Good	22 (100.0)	2 (100.0)	7 (100.0)	1 (100.0)	44 (100.0)

Year 1	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Missing n (%)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 8					
Good	22 (100.0)	2 (100.0)	4 (100.0)	0 (0.0)	31 (100.0)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 9					
Good	20 (100.0)	2 (100.0)	4 (100.0)	0 (0.0)	25 (100.0)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 10					
Good	12 (100.0)	2 (100.0)	5 (100.0)	0 (0.0)	29 (100.0)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 11					
Good	11 (100.0)	1 (100.0)	2 (100.0)	0 (0.0)	27 (100.0)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 12					
Good	5 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	23 (100.0)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Year 13					
Good	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	20 (95.2)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8)
Year 14					
Good	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	7 (100.0)
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Year 1	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Missing n (%)
Not assessed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: n, Number of patients in each group
Note: For patients with more than one visit for a given year the worst case of overall clinical condition is used.

10.4.2.12.3 Overall clinical condition versus treatment compliance

The treatment compliance seemed to decline over the treatment time (Section 10.4.2.10.1). When cross-tabulating the overall clinical condition with the treatment compliance, no clear relation between these variables could be seen but most patients had a good clinical condition regardless the outcome of the treatment compliance assessments (Table 33). It can also be seen that a substantial number of patients had an "unknown" treatment compliance.

Similar results were seen in the complete set (data not shown).

Table 33 Cross-tabulation of overall clinical condition by treatment compliance, by year after start of Orfadin treatment (index set)

Overall clinical condition	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Total n
Treatment compliance					
Year 1					
Very good	56 (91.8)	1 (1.6)	4 (6.6)	0 (0.0)	61
Good	10 (83.3)	0 (0.0)	2 (16.7)	0 (0.0)	12
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Very poor	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1
Unknown	30 (75.0)	5 (12.5)	4 (10.0)	1 (2.5)	40
Year 2					
Very good	50 (98.0)	1 (2.0)	0 (0.0)	0 (0.0)	51
Good	13 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	13
Poor	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	33 (89.2)	3 (8.1)	1 (2.7)	0 (0.0)	37
Year 3					
Very good	46 (97.9)	0 (0.0)	1 (2.1)	0 (0.0)	47
Good	15 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	15
Poor	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	40 (97.6)	1 (2.4)	0 (0.0)	0 (0.0)	41
Year 4					
Very good	44 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	44

Overall clinical condition	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Total n
Good	15 (93.8)	1 (6.3)	0 (0.0)	0 (0.0)	16
Poor	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	3
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	31 (93.9)	1 (3.0)	0 (0.0)	1 (3.0)	33
Year 5					
Very good	47 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	47
Good	15 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	15
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	22 (95.7)	1 (4.3)	0 (0.0)	0 (0.0)	23
Year 6					
Very good	49 (98.0)	1 (2.0)	0 (0.0)	0 (0.0)	50
Good	17 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	17
Poor	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	23 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	23
Year 7					
Very good	38 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	38
Good	16 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	16
Poor	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	21 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	21
Year 8					
Very good	34 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	34
Good	10 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	10
Poor	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	3
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	12 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	12
Year 9					
Very good	41 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	41
Good	8 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	8
Poor	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
Year 10					
Very good	32 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	32
Good	15 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	15
Poor	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1

Overall clinical condition	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Total n
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Year 11					
Very good	27 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	27
Good	11 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	11
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	3
Year 12					
Very good	22 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	22
Good	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	4
Poor	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	3
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
Year 13					
Very good	16 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	16
Good	5 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	5
Poor	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1
Year 14					
Very good	5 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	5
Good	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	3
Poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Very poor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Unknown	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1

Abbreviations: n, Number of patients in each group
Note: For patients with more than one visit for a given year the worst case of overall clinical condition is used.

10.4.2.12.4 Summary of overall clinical condition in the once daily Orfadin treatment subgroups

In the treatment-naïve patients on once daily dosing, the overall clinical condition improved from Treatment Year 1 to Treatment Year 2 as expected and thereafter there was a consistently high proportion of patients with good overall condition rated throughout the study (Table 34). In the treatment-experienced patients, there was a consistently high proportion of patients with good overall condition rated already from the start of the study and throughout the study (Table 35).

Table 34 Overall clinical condition by treatment year for treatment-naïve patients on once daily dosage (index set)

Treatment year	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Total n
Year 1	2 (33.3)	1 (16.7)	2 (33.3)	1 (16.7)	6
Year 2	5 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	5
Year 3	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	4
Year 4	4 (80.0)	1 (20.0)	0 (0.0)	0 (0.0)	5
Year 5	8 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	8
Year 6	8 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	8
Year 7	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	3
Year 8	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2
Year 9	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2
Year 10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Year 11	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1

Abbreviations: n, Number of patients in each group.
Note: For patients with more than one visit for a given year the worst case of overall clinical condition is used.
Treatment-naïve: Patients who have previously not been treated with Orfadin and start their Orfadin treatment with once daily dosage (as confirmed by the first dose recorded for each patient).

Table 35 Overall clinical condition by treatment year for treatment-experienced patients after initiation of once daily dosing (index set)

Treatment year	Good n (%)	Poor n (%)	Very poor n (%)	Not assessed n (%)	Total n
Year 1	11 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	11
Year 2	14 (93.3)	0 (0.0)	1 (6.7)	0 (0.0)	15
Year 3	12 (92.3)	1 (7.7)	0 (0.0)	0 (0.0)	13
Year 4	14 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	14
Year 5	12 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	12
Year 6	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	6
Year 7	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1

Abbreviations: n, Number of patients in each group.
Note: For patients with more than one visit for a given year the worst case of overall clinical condition is used.
Treatment-experienced: Patients who have switched to once daily dosage after twice daily dosage (as confirmed by a twice daily regimen reported before the once daily dosing).

10.5 Other analyses

There were no other analyses performed.

10.6 Adverse events/adverse reaction

The primary endpoint of this study was the occurrence of defined AEs (defined in Section 9.4.1) and the occurrence of other AEs was a secondary endpoint (defined in Section 9.4.2.7). Therefore, key AE results have already been reported in the Section 10.4.

In this section, all AE data are presented to provide a complete picture of the AEs reported during the study.

10.6.1 Brief summary of adverse events

A summary of AEs, SAEs, AEs leading to death, AEs related to study medication and AEs leading to discontinuation of study medication is provided in Table 36. During the study, 91 patients (28.9 %) in the complete set reported at least one AE. In total, 45 patients in the complete set (14.3 %) experienced an SAE and 53 patients (16.8 %) had an AE that was assessed by the investigator as related to the study medication. Only 3 patients (1.0 %) discontinued the study medication due to an AE (all 3 patients had liver transplantations).

Similar results were obtained in the index set.

Table 36 Summary of Adverse Events

	Complete set[a]	Index set
	Patients (N=315) n (%)	**Patients (N=203) n (%)**
Any AE	91 (28.9)	50 (24.6)
Any SAE	45 (14.3)	26 (12.8)
AEs leading to death	0 (0.0)	0 (0.0)
AEs related to study medication	53 (16.8)	29 (14.3)
AEs leading to discontinuation of study medication	3 (1.0)	3 (1.5)

Abbreviations: AE, Adverse event; N, Total number of patients; n, Number of patients with at least one event; SAE, Serious adverse event.
[a]Only assessments on or after the index date (February 21, 2005) are included.

10.6.2 Most frequently reported adverse events

All AEs reported during the study in the complete set are summarized by SOCs and PTs in Table 53. The most frequently reported AEs (i.e., AEs with a PT incidence of ≥1.0 %) are presented in Table 37.

The most commonly reported events by SOCs were Investigations (10.8 %), Eye disorders (4.8 %), Nervous system disorders (4.4 %), General disorders and administration site condition (3.8 %) and Gastrointestinal disorders (3.5 %) (Table 53). The most commonly reported events by PTs were amino acid level increased (6.3 %) and drug level decreased (2.5 %) (both events within the SOC Investigations) (Table 37).

Similar results were obtained in the index set (data not shown).

Table 37 Most frequently (preferred term incidence cut-off ≥1.0 %) reported adverse events by system organ class and preferred term (complete set)

SOC/ Preferred term	Patients with at least one event (N=315) n (%)	Number of events
Any AE	91 (28.9)	192
Eye disorders	15 (4.8)	19
Eye pain	4 (1.3)	4
Gastrointestinal disorders	11 (3.5)	18
Abdominal pain	3 (1.0)	3
Vomiting	4 (1.3)	6
General disorders and administration site conditions	12 (3.8)	12
Treatment noncompliance	5 (1.6)	5
Injury, poisoning and procedural complications	8 (2.5)	9
Maternal exposure during pregnancy	5 (1.6)	5
Investigations	34 (10.8)	57
Alpha 1 foetoprotein increased	4 (1.3)	4
Amino acid level decreased[a]	5 (1.6)	5
Amino acid level increased[b]	20 (6.3)	28
Drug level decreased	8 (2.5)	8
Neoplasms benign, malignant and unspecified (including cysts and polyps)	4 (1.3)	4
Hepatic cancer	3 (1.0)	3
Nervous system disorders	14 (4.4)	16
Cognitive disorder	3 (1.0)	3
Epilepsy	3 (1.0)	3
Social circumstances	4 (1.3)	4
Diet noncompliance	3 (1.0)	3
Surgical and medical procedures	5 (1.6)	5
Liver transplant	5 (1.6)	5

Source: Modified from Table 53.
Abbreviations: N, Total number of patients; n, Number of patients with at least one corresponding event; AE, Adverse event; SOC, System Organ Class.
Note: Only assessments on or after the index date (February 21, 2005) are included.
[a]Includes decreased levels of p-Phe. [b]Includes increased levels of p-Tyr and p-Phe, the majority being p-Tyr.

10.6.3 Deaths and other serious adverse events

There were no deaths during the PASS (Table 36). However, 3 patients died during OAS and these are included in the extended mortality set and the extended mortality/liver transplantations set (Table 4). These patients died 12.3, 0.1 and 1.2 years after the start of Orfadin treatment.

In total, 45 patients (14.3 %) in the complete set experienced 78 SAEs (Table 54). Most events only occurred once during the study except for liver transplantation, vomiting, hepatic cancer, epilepsy, porphyria, pyrexia, treatment non-compliance, hepatic failure, amino acid level increased, drug level decreased, cognitive disorder and diet non-compliance (Table 38).

Table 38 Serious adverse events (preferred term) occurring in >1 patient (complete set)

SOC/ Preferred term	Patients with at least one event (N=315) n (%)	Number of events
Congenital, familial and genetic disorders		
Porphyria	2 (0.6)	2
Gastrointestinal disorders		
Vomiting	3 (1.0)	5
General disorders and administration site conditions		
Pyrexia	2 (0.6)	2
Treatment noncompliance	2 (0.6)	2
Hepatobiliary disorders		
Hepatic failure	2 (0.6)	2
Investigations		
Amino acid level increased	2 (0.6)	2
Drug level decreased	2 (0.6)	2
Neoplasms benign, malignant and unspecified (including cysts and polyps)		
Hepatic cancer	3 (1.0)	3
Nervous system disorders		
Cognitive disorder	2 (0.6)	2
Epilepsy	3 (1.0)	3
Social circumstances		
Diet noncompliance	2 (0.6)	2
Surgical and medical procedures		
Liver transplant	5 (1.6)	5

Source: Modified from Table 54.
Abbreviations: AE, Adverse event; N, Total number of patients; n, Number of patients with at least one corresponding event; SOC, System Organ Class.
Note: Only assessments on or after the index date (February 21, 2005) are included.

Most SAEs were assessed as not related to study medication (Table 54). The events assessed by the investigator as related to study medication occurred mostly as single events except for drug level decreased and cognitive disorder (Table 39).

Table 39 Serious adverse events (preferred term) assessed as related to study medication by the investigator (complete set)

SOC/ Preferred term	Patients with at least one event (N= 315) n (%)	Number of events
Congenital, familial and genetic disorders		
Porphyria	1 (0.3)	2
Eye disorders		
Corneal erosion	1 (0.3)	1
Ulcerative keratitis	1 (0.3)	1
Gastrointestinal disorders		
Duodenal ulcer	1 (0.3)	1
Pancreatitis	1 (0.3)	1
General disorders and administration site conditions		
Developmental delay	1 (0.3)	1
Mucosal inflammation	1 (0.3)	1
Pseudocyst	1 (0.3)	1
Treatment noncompliance	1 (0.3)	1
Hepatobiliary disorders		
Hepatic lesion	1 (0.3)	1
Investigations		
Amino acid level increased	1 (0.3)	1
Drug level decreased	2 (0.6)	2
Nervous system disorders		
Cognitive disorder	2 (0.6)	2
Speech disorder developmental	1 (0.3)	1
Psychiatric disorders		
Aggression	1 (0.3)	1
Autism spectrum disorder	1 (0.3)	1
Confusional state	1 (0.3)	1
Hallucination, auditory	1 (0.3)	1
Psychotic disorder	1 (0.3)	1
Renal and urinary disorders		
Acute kidney injury	1 (0.3)	1
Skin and subcutaneous tissue disorders		

SOC/ Preferred term	Patients with at least one event (N= 315) n (%)	Number of events
Pruritus	1 (0.3)	1
Rash	1 (0.3)	1
Social circumstances		
Diet noncompliance	1 (0.3)	1
Surgical and medical procedures		
Liver transplant	1 (0.3)	1

Source: Modified from Table 54.
Abbreviations: AE, Adverse event; N, Total number of patients; n, Number of patients with at least one corresponding event; SOC, System Organ Class.
Note: Only assessments on or after the index date (February 21, 2005) are included.

Similar results were obtained in the index set (data not shown).

10.6.4 Pregnancies

A total of 6 patients became pregnant during the study. The information about these pregnancies including outcomes is limited and summarized below:

- One patient was started on Orfadin treatment at the age of 3.5 years. The patient became at the age of 20 years. A healthy baby was born after an uneventful delivery. 2 years later, the patient became pregnant again and a baby was born. The patient had a third pregnancy (twin pregnancy) ending in stillbirth in week 20 at the age of 23 years. The patient had a fourth pregnancy one year later and a baby was born.
- One patient was started on Orfadin treatment within one month of birth. The patient became pregnant at the age of 21 years. The outcome of this pregnancy is unknown. The estimated delivery is after study end.
- One patient was started on Orfadin treatment during childhood. The patient became pregnant at the age of 24 years. The outcome of this pregnancy is unknown, and the estimated delivery was reported after study end.
- One patient was started on Orfadin treatment at the age of 3 months. The patient became pregnant at the age of 17 years. The outcome of this pregnancy is unknown. Estimated delivery is after study end.
- One patient had first reported Orfadin treatment at the age of 20 years. The patient became pregnant at the age of 25 years and a healthy baby was born.
- One patient was started on Orfadin treatment at the age of 8 months. The patient became pregnant at the age of 17 years and a healthy baby was born.

