

# Nitisinone: a review

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**Abstract:** Nitisinone (2-[2-nitro-4-trifluoromethylbenzoyl]cyclohexane-1,3-dione), an effective triketone herbicide, is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, the second enzyme in the tyrosine catabolic pathway. Since 1992, the drug has become an effective pharmacological treatment for hereditary tyrosinemia type 1 (HT1). Nitisinone can prevent the development of liver disease, reverse and prevent the renal tubular dysfunction, severe neurological crisis and cardiomyopathy and significantly reduce the risk of developing hepatocellular carcinoma in HT1 patients. Its mode of action, with few side effects reported, make the drug a potential candidate for the treatment of other disorders of tyrosine metabolism, including alkaptonuria, with successful reduction in homogentisic acid production to prevent long-term complications. Nitisinone could also be a promising agent in the treatment of tumors with active tyrosine metabolic pathways. In this review, we discuss the effects of nitisinone for various tyrosine pathway disorders.

**Keywords:** nitisinone, hereditary tyrosinemia type 1, alkaptonuria, tyrosine

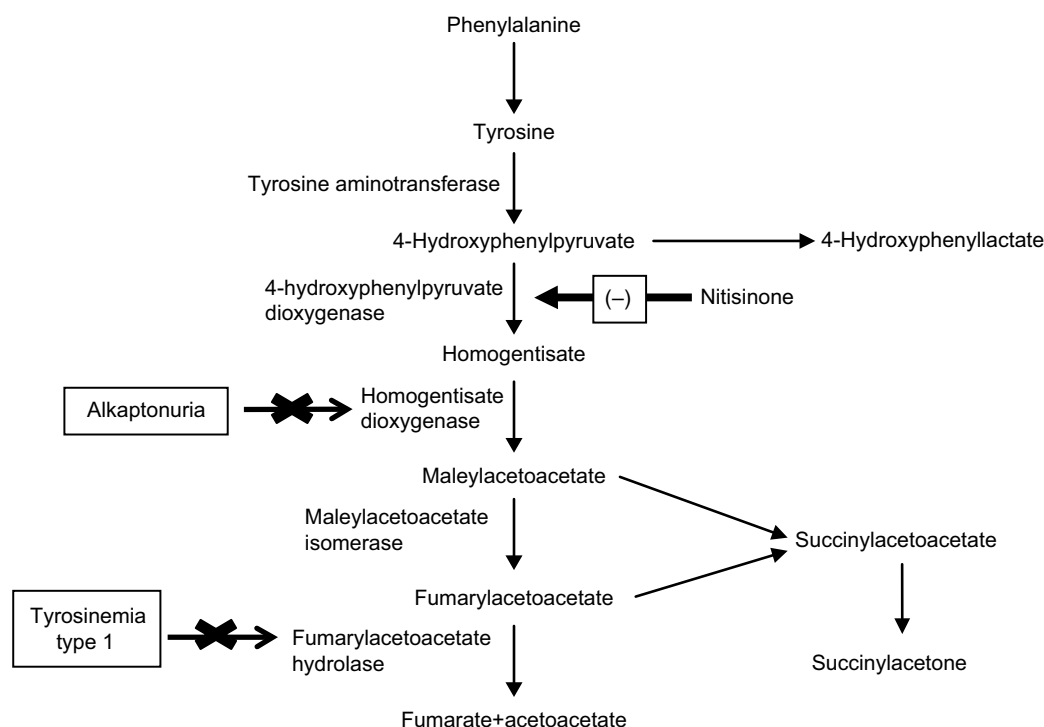
## Introduction

The development of nitisinone (2-[2-nitro-4-trifluoromethylbenzoyl]cyclohexane-1,3-dione, NTBC) has dramatically changed the natural course of hereditary tyrosinemia type 1 (HT1). Discovery of its mode of action, with a few side effects reported, resulted in clinical use of this drug in treating various clinical disorders in the tyrosine (Tyr) pathway. In this review, we focus on the mode of action and clinical effects of nitisinone for various Tyr pathway disorders.

## The history of nitisinone

Nitisinone is a triketone herbicide derived from leptospermone produced by the bottle-brush plant.<sup>1</sup> It was first discovered by Zeneca Agrochemicals in 1982.<sup>1</sup> The members of this family occur naturally in plant oils. Treating plants with triketone herbicides causes bleaching symptomatology due to reduced chlorophyll and carotenoid synthesis and prevents the growth of competing plants around.<sup>2</sup> Nitisinone, despite belonging to this wide family, revealed a novel mechanism of action by inhibiting the enzyme 4-hydroxyphenyl pyruvate dioxygenase (4-HPPD) which catalyzes the conversion of 4-hydroxyphenyl pyruvate (4-HPP) to homogentisic acid (HGA) as part of the Tyr degradation pathway (Figure 1).<sup>3-5</sup> The same mechanism was also confirmed in animal toxicology studies in the rat.<sup>6</sup> Inhibition of 4-HPPD led to a profound increase in plasma Tyr levels in rats and corneal lesions similar to those detected in tyrosinemia type-2 patients.<sup>6</sup> This discovery led to the hypothesis that the drug could decrease the

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**Figure 1** Tyrosine metabolism and nitisinone.

production of toxic metabolites (fumarylacetoacetate [FAA], maloylacetoacetate [MAA] and succinylacetone [SA]) seen in HT1 by effective blockage of the Tyr catabolic pathway. In 1992, Lindstedt et al presented a new principle for treatment of HT1 based on the inhibition of 4-HPPD with nitisinone.<sup>7</sup> The first patient showed a dramatic response to treatment with an immediate biochemical response. SA excretion was reduced with a dose of 0.05 mg/kg/day of nitisinone as early as 12 hours after the initial dose and became undetectable as the dose was increased.<sup>8</sup> Upon marked improvement in clinical conditions and biochemical response, four more patients were also treated with nitisinone with similar response during 7–9 months of follow-up.<sup>7</sup> Finally, the Gothenburg group<sup>9</sup> coordinated an international study of nitisinone treatment in HT1.

The drug has received approval from both the US FDA and the EU Drug Agency in January 2002 and February 2005, respectively, without formal phase II, III, and IV clinical trials,<sup>9–11</sup> and is marketed as Orfadin<sup>®</sup> (Swedish Orphan Biovitrum, Stockholm, Sweden).

## Pharmacological properties and mechanism of action

The structure of nitisinone has some similarities to 4-HPP which is a substrate for 4-HPPD. The drug is a potent time-dependent (tight-binding) reversible inhibitor of mouse, rat, and human liver 4-HPPD *in vitro*.<sup>3–6</sup> 4-HPPD is a non-heme Fe(II) enzyme that catalyzes the second step in the Tyr

degradation pathway, involving the conversion of 4-HPP to HGA (Figure 1).<sup>5</sup> The binding of nitisinone to HPPD, Fe(II), was shown to occur in two phases and comprise three steps: a reversible binding proximal to the active site of metal ion, bidentate association with the Fe<sup>+2</sup> center and an irreversible deprotonation of the enol.<sup>4</sup> Hanuske-Abel et al also suggested a tetradentate association, obtained from crystallographic and computational data on nitisinone binding.<sup>12</sup>

Studies on the rat liver cytosol extracts by Ellis and Laschi revealed an effective time-dependent reversible inhibition of liver HPPD with median inhibitory concentrations (IC<sub>50</sub>) of 40 and 173 nM and a very slow dissociation of the NTBC–enzyme complex.<sup>6,13</sup> A single oral dose of 0.1 mg/kg nitisinone produced complete inhibition of hepatic HPPD within 1 hour of administration and only 40%–50% of the enzyme returned normal 4 days after dosing. Thus, the interaction of nitisinone with HPPD is characterized by a rapid inhibition step to form an enzyme–inhibitor complex that can dissociate slowly with recovery of enzyme activity.<sup>12</sup> Analysis of the key hepatic enzymes involved in Tyr catabolism in rodents showed despite inhibition of HPPD soon after dosing, the activity of tyrosine aminotransferase (TAT) in the liver was induced by about twofold presumably due to enzyme induction and homogentisic acid oxidase (HGO) was not affected.<sup>14</sup> There is no information about the effects of nitisinone on renal HPPD or the kinetics of HPPD inhibition in human liver or kidney tissue.