11 Discussion

11.1 Key results

- The results of the primary endpoint of assessing long-term safety of Orfadin used in standard clinical practice to treat patients with HT-1 did not reveal any new safety concerns and the previously known safety profile of Orfadin was confirmed also after exposure of the drug for up to 15 years:
    - The incidences of hepatic, renal, ophthalmic, hematological, or cognitive/developmental function AEs were low for all AE categories.
    - However, for hepatic function and hematological status AEs, there was a trend of increased incidence of these events with higher age at start of Orfadin treatment.
    - For renal function AEs, the only event was reported for a patient in the ≥12 months age at start of Orfadin treatment group.
- 36 patients (10.6 %) in the extended mortality/liver transplantation set either died or were liver transplanted and the occurrence of death or liver transplantation was more frequent the later the treatment was initiated.
- 4 patients (1.3 %) had reports of hepatic malignancy. No other malignancies were reported.
- There were no reported events of incorrect administration of oral suspension of Orfadin.
- AEs other than those related to the primary endpoint were reported with low frequency and were in line with the known safety profile of Orfadin.
- 11 patients (3.5 %) discontinued Orfadin treatment and 8 of these were due to liver transplantations, mostly in patients with late (≥6 months of age) treatment start.
- The results from the laboratory investigations confirmed increased values of p-Phe and p-Tyr over time, which seemed to correlate with data indicating a declined diet compliance. Even though mean p-Tyr was >500 µmol/L from Treatment Year 4 and onwards, less than 5 % (15 patients) experienced ophthalmic AEs. At most treatment years there were no patients with clinically relevant abnormal SA values, as judged by the Investigator.
- 88.6 % of the patients were assessed as having either “very good” or “good” treatment compliance throughout the study. All patients were on a diet low in phenylalanine and tyrosine at study start and the proportion remained high during the study. The compliance with Orfadin treatment and diet seemed to decline as patients grew older.
- The cumulative exposure to Orfadin during the study was 3172.7 patient years. The mean daily weight-based dose of Orfadin decreased over treatment time and was highest in the infants and lowest in the adults.
- Most patients were reported to have “good” (highest level of available categories for reporting) overall clinical condition throughout the study indicating a sustained effect of Orfadin during long-term exposure up to 15 years.

11.2 Limitations

11.2.1 Uncontrolled study design

An uncontrolled study design was the only feasible design in this non-intervention study because a comparator group could not be introduced in a study population where all patients are treated with Orfadin, and this treatment cannot be substituted. Accordingly, the study design carries the general limitations inherent in an uncontrolled design.

Furthermore, due to the real-life design of this long-term study in the normal clinical setting, many patients did not perform all assessments each year which means that the studied populations differed slightly from one treatment year to the other. Furthermore, because laboratory assessments for p-Tyr, p-Phe, SA, p-AFP and nitisinone plasma concentration were not assessed regularly in OAS but added to the PASS protocol, only a limited number of patients had these laboratory variables assessed during the study.

It should be remembered that the protocol of the study was developed in accordance with EMA guidelines (9) and approved by the EMA. The chosen design is thus deemed acceptable for a study with the primary objective to assess long-term safety of Orfadin used in HT-1 patients under standard clinical care.

11.2.2 Study monitoring and protocol deviations

There was no on-site study monitoring in this study. The quality of the data reported in the CRF were therefore solely under the investigator's responsibility as no source data verification were performed. The lack of on-site study monitoring also carries the risk for under-reporting of AEs. However, the study was designed to trigger AE reporting by the investigators and thus expected to result in a higher reporting of AEs compared with if only spontaneous AEs were to be reported.

During the study, protocol deviations related to investigators occasionally reporting AEs directly into the Sobi Drug Safety Database instead of via the eCRF were noted. Also, during the study close-out activities, it was identified that ICFs were not appropriately collected and documented for all patients. By a targeted manual data review of date for consenting in the eCRF, several cases of missing dates were identified and followed up until DBL. The issues had not been noted earlier because of not having any on-site monitoring in the study.

A total of 11 deviations concerning ICFs were identified. For 7 patients, the ICFs were dated after first data entry. For another 2 patients, the Investigator had only collected a verbal consent at study start, a written consent was collected retrospectively for both patients prior to DBL. At DBL, there were 2 patients for whom the Sponsor had no evidence on whether an ICF was signed, and the sites were unresponsive to questions from Sponsor representatives. As a result, the Sponsor decided to permanently delete the entered eCRF data for these 2 patients from the study database prior to extracting data for analysis and archiving. Data from these 2 patients are thus not included in this study report.

11.2.3 Data collection

AE data reported during the preceding OAS (prior to the PASS start in 2013) were included in this PASS report only for patients who provided informed consent to participate in the PASS. OAS data for patients who did not consent to join the PASS were not included in this study report. Thus, some AEs from OAS which have previously been reported in yearly PSURs for Orfadin may not have been included in the present study report. However, events of liver transplantation or death were incorporated for the OAS patients if available, for use with the extended population sets.

Due to the partially retrospective data set-up, some data queries could not be resolved before DBL and remained unresolved in the study database.

11.3 Interpretation

Before 1991, treatment of HT-1 was mainly based on diet to lower the tyrosine intake, and eventually liver transplantation. However, the prognosis was generally very poor (1, 2) and patients with the acute form presented with liver failure before the age of 6 months. Patients with subacute HT-1 usually suffered from liver disease before one year of age, whereas patients with chronic HT-1 usually suffered from liver cirrhosis and/or kidney disease. Because of the poor prognosis, it was previously not possible to follow these patients over an extended period of time. However, with the advent of the EMA approval of Orfadin in 2005 and its acceptance as standard of care for HT-1 (4), the prognosis of the disease has improved significantly and these patients now age with the disease.

The primary objective of the present PASS was to assess long-term safety of Orfadin used in standard clinical practice to treat patients with HT-1, by evaluation of occurrence of AEs related to hepatic, renal, ophthalmic, hematological functions, or cognitive/development, respectively. The results of the primary endpoint did not reveal any new safety concerns and the previously known safety profile of Orfadin, based on shorter-term studies as well as post-marketing surveillance, was confirmed also after exposure of the drug for up to 15 years.

Nitisinone is an enzymatic competitive inhibitor of 4-hydroxyphenylpyruvate dioxygenase and by inhibiting this enzyme, nitisinone prevents the accumulation of toxic intermediates of the tyrosine catabolic pathway such as SA. Successful treatment leads to rapid decrease in plasma and urine SA. Following this normalization of SA levels, other liver parameters such as transaminases, AFP and bilirubin will also normalize. The liver echogram will usually also improve. However, late initiation of treatment cannot rectify all liver damage and the risk of developing liver malignancy remains elevated. Therefore, the EU Orfadin SmPC states that "Treatment of all genotypes of the disease should be initiated as early as possible to increase overall survival and avoid complications such as liver failure, liver cancer and renal disease." In the present study, only few patients either died or were liver transplanted and the occurrence of death or liver transplantation was higher the older the patients were at the start of Orfadin treatment. Furthermore, only 4 patients had reports of hepatic malignancy and 2 of these patients were ≥12 months at start of Orfadin treatment. These results thus confirm that early treatment is of importance and increase overall survival and reduces the risk of liver damage and malignancy.

Orfadin treatment leads to elevated tyrosine concentrations and this was also seen in the present study and many patients had occasions of p-Tyr levels >500 µmol/L during the study. Elevated levels of tyrosine have been associated with eye-related adverse reactions, such as e.g., corneal opacities and hyperkeratotic lesions.

Restriction of tyrosine and phenylalanine in the diet should limit the toxicity associated with this type of tyrosinemia by lowering tyrosine concentrations. In the present study, it was observed that compliance to diet seems to decline as the patient grows older; however, no increase in eye-related AEs were observed even for patients with elevated p-Tyr levels >500 µmol/L.

As part of normal clinical monitoring of patients with HT-1, if a patient present with elevated SA levels, the treating physician would prescribe a higher dose of Orfadin to further suppress the SA level, so at standard clinical visits it can be expected that some patients report increased levels. In the present study, it was shown that about 88 % of the patients had SA levels ≤2 µmol/L at Treatment Year 1 and at the last assessment (Treatment Year 15), this proportion was 97 %. Previous studies have demonstrated SA levels of 1-2 µmol/L in healthy people (11-14). Furthermore, assessment of SA levels by clinical relevance demonstrated that the proportion of patients with clinically relevant abnormal SA values, as judged by the investigator, was very low during the study; e.g. at most treatment years there were no patients with clinically relevant abnormal SA values.

At birth, the average AFP level of cord serum is several 1000-fold higher than are adult levels (15). Normally, these levels progressively decline as the infant matures to reach adult levels usually within 1 year after birth. In HT-1, AFP is a marker of liver regeneration and is often elevated in newly diagnosed patients due to ongoing liver damage by formation of toxic tyrosine metabolites. Increase in AFP concentration in HT-1 patients may be a sign of inadequate treatment. In the present study, the p-AFP levels decreased from a median at Treatment Year 1 of 31 ng/mL to 4 ng/mL at Treatment Year 15. The number of patients with p-AFP levels >6.6 ng/mL also decreased, from 81.4 % at Treatment Year 1 to 27.6 % at Treatment Year 15. Although the number of patients with p-AFP levels >6.6 ng/mL may seem high, the clinical relevance of these findings can be challenged as the limit of 6.6 ng/mL possibly is too low to indicate any clinical relevance. The p-AFP cut-off level of 6.6 ng/mL was defined by the Sponsor as the threshold for identifying abnormal values. This limit is more conservative than in several hospital laboratories, e.g. Karolinska University Hospital, Sweden, where values >8 ng/mL are considered abnormal values.

In parallel with the decline in diet compliance, the Orfadin treatment compliance also seemed to decline over time, e.g. the proportion of patients who were assessed as having “very good” compliance decreased from 82.7 % at Treatment Year 1 to 43.2 % at Treatment Year 15 while the proportion of patients who were assessed as having “very poor” compliance increased from 1.3 % at Treatment Year 1 to 8.1 % at Treatment Year 15. The decline in diet and treatment compliance might reflect the patient population becoming older and self-managing their disease as well as when the patients are entering puberty there might be a wish not to be “different” from peers of the same age (16).

Despite the decline in diet and treatment compliance, there was a consistently high proportion of patients with “good” overall clinical condition throughout the study indicating a sustained effect of the drug during long-term exposure.

The favorable safety profile of Orfadin was also reflected in a low incidence of other AEs and SAEs. Furthermore, few patients (11 patients; 3.5 %) discontinued Orfadin treatment and most of the discontinuations were due to liver transplantations in patients starting Orfadin treatment late (≥6 months of age). These results again confirm the benefits of early treatment initiation.

Even though not included as a formal objective of the study but following a request from the FDA, the outcomes for Orfadin when used on a once daily basis was also studied. In total, 76 patients in the complete set were reported to have been treated with Orfadin in a once daily dosing frequency at least once during the study and there were about twice as many patients who switched from twice daily dosing regimen to once daily dosing regimen than who started on once daily dosing regimen directly. The use of Orfadin on a once daily basis did not result in any new safety issues. Furthermore, in contrast to the full study population, the treatment compliance did seem to improve in the two once daily subgroups, e.g. the proportion of patients who were assessed as having "very good" compliance was 100 % at the latest assessment in both subgroups. However, this could be due to a bias when selecting the patients for once daily treatment; possibly, patients with poorer compliance were not selected for the once daily treatment regimen.

11.4 Generalizability

The patients included in the current study were the majority of patients with HT-1 in their respective countries, and the >300 patients evaluated is considered a substantial number in a rare disease. Furthermore, a large number of countries and sites were included in the study.

Another factor which adds to the generalizability of the study is the fact that no specific exclusion criteria were applied but all HT-1 patients on Orfadin treatment in standard clinical care at study initiation as well as patients diagnosed and starting Orfadin treatment during the time of the study were included.

The study population is thus deemed as highly representative of patients with HT-1.

12 Other information

Not applicable.

13 Conclusions

- The results of the present long-term study confirmed the safety profile of Orfadin without any new safety findings.
- The pattern of AEs, including SAEs, were in line with the known safety profile of Orfadin.
- Thus, up to 15 years' treatment with Orfadin in HT-1 patients was well tolerated, with no overall increase in incidence rate of AEs, over time.

14 References

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15 Tables and figures

Table 40 Number of patients by country

	Complete set	Index set
Country	Patients (N= 315) n (%)	Patients (N= 203) n (%)
AUSTRIA	8 (2.5)	3 (1.5)
BELGIUM	17 (5.4)	9 (4.4)
CZECH REPUBLIC	7 (2.2)	3 (1.5)
DENMARK	8 (2.5)	4 (2.0)
FINLAND	9 (2.9)	8 (3.9)
FRANCE	50 (15.9)	32 (15.8)
GERMANY	36 (11.4)	23 (11.3)
HUNGARY	5 (1.6)	3 (1.5)
IRLAND	4 (1.3)	4 (2.0)
ITALY	33 (10.5)	22 (10.8)
NETHERLANDS	3 (1.0)	2 (1.0)
NORWAY	9 (2.9)	7 (3.4)
POLAND	12 (3.8)	9 (4.4)
PORTUGAL	3 (1.0)	3 (1.5)
SPAIN	62 (19.7)	40 (19.7)
SWEDEN	9 (2.9)	4 (2.0)
UNITED KINGDOM	40 (12.7)	27 (13.3)

Abbreviations: N, Total number of patients; n, Number of patients in each country.