The HPPD affinity was higher in humans than in rats with an  $IC_{50}$  at 5 nM on purified human enzyme.<sup>2</sup> In 10 healthy male volunteers aged 19–39 years, peak plasma concentrations were achieved rapidly following a single oral dose of 1 mg/kg body weight (BW) with a half-life of approximately 54 hours.<sup>15</sup> Pharmacokinetic studies in 9 infants with HT1 over a period of up to 24 hours also revealed that the administration of a single daily dose results in therapeutic drug levels and suppression of SA formation.<sup>16</sup> Nitisinone was rapidly and completely absorbed after oral administration and had a terminal half-life of about 9 hours in rats.<sup>17</sup> Absorption kinetics were also studied in either the liquid or capsule form in healthy volunteers, and the median time to reach the maximum plasma concentration was 15 minutes for the liquid and 3 hours for the capsule form.<sup>15</sup> The capsule forms should be refrigerated at a temperature range of 2°C–8°C because of heat instability, while the liquid forms should be stored and refrigerated between 2°C and 8°C prior to first use and at room temperature after first opening. For patients who have difficulty swallowing the capsules and for whom oral suspension is not suitable, opening the capsules and suspending the contents in a small amount of water, formula, or apple sauce immediately before use are recommended.<sup>17</sup> There are no pharmacokinetic studies on the effect of food on absorption nor on plasma protein binding.

Following oral administration of radiolabeled nitisinone in rats, the bioavailability was found to be high (>90%) with multiple organ distribution.<sup>18</sup> Administration of a single oral dose of [<sup>14</sup>C]-nitisinone in rats (0.3 µmol/kg [0.1 mg/kg] or 30 µmol/kg [10 mg/kg]) and in mice (30 µmol/kg [10 mg/kg]) led to selective retention of radiolabeled nitisinone in the liver (>90% associated reversibly with the cytosol fraction), in kidneys, and to a lesser extent in the Harderian gland.<sup>13,18</sup> Radioactivity was detected up to 7 days in these organs.

The route of elimination of nitisinone has been investigated in both animals and man.<sup>3,15,18–20</sup> In an autoradiographic study in rats, nitisinone and two hydroxylated metabolites were found to be excreted in the urine.<sup>19</sup> Animal and human studies indicate that nitisinone is excreted through both the feces and the urine at approximately equal levels.<sup>19,20</sup> The tentative identification of hydroxylated metabolites and 2-nitro-4-trifluoromethylbenzoic acid in urine points to the involvement of the cytochrome P450 (CYP) system in the metabolism of nitisinone.<sup>21</sup> Investigations on the potential for drug–drug interactions between nitisinone and other medicines that induce or inhibit CYP activity have been studied *in vitro* on human microsomes.<sup>21</sup> In summary, with the exception of CYP2C9 inhibition, nitisinone was found

to have little effect on human CYP enzymes as either an inhibitor or an inducer.

The inhibition of HPPD leads to a marked increase in plasma Tyr. Administration of a single dose of nitisinone in rats resulted in a marked increase in Tyr level in both plasma (about 2,500 nmol/mL) and ocular fluids (3,500–4,000 nmol/mL).<sup>14,18</sup> Tyrosinemia was both time and dose dependent with a maximum Tyr level at 24 hours after dosing. Plasma Tyr level returned to normal level by 48 hours, indicating that only a small recovery of the enzyme activity was able to clear Tyr from plasma.<sup>3,14,18</sup> On the other hand, it took 3 days to normalize for the larger increase of Tyr in aqueous humor of the eye.<sup>3</sup> Human fibroblast cultures treated with nitisinone also revealed clearly that Tyr accumulation during administration of nitisinone appears to be dose-dependent.<sup>13</sup>

Tyr accumulation in ocular fluids can lead to eye lesions both in human and in some animals.<sup>14,18,22</sup> Administration of a dose of 10 mg/kg/day to mice for 6 weeks increased plasma Tyr level to 650 nmol/L and to 2,300 nmol/L in aqueous humor without any eye lesions.<sup>14</sup> This finding was thought to be due to higher activity of TAT in mouse liver which is the first enzyme of the Tyr degradation pathway, which can clear Tyr as hydroxyphenyllactic acid and HPP. Also, there is a threshold plasma Tyr level, above which it leads to eye lesions.<sup>14,18,22</sup>

The first report of nitisinone lethal dose 50% ( $LD_{50}$ ) values on human primary fibroblasts following both acute (24 hour) and chronic (144 hour) treatment revealed that the short-term  $LD_{50}$  was considered to be >1 µM, while long-term  $LD_{50}$  was about 1 µM.<sup>13</sup> A long-term  $LD_{50}$  value lower than the short-term  $LD_{50}$  was reported to support the hypothesis that the toxicity of nitisinone was linked to its mode of action.<sup>13,22–25</sup>

## Nitisinone and hereditary tyrosinemia type I

Phenylalanine and Tyr, from the diet and endogenous protein turnover *in vivo*, are used to synthesize new proteins, hormones (eg, thyroxine and catecholamines) and melanin. The majority of Tyr is metabolized to malate, acetoacetate, and products that enter the intermediary metabolism (Figure 1).

HT1 is an autosomal recessive inborn error of Tyr metabolism caused by deficiency of the last enzyme in the Tyr degradation pathway, “FAH”. Reduced activity of FAH leads to the accumulation of upstream metabolites such as FAA and MAA, which in turn are converted to SA.<sup>26</sup> Toxic metabolites FAA, MAA, SA and succinylacetoacetate (SAA) are highly reactive electrophilic toxic compounds and bind to sulfhydryl

groups. These toxic metabolites are thought to be responsible for progressive hepatic, renal, and neurological findings.<sup>26–29</sup> The human FAH complementary DNA has been mapped to chromosome 15q, and various mutations have been identified in the human *FAH* gene.<sup>26,30–34</sup> FAH is mainly expressed in liver and kidney cells, although a basal level of expression has been detected in many tissues.<sup>26</sup> No clinical correlation has been detected between genotype and phenotype.<sup>26,30–34</sup> The clinical manifestations of HT1 are heterogeneous even within the members of the same family. HT1 can be classified based on the age at symptom onset: acute HT1 (<6 months), subacute HT1 (6–12 months), chronic HT1 (>12 months).<sup>35,36</sup> Acute or chronic liver disease, renal dysfunction with Fanconi syndrome and hypophosphatemic rickets, polyneuropathy, and abdominal pain resembling acute porphyria are the main clinical findings.<sup>26,32,34–37</sup> Cardiomyopathy and hyperinsulinism have also been described.<sup>38–40</sup> Most patients present with the acute and subacute form of the disease characterized by clinical findings of acute–subacute liver failure with poor growth, vomiting, ascites, coagulopathy, hypoglycemia, hypoalbuminemia, and hyperbilirubinemia. Patients with the chronic form have growth failure; renal tubular dysfunction including Fanconi syndrome; and neurologic crises with pain and paresthesias associated with evidence of liver dysfunction, including hepatomegaly, transaminasemia, hyperbilirubinemia, and coagulopathy.<sup>8,29,35–37,41</sup> An odor resembling boiled cabbage may be present due to increased methionine metabolites.<sup>36,37</sup>