Table 41 Demographics and baseline characteristics of treatment-naïve patients on once daily dosage and treatment-experienced patients after initiation of once daily dosage (index set)

	Treatment-naïve	Treatment-experienced
	Patients (N=11)	Patients (N=21)
Sex, n (%)		
Female	5 (45.5)	13 (61.9)
Male	6 (54.5)	8 (38.1)
Age at diagnosis (months)		
n	11	21
Mean	10.6	12.1
SD	9.32	16.04
Median	7.5	4.8

	Treatment-naïve	Treatment-experienced
	Patients (N=11)	**Patients (N=21)**
Range	0.5 - 26.5	0.1 - 54.3
Diagnosed by newborn screening, n (%)		
Yes	2 (18.2)	4 (19.0)
No	9 (81.8)	12 (57.1)
Unknown	0	5 (23.8)
Age at Orfadin treatment start (months)		
n	11	21
Mean	76.7	34.0
SD	93.30	58.85
Median	22.9	9.8
Range	1.7 - 257.7	0.1 - 239.0
Age category at Orfadin treatment start, n (%)		
Newborn (<28 days)	0	4 (19.0)
>=28 days - <6 months	1 (9.1)	6 (28.6)
>=6 months -<12 months	2 (18.2)	2 (9.5)
>=12 months	8 (72.7)	9 (42.9)
Age category at initiation of OD, n (%)		
0 - <2 years	6 (54.5)	5 (23.8)
2 - <5 years	1 (9.1)	3 (14.3)
5 - <12 years	1 (9.1)	11 (52.4)
12 - <18 years	2 (18.2)	1 (4.8)
>= 18 years	1 (9.1)	1 (4.8)
Years on Orfadin treatment prior to initiation of the OD regimen		
n	N/A	21
Mean	N/A	3.7
SD	N/A	3.11
Median	N/A	3.3
Range	N/A	0.2 - 10.5

Abbreviations: N, Total number of patients; n, Number of patients in each group; N/A, not applicable; SD, Standard deviation; OD, Once daily dosing regimen.
Note: Treatment-naive: Patients who have previously not been treated with Orfadin and start their Orfadin treatment with once daily dosage (as confirmed by the first dose recorded for each patient). Treatment-experienced: Patients who have switched to once daily dosage after twice daily dosage (as confirmed by a twice daily regimen reported before the once daily dosing). Only data during once daily treatment is included.

Table 42 Number of patients with hepatic, renal, ophthalmic, hematological, cognitive/development function adverse events with event counts and incidence rates per 100 patient years (index set)

	Patients (N= 203) n	Percentage (95% CI)	Events f	Incidence rate per 100 patient years (95% CI)
Hepatic function	11	5.4 (2.7-9.5)	11	0.7 (0.4-1.3)
Renal function	0	-	0	-
Ophthalmic function	7	3.4 (1.4-7.0)	9	0.6 (0.3-1.1)
Hematological status	5	2.5 (0.8-5.7)	6	0.4 (0.2-0.9)
Cognitive/developmental function	5	2.5 (0.8-5.7)	6	0.4 (0.2-0.9)
Total	24	11.8 (7.7-17.1)	32	2.1 (1.5-2.9)

Abbreviations: N, Total number of patients; n, Number of patients with at least one AE in the pre-defined group; f, Total number of AEs in the pre-defined group; CI, Confidence interval; AE, Adverse event.
Note: Patient years of exposure = 1558.6.

Table 43 Number of patients with hepatic, renal, ophthalmic, hematological, cognitive/development function adverse events with event counts and incidence rates per 100 patient years by age at start of Orfadin treatment (index set)

Adverse Event category/ Age at start of Orfadin treatment	N	Patients with events n	Percentage (95% CI)	Events f	Incidence rate per 100 patient years (95% CI)
Hepatic function	203	11	5.4 (2.7-9.5)	11	0.7 (0.4-1.3)
<28 days	48	2	4.2 (0.5-14.3)	2	0.5 (0.1-2.1)
>=28 days - <6 months	54	2	3.7 (0.5-12.7)	2	0.4 (0.1-1.6)
>=6 months - <12 months	26	2	7.7 (0.9-25.1)	2	1.0 (0.3-4.1)
>=12 months	75	5	6.7 (2.2-14.9)	5	1.0 (0.4-2.5)
Renal function	203	0	-	0	-
<28 days	48	0	-	0	-
>=28 days - <6 months	54	0	-	0	-
>=6 months - <12 months	26	0	-	0	-
>=12 months	75	0	-	0	-
Ophthalmic function	203	7	3.4 (1.4-7.0)	9	0.6 (0.3-1.1)
<28 days	48	3	6.3 (1.3-17.2)	5	1.3 (0.5-3.1)
>=28 days - <6 months	54	1	1.9 (0.0-9.9)	1	0.2 (0.0-1.4)
>=6 months - <12 months	26	0	-	0	-
>=12 months	75	3	4.0 (0.8-11.2)	3	0.6 (0.2-1.9)
Hematological status	203	5	2.5 (0.8-5.7)	6	0.4 (0.2-0.9)
<28 days	48	0	-	0	-
>=28 days - <6 months	54	0	-	0	-

Adverse Event category/ Age at start of Orfadin treatment	N	Patients with events n	Percentage (95% CI)	Events f	Incidence rate per 100 patient years (95% CI)
>=6 months - <12 months	26	3	11.5 (2.4-30.2)	3	1.5 (0.5-4.8)
>=12 months	75	2	2.7 (0.3-9.3)	3	0.6 (0.2-1.9)
Cognitive/developmental function	203	5	2.5 (0.8-5.7)	6	0.4 (0.2-0.9)
<28 days	48	1	2.1 (0.1-11.1)	2	0.5 (0.1-2.1)
>=28 days - <6 months	54	3	5.6 (1.2-15.4)	3	0.6 (0.2-1.9)
>=6 months - <12 months	26	0	-	0	-
>=12 months	75	1	1.3 (0.0-7.2)	1	0.2 (0.0-1.5)
Total	203	24	11.8 (7.7-17.1)	32	2.1 (1.5-2.9)
<28 days	48	5	10.4 (3.5-22.7)	9	2.3 (1.2-4.5)
>=28 days - <6 months	54	6	11.1 (4.2-22.6)	6	1.2 (0.5-2.7)
>=6 months - <12 months	26	3	11.5 (2.4-30.2)	5	2.6 (1.1-6.2)
>=12 months	75	10	13.3 (6.6-23.2)	12	2.5 (1.4-4.4)

Abbreviations: AE, Adverse event; CI, Confidence interval; f, Total number of AEs in the pre-defined group; N, Total number of patients; n, Number of patients with at least one AE in the pre-defined group.
Note: Patient years of exposure in total = 1558.6, and by age at start of Orfadin treatment: <28 days = 385.4, ≥28 days - <6 months = 498.9, 6 - <12 months = 195.1, ≥12 month = 479.2.

Table 44 Occurrence of hepatic malignancy, by age at start of Orfadin treatment (index set)

	Patients (N= 203) n	Percentage (95% CI)
Total	2	1.0 (0.1-3.5)
Age at start of Orfadin treatment		
>=12 months	2	1.0 (0.1-3.5)

Abbreviations: N, Total number of patients at risk; n, Number of patients with hepatic malignancy in each age group; CI, Confidence interval.

Table 45 Incorrect administration of oral suspension formulation in subgroup of patients with at least one administration of oral suspension of Orfadin (complete set)

	Number of events	Patients with at least one event (N=39) n (%)
Incorrect administration	0	0 (0.0)

Abbreviations: N, Total number of patients; n, Number of patients with at least one event.
Note: Includes administrations of oral suspensions from 19 June 2015 and onwards.

Table 46 Occurrence of other adverse events by system organ class and preferred term (complete set)

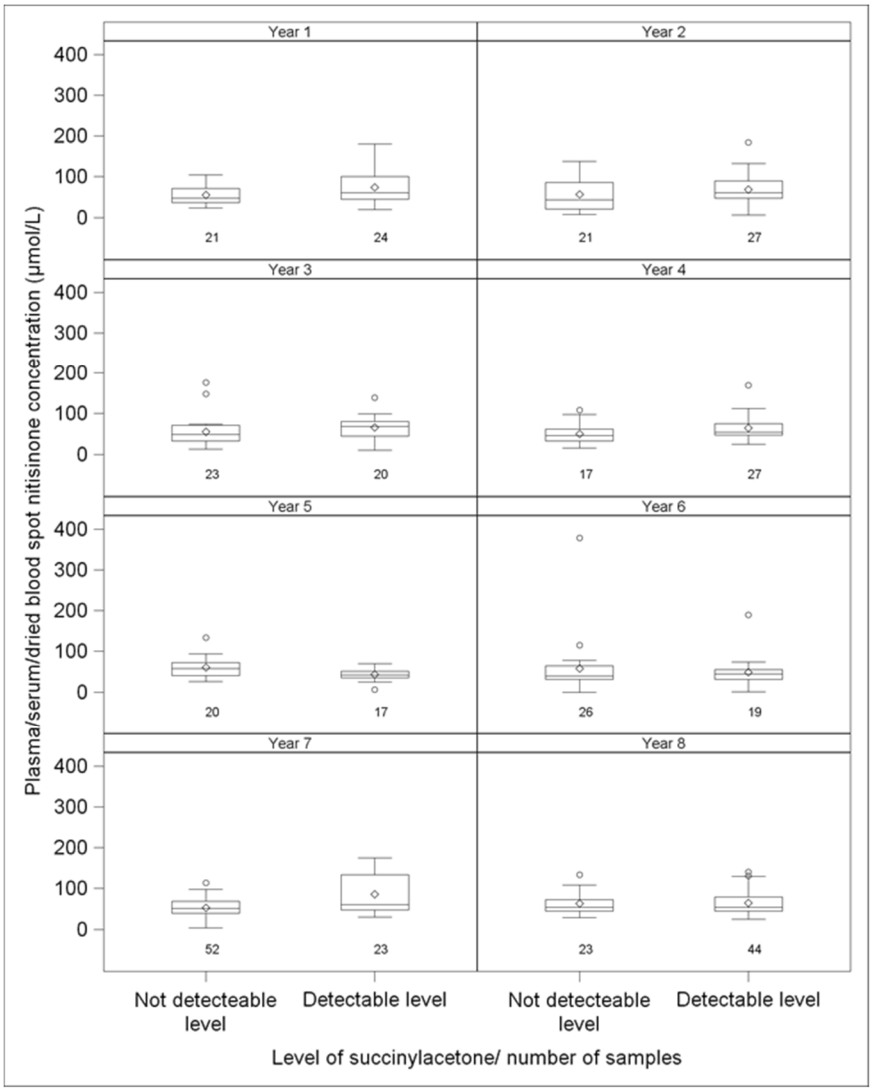
SOC/ Preferred term	Patients (N= 315) n	Percentage of patients with event (95% CI)	Incidence rate per 100 patient years (95% CI)
Congenital, familial and genetic disorders	4	1.3 (0.3-3.2)	0.1 (0.0-0.3)
Arnold-Chiari malformation	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Porphyria	2	0.6 (0.1-2.3)	0.1 (0.0-0.3)
Porphyria acute	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Gastrointestinal disorders	11	3.5 (1.8-6.2)	0.6 (0.4-0.9)
Abdominal pain	3	1.0 (0.2-2.8)	0.1 (0.0-0.3)
Ascites	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Constipation	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Diarrhoea	2	0.6 (0.1-2.3)	0.1 (0.0-0.3)
Duodenal ulcer	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Irritable bowel syndrome	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Melaena	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Nausea	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Pancreatitis	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Vomiting	4	1.3 (0.3-3.2)	0.2 (0.1-0.4)
General disorders and administration site conditions	6	1.9 (0.7-4.1)	0.2 (0.1-0.4)
Adverse event	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Mucosal inflammation	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Nodule	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Pseudocyst	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Pyrexia	2	0.6 (0.1-2.3)	0.1 (0.0-0.3)
Immune system disorders	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Transplant rejection	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Infections and infestations	6	1.9 (0.7-4.1)	0.2 (0.1-0.5)
Bronchitis	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Ear infection	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Gastroenteritis	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Pilonidal cyst	1	0.3 (0.0-1.8)	0.1 (0.0-0.3)
Tonsillitis	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Upper respiratory tract infection	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Injury, poisoning and procedural complications	8	2.5 (1.1-4.9)	0.3 (0.1-0.5)
Accident	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)

SOC/ Preferred term	Patients (N= 315) n	Percentage of patients with event (95% CI)	Incidence rate per 100 patient years (95% CI)
Clavicle fracture	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Maternal exposure during pregnancy	5	1.6 (0.5-3.7)	0.2 (0.1-0.4)
Post procedural haemorrhage	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Radius fracture	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Investigations	31	9.8 (6.8-13.7)	1.4 (1.1-1.9)
Amino acid level	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Amino acid level decreased[a]	5	1.6 (0.5-3.7)	0.2 (0.1-0.4)
Amino acid level increased[b]	20	6.3 (3.9-9.6)	0.9 (0.6-1.3)
Blood phosphorus increased	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Drug level decreased	8	2.5 (1.1-4.9)	0.3 (0.1-0.5)
Succinylacetone increased	2	0.6 (0.1-2.3)	0.1 (0.0-0.3)
Vitamin D decreased	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Metabolism and nutrition disorders	5	1.6 (0.5-3.7)	0.2 (0.1-0.4)
Dehydration	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Hyponatraemia	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Poor feeding infant	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Vitamin A deficiency	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Vitamin D deficiency	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Musculoskeletal and connective tissue disorders	2	0.6 (0.1-2.3)	0.1 (0.0-0.3)
Joint swelling	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Scoliosis	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Nervous system disorders	8	2.5 (1.1-4.9)	0.3 (0.1-0.5)
Epilepsy	3	1.0 (0.2-2.8)	0.1 (0.0-0.3)
Generalised tonic-clonic seizure	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Headache	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Migraine without aura	2	0.6 (0.1-2.3)	0.1 (0.0-0.3)
Seizure	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Tremor	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Pregnancy, puerperium and perinatal conditions	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Pregnancy	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Psychiatric disorders	6	1.9 (0.7-4.1)	0.3 (0.1-0.5)
Acute psychosis	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Aggression	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Confusional state	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Eating disorder	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)