The natural course of the disease usually results in death.<sup>35</sup> The causes of death are primarily liver failure, hepatocellular carcinoma (HCC) and respiratory failure from neurological crisis.<sup>32</sup> The prognosis gets poorer if the presentation is earlier.<sup>8,35,41,42</sup> The leading causes of death are fulminant liver failure, porphyria-like neurological crises, and metastatic HCC, especially if left untreated.<sup>35</sup> Micro-macronodular cirrhosis and HCC are the long-term complications. HT1 is the inborn error of metabolism with the highest incidence of progression to HCC due to mutagenic effects of accumulated toxic metabolites.<sup>26,35</sup>

Liver transplantation (LT) was the only choice of treatment and a life-saving procedure, although renal dysfunction remained, as low Tyr diet was not effective in the acute form and had limited success in the chronic form.<sup>8,35</sup> Still, LT carries its own risks with a mortality of 10%–15% and side effects associated with direct and indirect costs.<sup>43</sup> The introduction of nitisinone in 1992 has radically improved the natural course of the disease. The drug was found to be effective not only in preventing the acute life-threatening

complications of the disease (eg, acute hepatic failure and neurological crises) but also in reducing the need for LT, improving the survival and the quality of life in HT1 patients, especially when started in the newborn period.<sup>8,33,44</sup> The starting dose is 1–2 mg/kg BW per day.<sup>44</sup> The initial dose can be titrated down by monitoring nitisinone levels without risking metabolic control as judged from plasma SA levels and SA excretion.<sup>45,46</sup> A few anecdotal patients were reported who did well under doses lower than 1 mg/kg/day. El-Karakasy et al described 4 patients and D'Eufemia et al described one patient for whom doses of 0.55–0.65 mg/kg/day were sufficient to prevent liver dysfunction.<sup>47,48</sup>

NTBC should be quantified in plasma and blood spot samples to identify the optimal dose for an individual. A column liquid chromatographic method, a liquid chromatographic–tandem mass spectrometry (LC–MS/MS); a capillary electrophoretic method with photometric detection and a high-performance liquid chromatographic–tandem mass spectrometric method have been applied for the detection and quantitative determination of nitisinone in serum, plasma and dried blood spot samples.<sup>49–54</sup> Although the mean plasma nitisinone concentration which is theoretically sufficient to produce a 99.9% inhibition of HPPD based on *in vitro* studies has been determined to be 35  $\mu\text{mol/L}$ , it is quite common that some HT1 patients require higher plasma levels (<70  $\mu\text{mol/L}$ ) to achieve complete elimination of urinary SA excretion.<sup>8,11</sup> As plasma SA is protein bound, plasma SA level and  $\delta$ -aminolevulinic acid (ALA) dehydratase inhibition take longer to normalize.<sup>8</sup> Different plasma nitisinone levels are proposed to keep urinary SA below the limit of detection. Schlune et al proposed plasma nitisinone levels of 45–50  $\mu\text{mol/L}$ , Herebian et al proposed plasma nitisinone levels of 50–150  $\mu\text{mol/L}$  and Prieto et al proposed plasma nitisinone levels of 15–40  $\mu\text{mol/L}$ .<sup>16,49,50</sup>

Administration of nitisinone in two divided daily doses is recommended but administration of a single daily dose which is found to be well tolerated and safe, potentially improving compliance with therapy, also results in therapeutic plasma drug levels and effective suppression of SA formation.<sup>8,16,45</sup>

## Effects of nitisinone on the hepatic manifestations of HT1

Infants presenting with acute hepatic dysfunction can rapidly develop severe liver dysfunction with a high risk of mortality.<sup>9,35</sup> Nitisinone administration results in a remarkable clinical response, where 90% of the patients respond completely.<sup>54,55</sup> SA and  $\delta$ -ALA decrease within the hours after the first dose of nitisinone.<sup>7,8</sup> The majority of infants who present

clinically already have established cirrhosis, and treatment could convert them to a stable chronic liver disease.<sup>9</sup> In most cases, liver function and coagulopathy improve within 1 week of treatment.<sup>7,8,56,57</sup> In those who are unresponsive, the nitisinone dosage should be increased and in those who are unresponsive even after higher doses, LT is indicated.<sup>9,45,57</sup> No new acute hepatic exacerbation has been reported after nitisinone initiation.<sup>23</sup>

Children with chronic tyrosinemia and those with acute/subacute tyrosinemia treated with nitisinone have generally established chronic liver disease and nodular cirrhosis. Nitisinone slows down the progression of chronic hepatic disease.<sup>9,11,33</sup> Even radiological regression of liver nodules has also been reported.<sup>58</sup> Nitisinone also improves growth, liver function, and portal hypertension in HT1 patients with chronic liver disease.<sup>57</sup> The effect of nitisinone on the hepatic findings depends on the drug use on a regular basis. FAH<sup>-/-</sup> mouse model studies revealed that nitisinone treatment prevented lethal liver dysfunction when started in the neonatal period but discontinuation of this treatment led to disruption of hepatic architecture depending on the length of time.<sup>20</sup>

The development of HCC is the most important long-term complication of HT1. Although improvement in liver function and portal hypertension can be achieved, nitisinone treatment cannot completely alter abnormal gene expression in the liver and collagen metabolism nor can it reverse established hepatic lesions such as oncogenic mutations, hepatic dysplasia, and fibrosis.<sup>12,59–62</sup> Late-treated HT1 patients can develop cirrhosis and HCC with progression in nodules.<sup>9,33,56,63,64</sup> Development of HCC mainly affects patients with chronic form or who have been treated after 2 years of age, in whom malignant transformation at the microscopic level is thought to have started even by the time nitisinone is started.<sup>56,63–65</sup> These patients usually have a slow decrease in  $\alpha$ -fetoprotein (AFP) levels without reaching normal levels despite treatment or persistent high levels of AFP.<sup>66</sup> Despite these findings, nitisinone decreases the risk of HCC compared to those before nitisinone treatment, as HCC incidence was reported to be 18%–37% among children who have survived past their second birthday.<sup>35,67</sup> Early treatment has been shown to decrease the risk of HCC.<sup>44,46,56</sup> The earlier the nitisinone treatment is commenced, the lower the risk a patient should develop HCC as the exposure of the liver to the mutagenic effects of FAA and MA is lowered.<sup>33</sup> Medium-term follow-up results of the effect of nitisinone treatment on clinical course of HT1 revealed that none of the patients treated from the neonatal period developed detectable liver disease.<sup>44</sup> In sharp contrast, all late-treated HT1 patients had chronic liver

abnormalities before receiving nitisinone and developed cirrhosis, and some developed HCC.<sup>23,44,63,68</sup> Larochelle et al reported that LT was performed in 71% of non-nitisinone treated patients and 26% of late-treated patients. The clinical indications include cirrhosis or cancer, acute hepatic failure, and previous neurologic crisis.<sup>33</sup> None of the early-treated patients required LT.<sup>33,44</sup> Today, indications for LT are limited to failure to respond to nitisinone or HCC.<sup>45,46</sup>