SOC/ Preferred term	Patients (N= 315) n	Percentage of patients with event (95% CI)	Incidence rate per 100 patient years (95% CI)
Enuresis	2	0.6 (0.1-2.3)	0.1 (0.0-0.3)
Hallucination, auditory	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Psychotic disorder	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Renal and urinary disorders	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Haematuria	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Respiratory, thoracic and mediastinal disorders	2	0.6 (0.1-2.3)	0.1 (0.0-0.3)
Respiratory distress	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Respiratory failure	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Tachypnoea	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Skin and subcutaneous tissue disorders	1	0.3 (0.0-1.8)	0.1 (0.0-0.3)
Pruritus	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Rash	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Social circumstances	4	1.3 (0.3-3.2)	0.1 (0.0-0.3)
Diet noncompliance	3	1.0 (0.2-2.8)	0.1 (0.0-0.3)
Refusal of treatment by relative	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Surgical and medical procedures	6	1.9 (0.7-4.1)	0.2 (0.1-0.5)
Liver transplant	6	1.9 (0.7-4.1)	0.2 (0.1-0.5)
Vascular disorders	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)
Jugular vein thrombosis	1	0.3 (0.0-1.8)	0.0 (0.0-0.2)

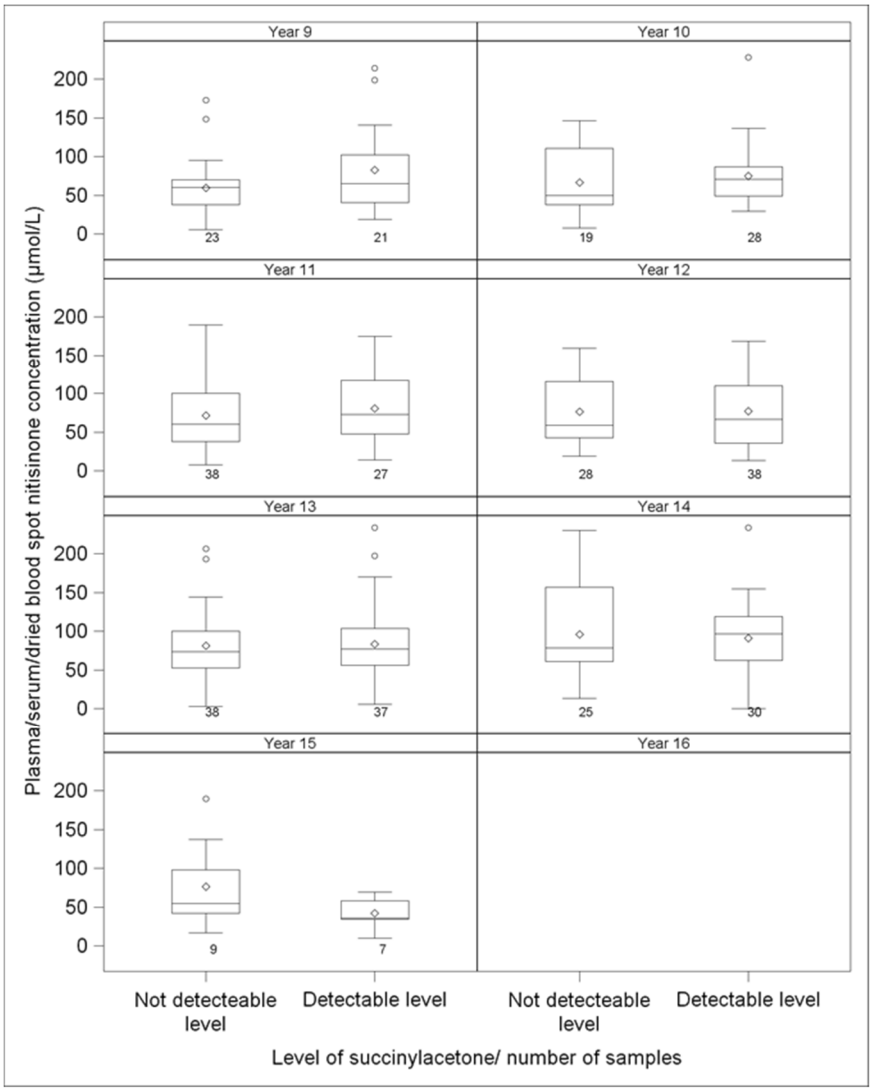
Abbreviations: N, Total number of patients; n, Number of patients with at least one corresponding event; CI, Confidence interval; AE, Adverse event; SOC, System Organ Class.
Note: The presentation excludes AEs included in other presentations of primary and secondary endpoints. Patient years of exposure = 3172.7.
[a]Includes decreased levels of p-Phe. [b]Includes increased levels of p-Tyr and p-Phe, the majority being p-Tyr.

Figure 9 Nitisinone by detectable level of succinylacetone in plasma, serum, dried blood spot or urine by treatment year, treatment year 1-8 (complete set)



Note: In the case a patient has more than one assay of nitisinone concentration during a year the mean result is displayed.

Figure 10 Nitisinone by detectable level of succinylacetone in plasma, serum, dried blood spot or urine by treatment year, treatment year 9-16 (complete set)



Note: In the case a patient has more than one assay of nitisinone concentration during a year the mean result is displayed.

Figure 11 Mean daily dose of Orfadin (mg/kg) by detectable level of succinylacetone in plasma, serum, dried blood spot or urine by treatment year, treatment year 1-8 (complete set)

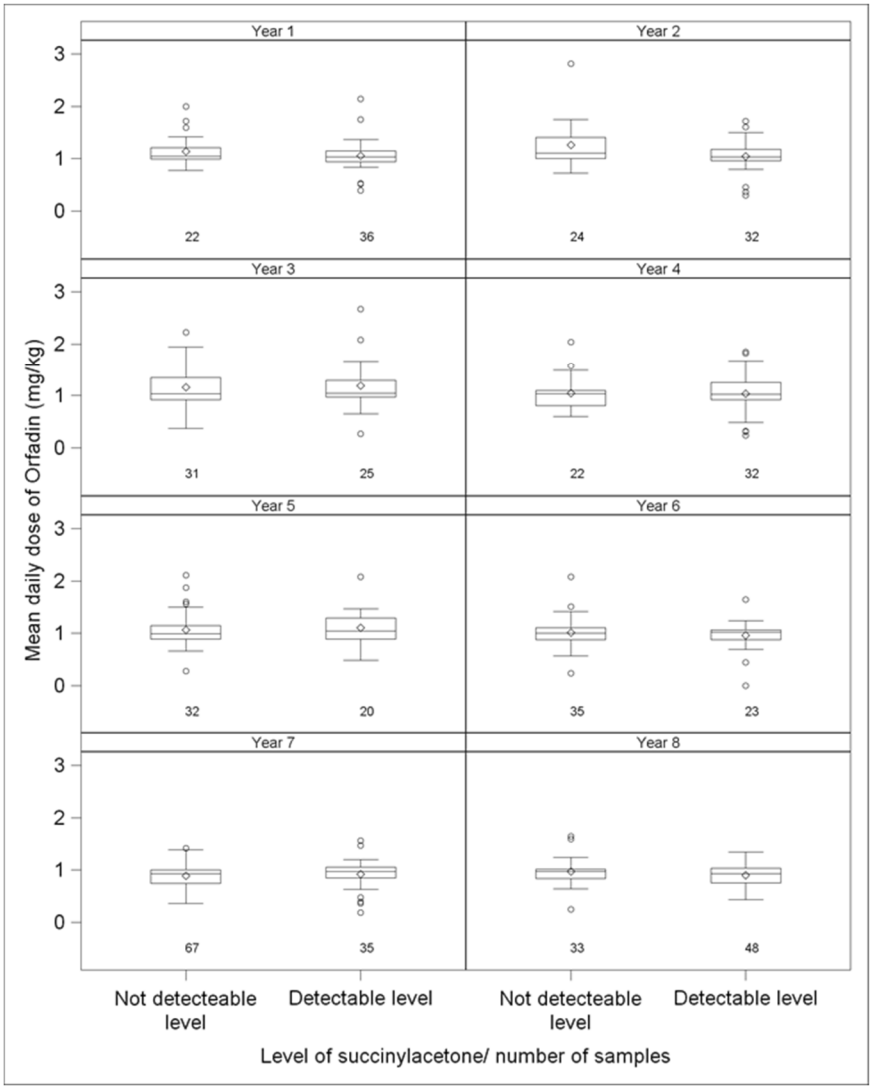


Figure 12 Mean daily dose of Orfadin (mg/kg) by detectable level of succinylacetone in plasma, serum, dried blood spot or urine by treatment year, treatment year 9-16 (complete set)

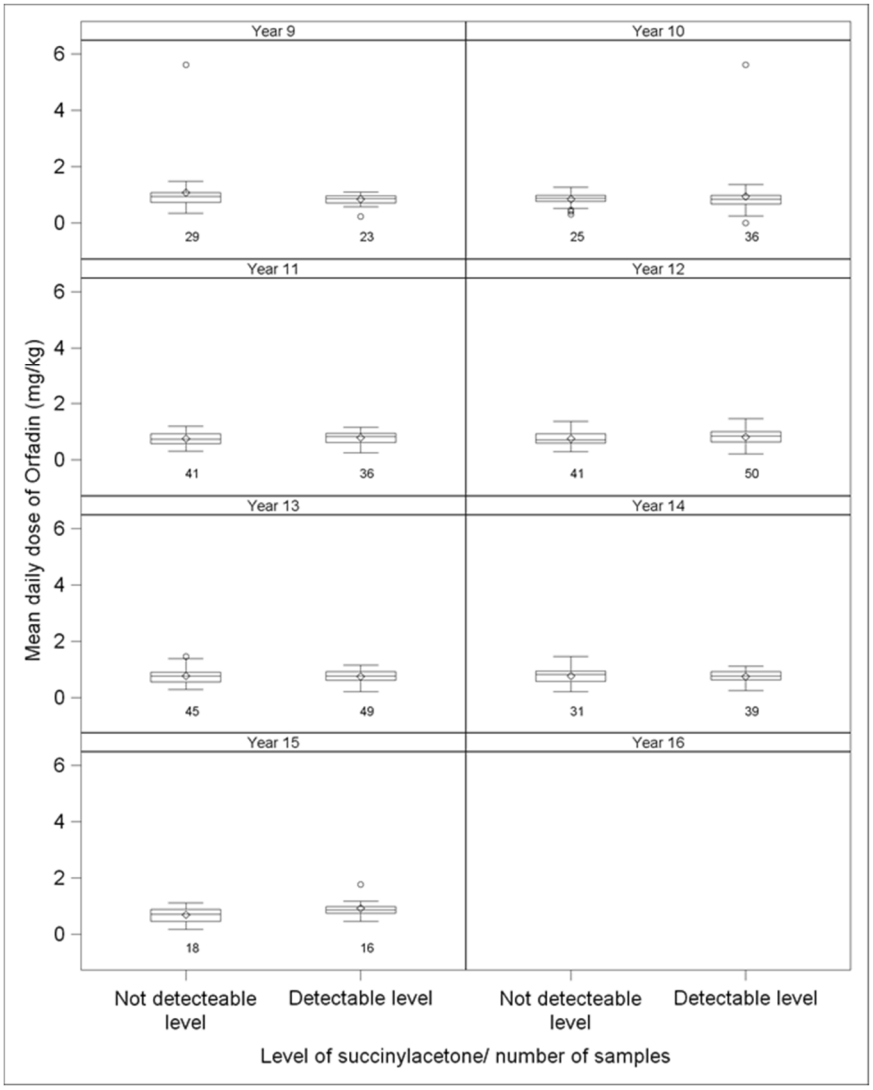


Table 47 Number of treatment-naïve patients on once daily dosage with detectable level of succinylacetone in plasma, serum, dried blood spot or urine (index

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Year 1				
Total	2	1 (50.0)	0 (0.0)	1 (50.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	2	1 (50.0)	0 (0.0)	1 (50.0)
Year 2				
Total	6	2 (33.3)	0 (0.0)	0 (0.0)
Plasma/serum	2	2 (100.0)	0 (0.0)	0 (0.0)
Dried blood spot	3	0 (0.0)	0 (0.0)	0 (0.0)
Urine	4	1 (25.0)	0 (0.0)	0 (0.0)
Year 3				
Total	4	1 (25.0)	0 (0.0)	0 (0.0)
Plasma/serum	1	1 (100.0)	0 (0.0)	0 (0.0)
Dried blood spot	2	0 (0.0)	0 (0.0)	0 (0.0)
Urine	2	1 (50.0)	0 (0.0)	0 (0.0)
Year 4				
Total	4	2 (50.0)	0 (0.0)	0 (0.0)
Plasma/serum	1	1 (100.0)	0 (0.0)	0 (0.0)
Dried blood spot	2	1 (50.0)	0 (0.0)	0 (0.0)
Urine	3	1 (33.3)	0 (0.0)	0 (0.0)
Year 5				
Total	4	1 (25.0)	0 (0.0)	0 (0.0)
Plasma/serum	1	1 (100.0)	0 (0.0)	0 (0.0)
Dried blood spot	1	0 (0.0)	0 (0.0)	0 (0.0)
Urine	4	1 (25.0)	0 (0.0)	0 (0.0)
Year 6				
Total	3	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	1	0 (0.0)	0 (0.0)	0 (0.0)
Urine	3	0 (0.0)	0 (0.0)	0 (0.0)
Year 7				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 8				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 9				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 10				
Total	1	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	1	0 (0.0)	0 (0.0)	0 (0.0)
Year 11				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 12				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 13				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 14				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Year 15				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: n, Number of patients.

Note: Percentage based on number of patients with at least one assay result. Treatment-naïve, Patients who have previously not been treated with Orfadin and start their Orfadin treatment with once daily dosage (as confirmed by the first dose recorded for each patient).

Table 48 Number of treatment-experienced patients with detectable level of succinylacetone in plasma, serum, dried blood spot or urine after initiation of once daily dosing (index set)

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Year 1				
Total	6	3 (50.0)	0 (0.0)	0 (0.0)
Plasma/serum	3	3 (100.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	6	1 (16.7)	0 (0.0)	0 (0.0)
Year 2				
Total	7	6 (85.7)	0 (0.0)	0 (0.0)
Plasma/serum	4	4 (100.0)	0 (0.0)	0 (0.0)
Dried blood spot	1	1 (100.0)	0 (0.0)	0 (0.0)
Urine	4	1 (25.0)	0 (0.0)	0 (0.0)
Year 3				
Total	9	5 (55.6)	0 (0.0)	0 (0.0)
Plasma/serum	1	1 (100.0)	0 (0.0)	0 (0.0)
Dried blood spot	5	4 (80.0)	0 (0.0)	0 (0.0)
Urine	6	1 (16.7)	0 (0.0)	0 (0.0)
Year 4				
Total	9	6 (66.7)	0 (0.0)	0 (0.0)
Plasma/serum	2	2 (100.0)	0 (0.0)	0 (0.0)

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Dried blood spot	5	4 (80.0)	0 (0.0)	0 (0.0)
Urine	5	2 (40.0)	0 (0.0)	0 (0.0)
Year 5				
Total	4	4 (100.0)	1 (25.0)	0 (0.0)
Plasma/serum	1	1 (100.0)	0 (0.0)	0 (0.0)
Dried blood spot	3	3 (100.0)	1 (33.3)	0 (0.0)
Urine	3	1 (33.3)	0 (0.0)	0 (0.0)
Year 6				
Total	2	1 (50.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	2	1 (50.0)	0 (0.0)	0 (0.0)
Urine	1	0 (0.0)	0 (0.0)	0 (0.0)
Year 7				
Total	1	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	1	0 (0.0)	0 (0.0)	0 (0.0)
Urine	1	0 (0.0)	0 (0.0)	0 (0.0)
Year 8				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 9				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 10				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 11				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)

			Patients with abnormal values	
Treatment year	**Patients with at least one assay result n**	**Patients with detectable succinyl-acetone n (%)**	**Clinically relevant values n (%)**	**Unknown values n (%)**
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 12				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 13				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 14				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)
Year 15				
Total	0	0 (0.0)	0 (0.0)	0 (0.0)
Plasma/serum	0	0 (0.0)	0 (0.0)	0 (0.0)
Dried blood spot	0	0 (0.0)	0 (0.0)	0 (0.0)
Urine	0	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: n, Number of patients.
Note: Percentage based on number of patients with at least one assay result. Treatment-experienced, Patients who have switched to once daily dosage after twice daily dosage (as confirmed by a twice daily regimen reported before the once daily dosing). Only data during once daily treatment is included.

Table 49 Succinylacetone (µmol/L) by treatment year in treatment-naïve patients on once daily dosage (index set)

	n	Mean	SD	Median	Min, Max
Year 1	1	0.00	.	0.0	0.0, 0.0
Year 2	6	0.33	0.516	0.0	0.0, 1.0
Year 3	4	0.05	0.100	0.0	0.0, 0.2
Year 4	4	0.73	1.141	0.3	0.0, 2.4
Year 5	4	0.08	0.150	0.0	0.0, 0.3
Year 6	3	0.00	0.000	0.0	0.0, 0.0
Year 7	0	.	.	.	-
Year 8	0	.	.	.	-
Year 9	0	.	.	.	-
Year 10	1	0.00	.	0.0	0.0, 0.0
Year 11	0	.	.	.	-
Year 12	0	.	.	.	-
Year 13	0	.	.	.	-
Year 14	0	.	.	.	-
Year 15	0	.	.	.	-

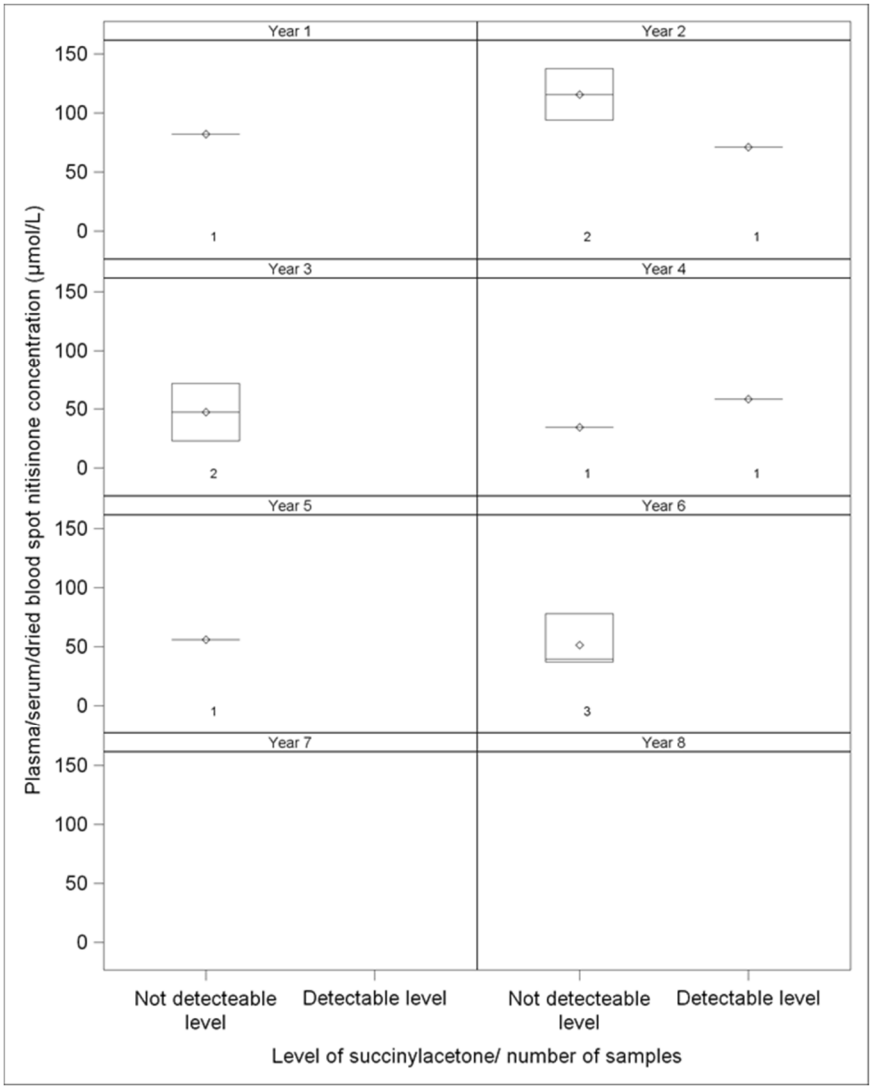
Abbreviations: n, Total number of patients with at least one assessment of succinylacetone in plasma, serum, dried blood spot or urine during the corresponding year; SD, Standard deviation
Note: For patients with more than one assay of succinylacetone in plasma, serum, dried blood spot or urine during a year, the maximum value is displayed. Only assessments with numeric values included, i.e., TRACE findings are excluded. Treatment-naïve, Patients who have previously not been treated with Orfadin and start their Orfadin treatment with once daily dosage (as confirmed by the first dose recorded for each patient).

Table 50 Succinylacetone (µmol/L) by treatment year in treatment-experienced patients after initiation of once daily dosing (index set)

	n	Mean	SD	Median	Min, Max
Year 1	6	0.25	0.397	0.1	0.0, 1.0
Year 2	7	0.68	0.643	0.5	0.0, 1.8
Year 3	9	0.68	1.098	0.3	0.0, 2.7
Year 4	9	0.69	0.882	0.3	0.0, 2.4
Year 5	4	1.15	0.840	1.1	0.3, 2.1
Year 6	2	0.46	0.643	0.5	0.0, 0.9
Year 7	1	0.00	.	0.0	0.0, 0.0
Year 8	0	.	.	.	-
Year 9	0	.	.	.	-
Year 10	0	.	.	.	-
Year 11	0	.	.	.	-
Year 12	0	.	.	.	-
Year 13	0	.	.	.	-
Year 14	0	.	.	.	-
Year 15	0	.	.	.	-

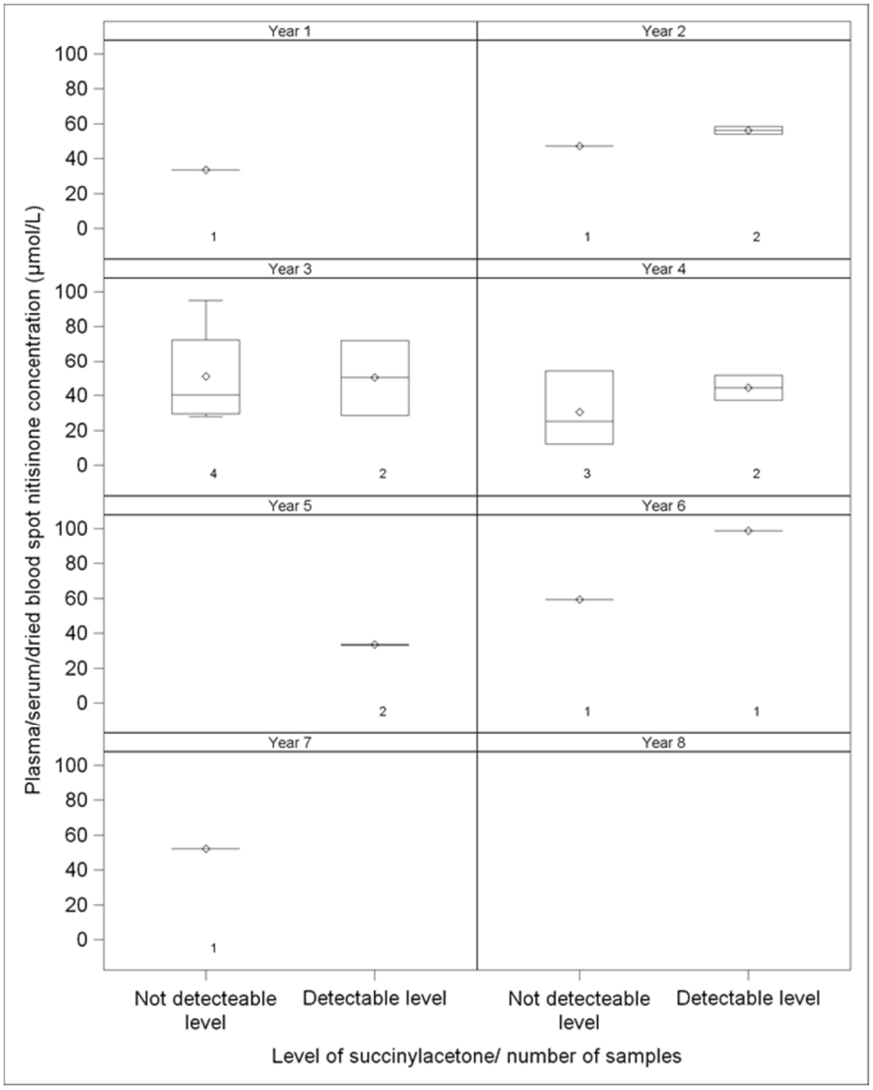
Abbreviations: n, Total number of patients with at least one assessment of succinylacetone in plasma, serum, dried blood spot or urine during the corresponding year; SD, Standard deviation
Note: For patients with more than one assay of succinylacetone in plasma, serum, dried blood spot or urine during a year, the maximum value is displayed. Treatment-experienced, Patients who have switched to once daily dosage after twice daily dosage (as confirmed by a twice daily regimen reported before the once daily dosing). Only data during once daily treatment is included.

Figure 13 Nitisinone by detectable level of succinylacetone in plasma, serum, dried blood spot or urine by treatment year, in treatment-naïve patients on once daily dosage, treatment year 1-8 (index set)



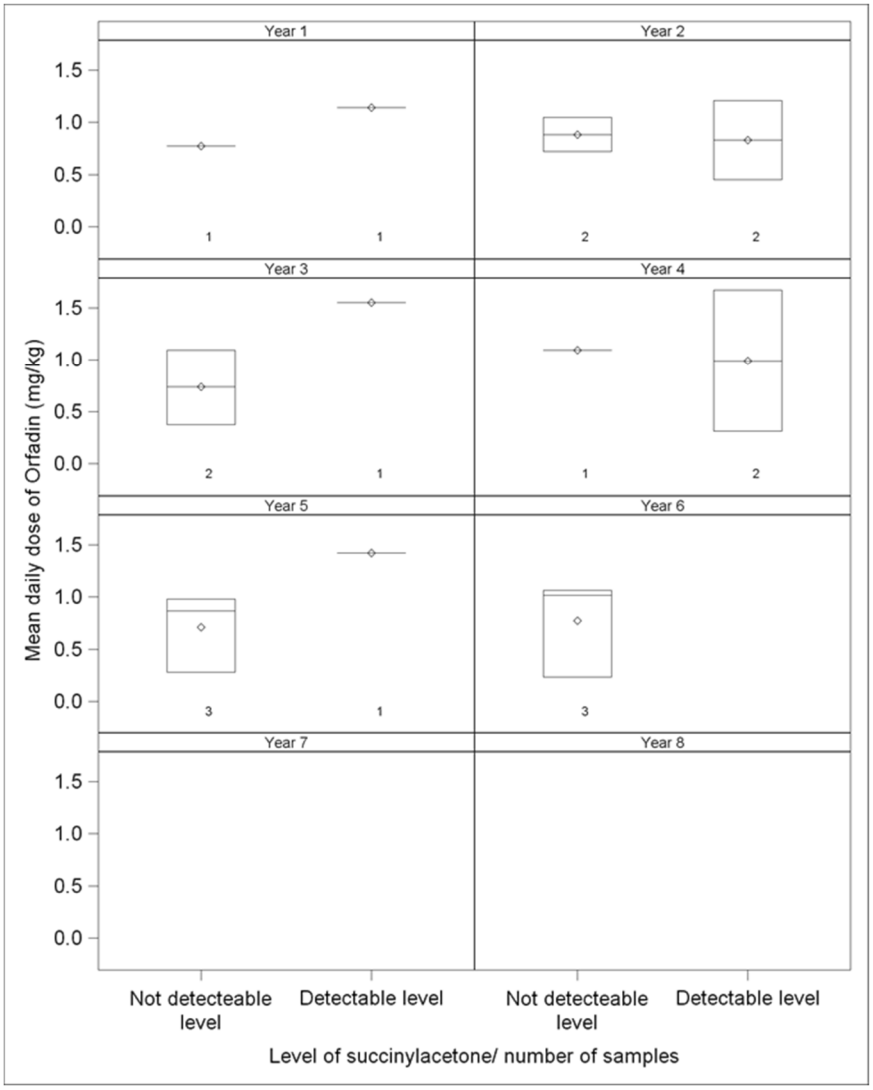
Note: In the case a patient has more than one assay of nitisinone concentration during a year the mean result is displayed. Treatment-naïve: Patients who have previously not been treated with Orfadin and start their Orfadin treatment with once daily dosage (as confirmed by the first dose recorded for each patient).

Figure 14 Nitisinone by detectable level of succinylacetone in plasma, serum, dried blood spot or urine by treatment year, in treatment-experienced patients after initiation of once daily dosing, treatment year 1-8 (index set)



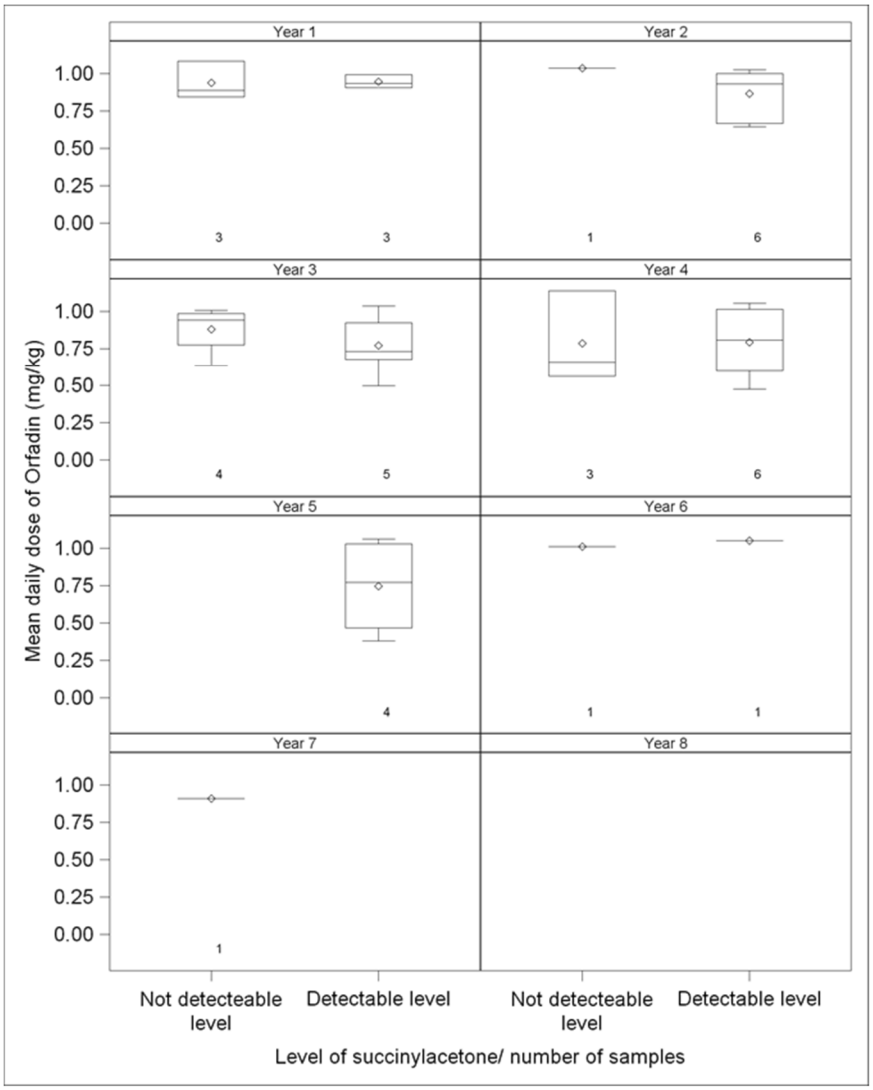
Note: In the case a patient has more than one assay of nitisinone concentration during a year the mean result is displayed. Treatment-experienced: Patients who have switched to once daily dosage after twice daily dosage (as confirmed by a twice daily regimen reported before the once daily dosing). Only data during once daily treatment is included.

Figure 15 Mean daily dose of Orfadin (mg/kg) by detectable level of succinylacetone in plasma, serum, dried blood spot or urine by treatment year, in treatment-naïve patients on once daily dosage, treatment year 1-8 (index set)



Note: Treatment-naïve: Patients who have previously not been treated with Orfadin and start their Orfadin treatment with once daily dosage (as confirmed by the first dose recorded for each patient).

Figure 16 Mean daily dose of Orfadin (mg/kg) by detectable level of succinylacetone in plasma, serum, dried blood spot or urine by treatment year, in treatment-experienced patients after initiation of once daily dosing, treatment year 1-8 (index set)



Note: Treatment-experienced: Patients who have switched to once daily dosage after twice daily dosage (as confirmed by a twice daily regimen reported before the once daily dosing). Only data during once daily treatment is included.

Figure 17 Age by treatment compliance and treatment year, treatment year 1-8 (complete set)

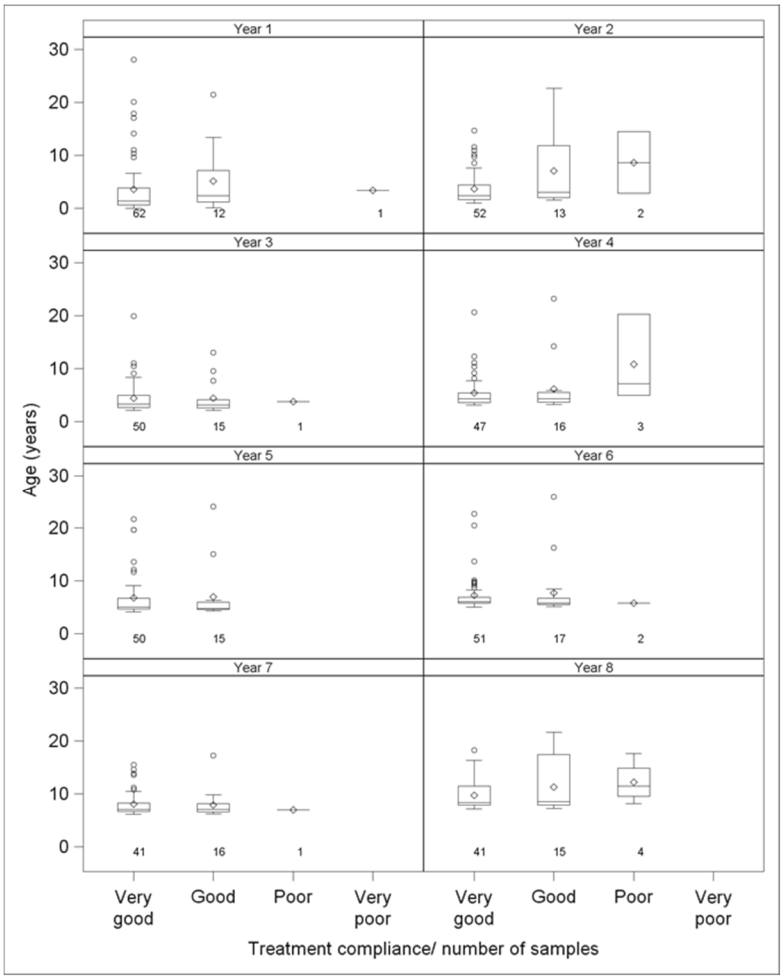


Figure 18 Age by treatment compliance and treatment year, treatment year 9-16 (complete set)

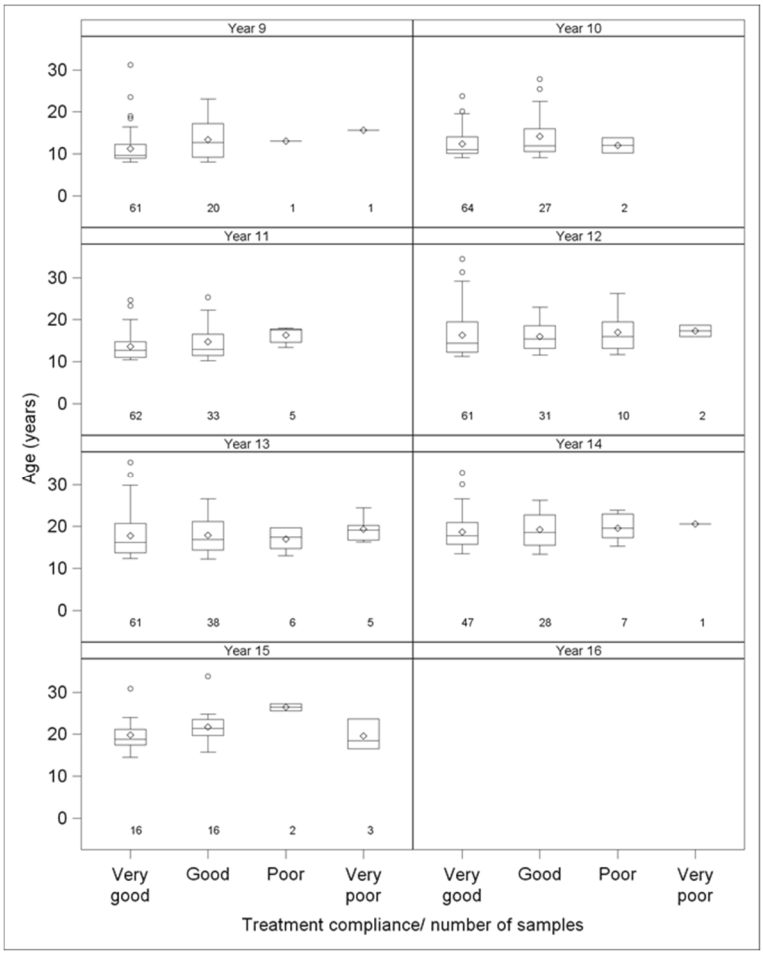


Table 51 Treatment compliance summarized over time in treatment-naïve patients and in treatment-experienced patients after initiation of once daily dosing (index set)

	Treatment-naïve patients	Treatment-experienced patients
	Patients (N=11) n (%)	**Patients (N=21) n (%)**
Very good	7 (63.6)	12 (57.1)
Good	4 (36.4)	6 (28.6)
Poor	0 (0.0)	1 (4.8)
Very poor	0 (0.0)	0 (0.0)
Missing	0 (0.0)	2 (9.5)

Abbreviations: N, Total number of patients; n, Number of patients in each group.
Note: Treatment-naïve: Patients who have previously not been treated with Orfadin and start their Orfadin treatment with once daily dosage (as confirmed by the first dose recorded for each patient). Only assessments after initiation

of once daily dosing regimen are included. Treatment-experienced: Patients who have switched to once daily dosage after twice daily dosage (as confirmed by a twice daily regimen reported before the once daily dosing). Only data during once daily treatment is included. For each patient the worst treatment compliance over time is presented.

Figure 19 Age by diet compliance and treatment year, treatment year 1-8 (complete set)

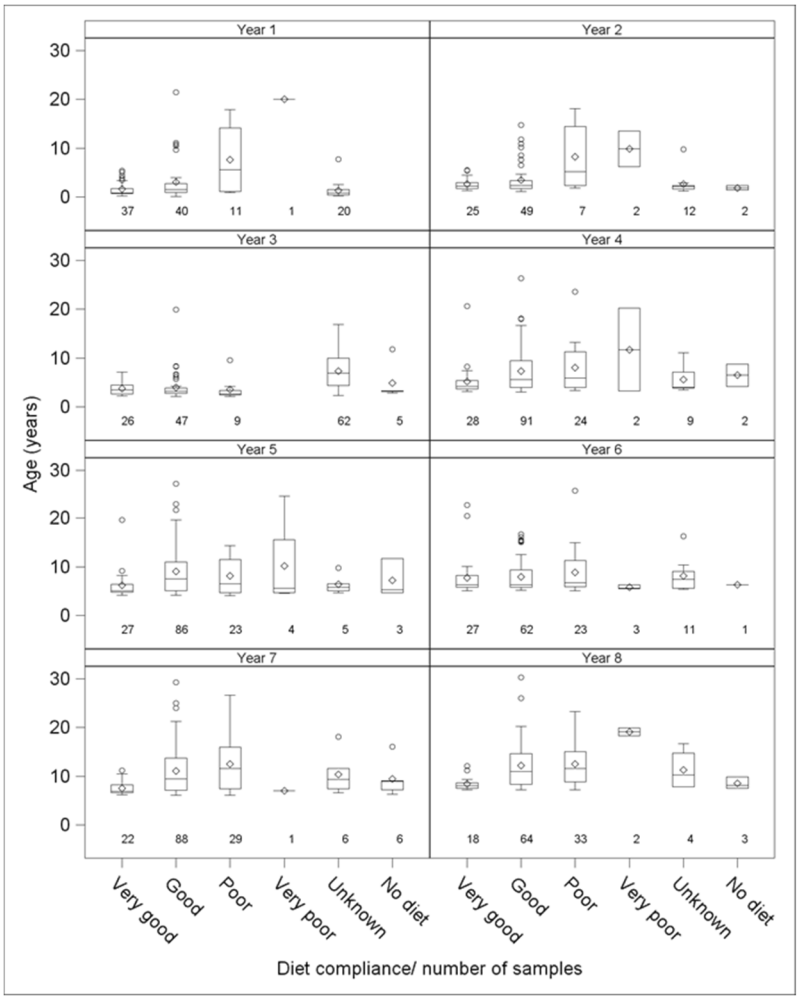


Figure 20 Age by diet compliance and treatment year, treatment year 9-16 (complete set)

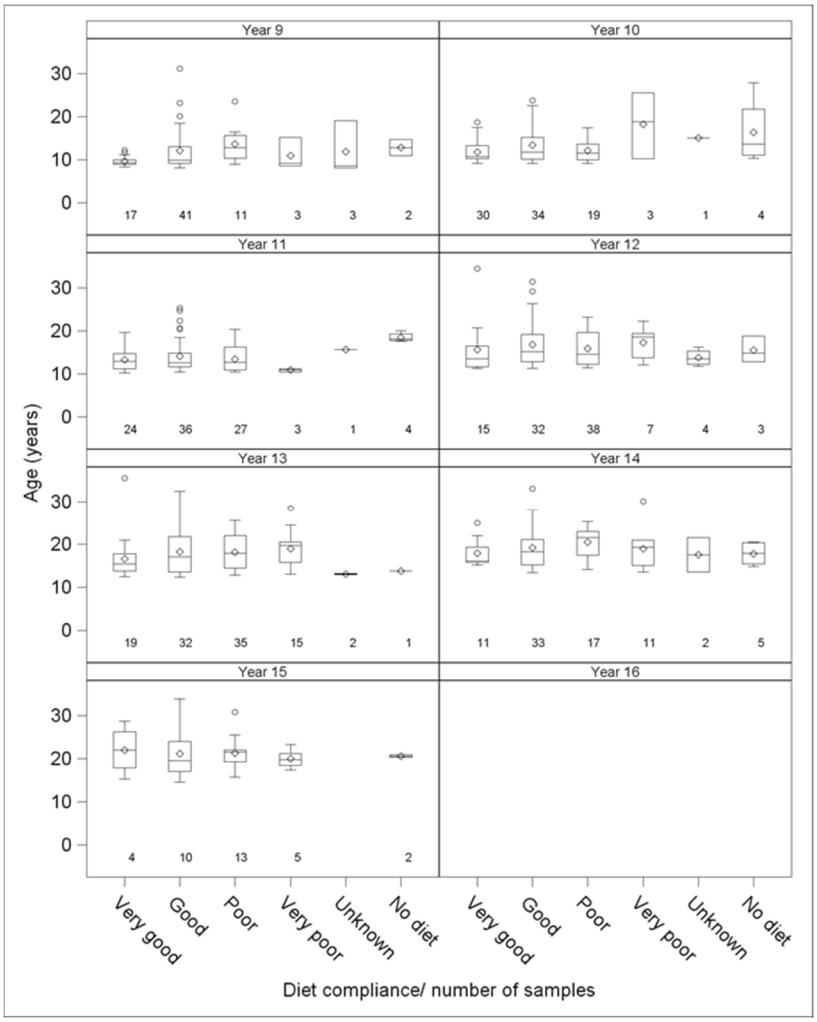
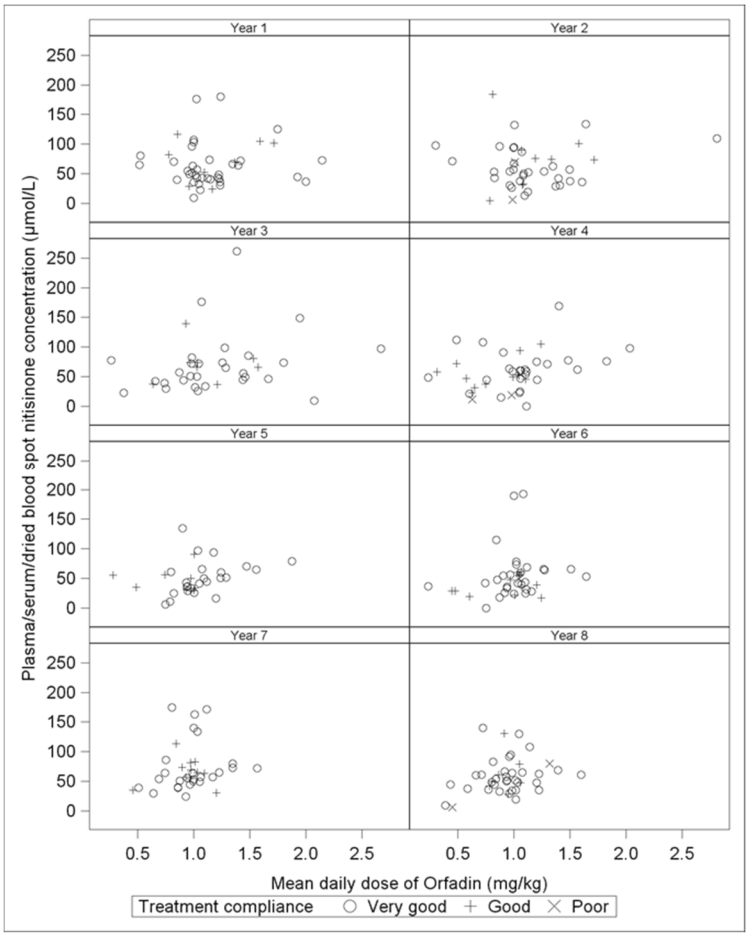
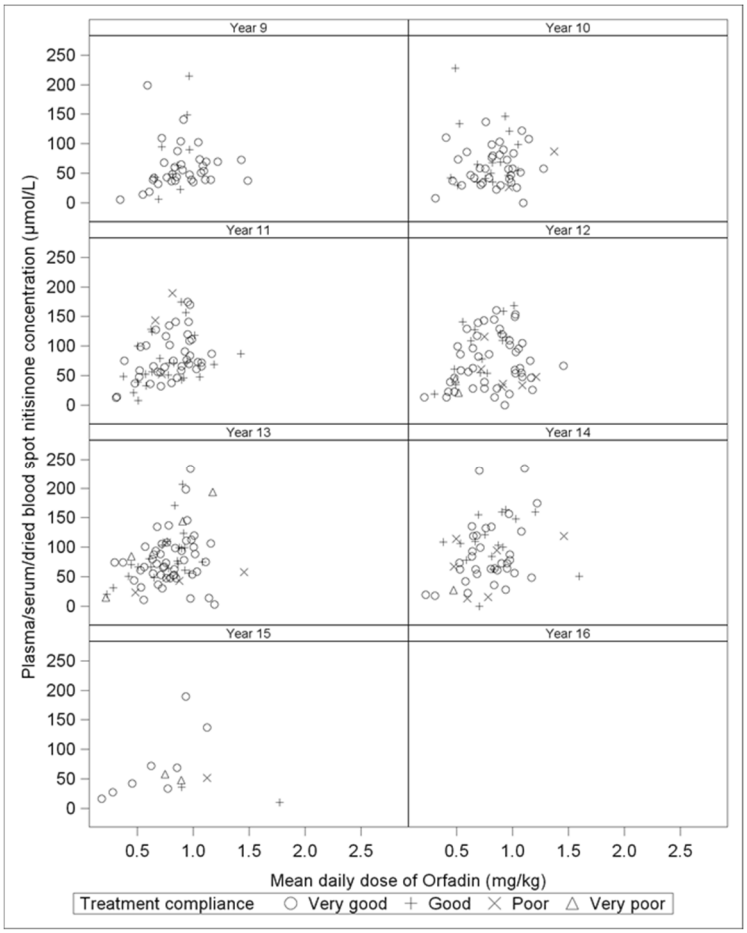


Figure 21 Plasma/serum/dried blood spot nitisinone concentration (µmol/L) versus mean daily dose of Orfadin (mg/kg), treatment year 1-8 (complete set)



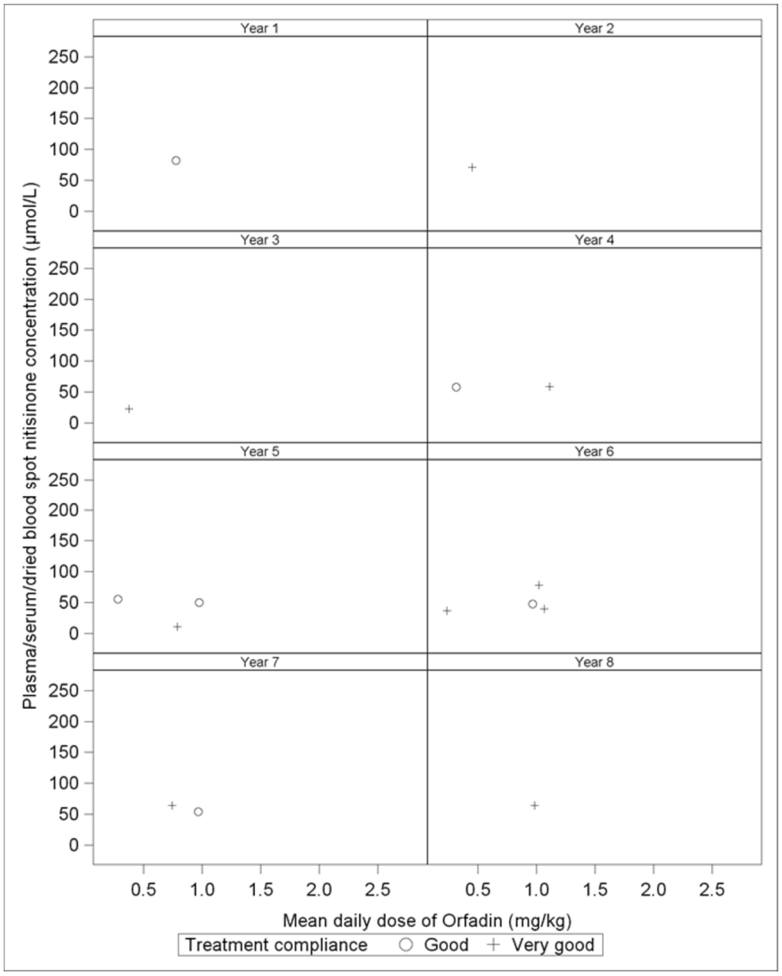
Note: In the case a patient has more than one assay of nitisinone concentration during a year the mean result is displayed.

Figure 22 Plasma/serum/dried blood spot nitisinone concentration (µmol/L) versus mean daily dose of Orfadin (mg/kg), treatment year 9-16 (complete set)



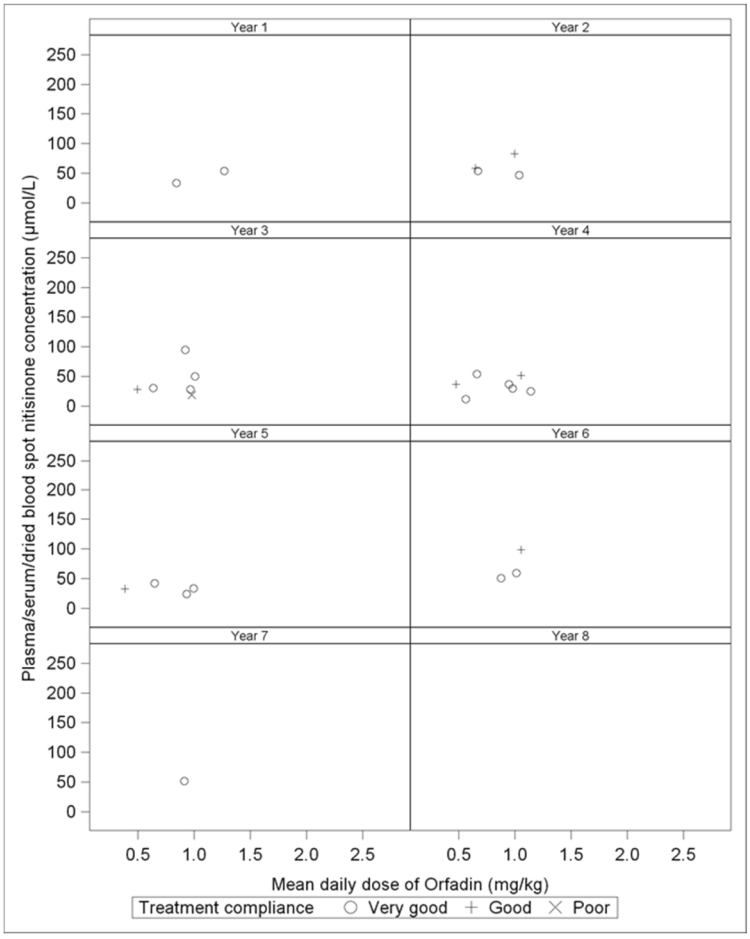
Note: In the case a patient has more than one assay of nitisinone concentration during a year the mean result is displayed.

Figure 23 Plasma/serum/dried blood spot nitisinone concentration (µmol/L) versus mean daily dose of Orfadin (mg/kg) in treatment-naïve patients on once daily dosage, treatment year 1-8 (index set)



Note: In the case a patient has more than one assay of nitisinone concentration during a year the mean result is displayed. Treatment-naïve: Patients who have previously not been treated with Orfadin and start their Orfadin treatment with once daily dosage (as confirmed by the first dose recorded for each patient).

Figure 24 Plasma/serum/dried blood spot nitisinone concentration (µmol/L) versus mean daily dose of Orfadin (mg/kg) in treatment-experienced patients, treatment year 1-8 after initiation of once daily dosing (index set)



Note: In the case a patient has more than one assay of nitisinone concentration during a year the mean result is displayed. Treatment-experienced: Patients who have switched to once daily dosage after twice daily dosage (as confirmed by a twice daily regimen reported before the once daily dosing). Only data during once daily treatment is included.

Table 52 Number of days since start of Orfadin treatment to assessment of overall clinical condition by treatment year (index set)

	n	Mean	Median	Q1, Q3	Min, Max
Year 1	45	265.4	280	214, 327	12, 364
Year 2	83	570.9	589	477, 673	366, 729
Year 3	90	932.9	955	835, 1029	735, 1094
Year 4	88	1284.5	1291	1222, 1368	1099, 1450
Year 5	78	1652.4	1654	1582, 1728	1483, 1823
Year 6	88	2028.2	2053	1927, 2117	1829, 2191
Year 7	73	2371.9	2380	2282, 2469	2194, 2549
Year 8	58	2739.7	2742	2646, 2829	2561, 2906
Year 9	51	3103.0	3098	3008, 3239	2923, 3267
Year 10	46	3478.7	3503	3361, 3573	3290, 3641
Year 11	41	3859.2	3860	3763, 3961	3669, 4014
Year 12	30	4236.6	4249	4159, 4321	4060, 4368
Year 13	23	4634.7	4655	4563, 4716	4390, 4746
Year 14	9	4921.0	4903	4881, 4927	4788, 5106

Abbreviations: n, Total number of patients with at least one assessment during the corresponding year; Q1, 25th percentile; Q3, 75th percentile.

Table 53 Adverse Events by system organ class and preferred term (complete set)

SOC/ Preferred term	Patients with at least one event (N= 315) n (%)	Number of events
Any AE	91 (28.9)	192
Blood and lymphatic system disorders	5 (1.6)	5
Immune thrombocytopenic purpura	1 (0.3)	1
Lymphadenopathy	1 (0.3)	1
Splenomegaly	2 (0.6)	2
Thrombocytopenia	1 (0.3)	1
Congenital, familial and genetic disorders	4 (1.3)	4
Arnold-Chiari malformation	1 (0.3)	1
Porphyria	2 (0.6)	2
Porphyria acute	1 (0.3)	1
Eye disorders	15 (4.8)	19
Corneal deposits	1 (0.3)	1
Corneal erosion	1 (0.3)	1
Corneal lesion	1 (0.3)	1
Corneal opacity	1 (0.3)	1
Eye disorder	2 (0.6)	2

SOC/ Preferred term	Patients with at least one event (N= 315) n (%)	Number of events
Eye irritation	2 (0.6)	2
Eye pain	4 (1.3)	4
Eye pruritus	1 (0.3)	1
Eye symptom	1 (0.3)	1
Keratitis	2 (0.6)	2
Myopia	1 (0.3)	1
Ulcerative keratitis	1 (0.3)	1
Vision blurred	1 (0.3)	1
Gastrointestinal disorders	11 (3.5)	18
Abdominal pain	3 (1.0)	3
Ascites	1 (0.3)	1
Constipation	1 (0.3)	1
Diarrhoea	2 (0.6)	2
Duodenal ulcer	1 (0.3)	1
Irritable bowel syndrome	1 (0.3)	1
Melaena	1 (0.3)	1
Nausea	1 (0.3)	1
Pancreatitis	1 (0.3)	1
Vomiting	4 (1.3)	6
General disorders and administration site conditions	12 (3.8)	12
Adverse event	1 (0.3)	1
Developmental delay	1 (0.3)	1
Mucosal inflammation	1 (0.3)	1
Nodule	1 (0.3)	1
Pseudocyst	1 (0.3)	1
Pyrexia	2 (0.6)	2
Treatment noncompliance	5 (1.6)	5
Hepatobiliary disorders	6 (1.9)	6
Hepatic failure	2 (0.6)	2
Hepatic lesion	1 (0.3)	1
Hepatomegaly	1 (0.3)	1
Hypertransaminasaemia	1 (0.3)	1
Liver disorder	1 (0.3)	1
Immune system disorders	1 (0.3)	1
Transplant rejection	1 (0.3)	1
Infections and infestations	6 (1.9)	7

SOC/ Preferred term	Patients with at least one event (N= 315) n (%)	Number of events
Bronchitis	1 (0.3)	1
Ear infection	1 (0.3)	1
Gastroenteritis	1 (0.3)	1
Pilonidal cyst	1 (0.3)	2
Tonsillitis	1 (0.3)	1
Upper respiratory tract infection	1 (0.3)	1
Injury, poisoning and procedural complications	8 (2.5)	9
Accident	1 (0.3)	1
Clavicle fracture	1 (0.3)	1
Maternal exposure during pregnancy	5 (1.6)	5
Post procedural haemorrhage	1 (0.3)	1
Radius fracture	1 (0.3)	1
Investigations	34 (10.8)	57
Alpha 1 foetoprotein decreased	1 (0.3)	1
Alpha 1 foetoprotein increased	4 (1.3)	4
Amino acid level	1 (0.3)	1
Amino acid level decreased[a]	5 (1.6)	5
Amino acid level increased[b]	20 (6.3)	28
Blood phosphorus increased	1 (0.3)	1
Coagulation factor V level decreased	1 (0.3)	1
Coagulation factor VII level decreased	1 (0.3)	1
Drug level decreased	8 (2.5)	8
International normalised ratio decreased	1 (0.3)	1
Prothrombin level decreased	1 (0.3)	1
Succinylacetone increased	2 (0.6)	2
Transaminases increased	1 (0.3)	1
Ultrasound liver abnormal	1 (0.3)	1
Vitamin D decreased	1 (0.3)	1
Metabolism and nutrition disorders	5 (1.6)	5
Dehydration	1 (0.3)	1
Hyponatraemia	1 (0.3)	1
Poor feeding infant	1 (0.3)	1
Vitamin A deficiency	1 (0.3)	1
Vitamin D deficiency	1 (0.3)	1
Musculoskeletal and connective tissue disorders	2 (0.6)	2
Joint swelling	1 (0.3)	1

SOC/ Preferred term	Patients with at least one event (N= 315) n (%)	Number of events
Scoliosis	1 (0.3)	1
Neoplasms benign, malignant and unspecified (including cysts and polyps)	4 (1.3)	4
Hepatic cancer	3 (1.0)	3
Hepatocellular carcinoma	1 (0.3)	1
Nervous system disorders	14 (4.4)	16
Cognitive disorder	3 (1.0)	3
Dyslexia	1 (0.3)	1
Epilepsy	3 (1.0)	3
Generalised tonic-clonic seizure	1 (0.3)	1
Headache	1 (0.3)	1
Lethargy	1 (0.3)	1
Migraine without aura	2 (0.6)	2
Seizure	1 (0.3)	1
Speech disorder developmental	2 (0.6)	2
Tremor	1 (0.3)	1
Pregnancy, puerperium and perinatal conditions	1 (0.3)	1
Pregnancy	1 (0.3)	1
Psychiatric disorders	7 (2.2)	9
Acute psychosis	1 (0.3)	1
Aggression	1 (0.3)	1
Autism spectrum disorder	1 (0.3)	1
Confusional state	1 (0.3)	1
Eating disorder	1 (0.3)	1
Enuresis	2 (0.6)	2
Hallucination, auditory	1 (0.3)	1
Psychotic disorder	1 (0.3)	1
Renal and urinary disorders	2 (0.6)	2
Acute kidney injury	1 (0.3)	1
Haematuria	1 (0.3)	1
Respiratory, thoracic and mediastinal disorders	2 (0.6)	3
Respiratory distress	1 (0.3)	1
Respiratory failure	1 (0.3)	1
Tachypnoea	1 (0.3)	1
Skin and subcutaneous tissue disorders	1 (0.3)	2
Pruritus	1 (0.3)	1

SOC/ Preferred term	Patients with at least one event (N= 315) n (%)	Number of events
Rash	1 (0.3)	1
Social circumstances	4 (1.3)	4
Diet noncompliance	3 (1.0)	3
Refusal of treatment by relative	1 (0.3)	1
Surgical and medical procedures	5 (1.6)	5
Liver transplant	5 (1.6)	5
Vascular disorders	1 (0.3)	1
Jugular vein thrombosis	1 (0.3)	1

Abbreviations: N, Total number of patients; n, Number of patients with at least one corresponding event; AE, Adverse event; SOC, System Organ Class.
Note: Only assessments on or after the index date (February 21, 2005) are included.
[a]Includes decreased levels of p-Phe. [b]Includes increased levels of p-Tyr and p-Phe, the majority being p-Tyr.

Table 54 Serious adverse events by system organ class, preferred term and causality (complete set)

SOC/ Preferred term/ Causality	Patients with at least one event (N= 315) n (%)	Number of events
Any AE	45 (14.3)	78
Blood and lymphatic system disorders	3 (1.0)	3
Immune thrombocytopenic purpura	1 (0.3)	1
Not related	1 (0.3)	1
Lymphadenopathy	1 (0.3)	1
Not related	1 (0.3)	1
Splenomegaly	1 (0.3)	1
Not related	1 (0.3)	1
Congenital, familial and genetic disorders	4 (1.3)	4
Arnold-Chiari malformation	1 (0.3)	1
Not related	1 (0.3)	1
Porphyria	2 (0.6)	2
Not related	1 (0.3)	1
Related	1 (0.3)	1
Porphyria acute	1 (0.3)	1
Not related	1 (0.3)	1
Eye disorders	2 (0.6)	2
Corneal erosion	1 (0.3)	1
Related	1 (0.3)	1

SOC/ Preferred term/ Causality	Patients with at least one event (N= 315) n (%)	Number of events
Ulcerative keratitis	1 (0.3)	1
Related	1 (0.3)	1
Gastrointestinal disorders	7 (2.2)	12
Ascites	1 (0.3)	1
Not related	1 (0.3)	1
Constipation	1 (0.3)	1
Not related	1 (0.3)	1
Diarrhoea	1 (0.3)	1
Not related	1 (0.3)	1
Duodenal ulcer	1 (0.3)	1
Related	1 (0.3)	1
Irritable bowel syndrome	1 (0.3)	1
Not related	1 (0.3)	1
Melaena	1 (0.3)	1
Not related	1 (0.3)	1
Pancreatitis	1 (0.3)	1
Related	1 (0.3)	1
Vomiting	3 (1.0)	5
Not related	3 (1.0)	5
General disorders and administration site conditions	7 (2.2)	7
Developmental delay	1 (0.3)	1
Related	1 (0.3)	1
Mucosal inflammation	1 (0.3)	1
Related	1 (0.3)	1
Pseudocyst	1 (0.3)	1
Related	1 (0.3)	1
Pyrexia	2 (0.6)	2
Not related	2 (0.6)	2
Treatment noncompliance	2 (0.6)	2
Not related	1 (0.3)	1
Related	1 (0.3)	1
Hepatobiliary disorders	6 (1.9)	6
Hepatic failure	2 (0.6)	2
Not related	2 (0.6)	2
Hepatic lesion	1 (0.3)	1
Related	1 (0.3)	1

SOC/ Preferred term/ Causality	Patients with at least one event (N= 315) n (%)	Number of events
Hepatomegaly	1 (0.3)	1
Not related	1 (0.3)	1
Hypertransaminasaemia	1 (0.3)	1
Not related	1 (0.3)	1
Liver disorder	1 (0.3)	1
Not related	1 (0.3)	1
Immune system disorders	1 (0.3)	1
Transplant rejection	1 (0.3)	1
Not related	1 (0.3)	1
Infections and infestations	1 (0.3)	2
Pilonidal cyst	1 (0.3)	2
Not related	1 (0.3)	2
Investigations	5 (1.6)	6
Alpha 1 foetoprotein increased	1 (0.3)	1
Not related	1 (0.3)	1
Amino acid level increased	2 (0.6)	2
Not related	1 (0.3)	1
Related	1 (0.3)	1
Drug level decreased	2 (0.6)	2
Related	2 (0.6)	2
Ultrasound liver abnormal	1 (0.3)	1
Not related	1 (0.3)	1
Metabolism and nutrition disorders	3 (1.0)	3
Dehydration	1 (0.3)	1
Not related	1 (0.3)	1
Hyponatraemia	1 (0.3)	1
Not related	1 (0.3)	1
Poor feeding infant	1 (0.3)	1
Not related	1 (0.3)	1
Neoplasms benign, malignant and unspecified (including cysts and polyps)	4 (1.3)	4
Hepatic cancer	3 (1.0)	3
Not related	3 (1.0)	3
Hepatocellular carcinoma	1 (0.3)	1
Not related	1 (0.3)	1
Nervous system disorders	8 (2.5)	9
Cognitive disorder	2 (0.6)	2

SOC/ Preferred term/ Causality	Patients with at least one event (N= 315) n (%)	Number of events
Related	2 (0.6)	2
Epilepsy	3 (1.0)	3
Not related	3 (1.0)	3
Generalised tonic-clonic seizure	1 (0.3)	1
Not related	1 (0.3)	1
Lethargy	1 (0.3)	1
Not related	1 (0.3)	1
Seizure	1 (0.3)	1
Not related	1 (0.3)	1
Speech disorder developmental	1 (0.3)	1
Related	1 (0.3)	1
Psychiatric disorders	5 (1.6)	7
Acute psychosis	1 (0.3)	1
Not related	1 (0.3)	1
Aggression	1 (0.3)	1
Related	1 (0.3)	1
Autism spectrum disorder	1 (0.3)	1
Related	1 (0.3)	1
Confusional state	1 (0.3)	1
Related	1 (0.3)	1
Eating disorder	1 (0.3)	1
Not related	1 (0.3)	1
Hallucination, auditory	1 (0.3)	1
Related	1 (0.3)	1
Psychotic disorder	1 (0.3)	1
Related	1 (0.3)	1
Renal and urinary disorders	1 (0.3)	1
Acute kidney injury	1 (0.3)	1
Related	1 (0.3)	1
Respiratory, thoracic and mediastinal disorders	1 (0.3)	1
Respiratory failure	1 (0.3)	1
Not related	1 (0.3)	1
Skin and subcutaneous tissue disorders	1 (0.3)	2
Pruritus	1 (0.3)	1
Related	1 (0.3)	1
Rash	1 (0.3)	1
Related	1 (0.3)	1

SOC/ Preferred term/ Causality	Patients with at least one event (N= 315) n (%)	Number of events
Social circumstances	2 (0.6)	2
Diet noncompliance	2 (0.6)	2
Not related	1 (0.3)	1
Related	1 (0.3)	1
Surgical and medical procedures	5 (1.6)	5
Liver transplant	5 (1.6)	5
Not related	4 (1.3)	4
Related	1 (0.3)	1
Vascular disorders	1 (0.3)	1
Jugular vein thrombosis	1 (0.3)	1
Not related	1 (0.3)	1

Abbreviations: AE, Adverse event; N, Total number of patients; n, Number of patients with at least one corresponding event; SOC, System Organ Class.
Note: Only assessments on or after the index date (February 21, 2005) are included.

Annex 1. List of stand-alone documents

Number	Title
1	List of principal investigators and their affiliations
2	List of Sobi study team members
3	List of responsible parties and names and affiliations of contractors involved in the conduct of the study
4	Statistical analysis plan
5	Tables, figures and listings

Annex 2. Conversion factors used for conversion from original laboratory units to standard units and for dried blood spot to serum/plasma nitisinone

Laboratory parameter	Original unit	Standard unit	Conversion factor
Plasma/serum Nitisinone	mmol/L	µmol/L	x 1000
Plasma/serum Nitisinone	µg/L	µmol/L	x 0.003037409
DBS Nitisinone (µmol/L) to serum/plasma Nitisinone (µmol/L)			x 2.4
Plasma tyrosine	nmol/mL	µmol/L	x 1
Plasma/serum tyrosine	mmol/mL	µmol/L	x 1000
Plasma/serum phenylalanine	mmol/L	µmol/L	x 1000
Plasma phenylalanine	mg/dL	µmol/L	x 60.54
Plasma/serum/DBS SA	mmol/L	µmol/L	x 1000
Urinary SA	mmol/L	µmol/L	x 1000
Urinary SA/creatinine	mg/g	mmol/mol	x 0.7152
Urinary SA/creatinine	µmol/mol	mmol/mol	x 0.001
Plasma/serum alpha-fetoprotein	IU/L	µg/L	x 0.00121
Plasma/serum alpha-fetoprotein	IU/mL	µg/L	x 1.21
Plasma/serum alpha-fetoprotein	kIU/L	ng/mL	x 1.21
Plasma/serum alpha-fetoprotein	µg/L	ng/mL	x 1
Plasma/serum alpha-fetoprotein	µg/dL	µg/L	x 0.1

Abbreviations: DBS, Dried blood spot; IU, International units; kIU, Kilo international unit; SA, Succinylacetone.