## Extrahepatic manifestations of HT1

Variable degree of renal dysfunction is detectable in most of the HT1 patients at presentation, ranging from mild tubular dysfunction to chronic renal disease.<sup>26</sup> As a result of mutagenic effects of FAA, MAA, and SA on intracellular metabolism of hepatic cells and renal cells, these cells undergo either death of apoptotic cell or adaptive alteration of gene expression.<sup>26</sup> Animal models suggest that while FAA is responsible for apoptosis, glomerulosclerosis and interstitial fibrosis, SA is responsible for renal tubular dysfunction.<sup>69,70</sup> Renal disease manifests with abnormal renal architecture (size and ultrasonographic echogenicity), tubular disorder (all or some components of Fanconi syndrome: aminoaciduria, phosphaturia, and renal tubular acidosis) and frank nephrocalcinosis.<sup>26,46,71</sup> Renal tubular function shows a rapid improvement after initiation of nitisinone therapy and returns to normalcy.<sup>64,71–73</sup> Maiorana et al revealed that almost all of the biomarkers of renal tubular dysfunction (including phosphaturia, glycosuria, and urinary  $\beta$ -2 microglobulin) normalized within 2 weeks in all HT1-affected children treated with nitisinone.<sup>73</sup> No child redeveloped tubular dysfunction at long-term follow-up after commencing nitisinone therapy.<sup>33,64,71,72</sup> On the contrary, long-term outcome in French patients showed that some tubular dysfunction remained in some patients despite nitisinone therapy, although no glomerular filtration abnormality was observed.<sup>23</sup> This finding was considered to be influenced by the age at the start of nitisinone therapy as tubular function remains normal in those treated prospectively from infancy.<sup>23,33,46</sup> Early nitisinone treatment not only reduces the need for LT but also improves post-transplant renal function.<sup>73–75</sup> Glomerular function seems to remain stable on long-term follow-up under nitisinone therapy.<sup>74,75</sup>

Porphyria-like neurologic crises (PLNCs) and recurrent peripheral neuropathy have been reported in HT1 patients.<sup>35,36,76,77</sup> PLNCs are among the major causes of death in untreated HT1 patients.<sup>35</sup> These crises include change in mental status, abdominal pain, peripheral neuropathy, and/or respiratory failure requiring mechanical ventilation, which last 1–7 days.<sup>76,77</sup> PLNC and neural damage in

HT1 patients are caused by inhibition of heme synthesis by SA. SA is the most potent inhibitor of the enzyme 5-ALA dehydratase known, resulting in the increased production of 5-ALA.<sup>77</sup> Normalization of SA in blood and correction of the complete inhibition of porphobilinogen synthase (PBGs) in erythrocytes during nitisinone treatment protect from the porphyric crisis.<sup>7</sup> PLNCs also respond dramatically to nitisinone therapy.<sup>68</sup> Interruption of the treatment can cause relapsing of severe crises that can lead to death in patients with HT1.<sup>64,78,79</sup>

Cardiomyopathy is a rare complication of HT1 and usually reported at the initial presentation as hypertrophic cardiomyopathy involving hypertrophy of the interventricular septum.<sup>39,40</sup> The pathogenesis is poorly understood and is thought to be related to the accumulation of toxic FAA in the cardiac tissues or to the elevated Tyr levels.<sup>40</sup> Cardiomyopathy resolves in the patients treated with nitisinone.<sup>39,40,80</sup>

## Nitisinone and alkaptonuria

Alkaptonuria (AKU; OMIM 203500) is a rare autosomal recessive disease caused by a deficiency of homogentisate 1,2-dioxygenase (HGD) that converts intermediate HGA to MAA in the Tyr metabolism pathway (Figure 1). The single-copy human HGD gene maps to chromosome 3q13.33, encompassing 14 exons and encoding a 445-mer protein.<sup>81</sup> AKU arises from homozygous or compound heterozygous loss-of-function mutations in the HGD gene, with 115 different human mutations currently identified.<sup>81,82</sup> There appears to be no correlation, currently, between the type of mutation and severity of the phenotype.<sup>83</sup>

Inability to metabolize HGA increases both the circulating concentration and urinary excretion of HGA, with at least 90% of the compound.<sup>24,84</sup> Despite increased urinary excretion, it is not sufficient to completely remove it from bodily fluids and tissues. HGA in urine polymerizes to benzoquinone acetic acid which forms a melanin-like polymeric pigment that leads to a characteristic early sign of the disorder: darkening of the urine on standing.<sup>85,86</sup> The melanin-like polymeric pigment *in vivo* also deposits in and binds to connective tissues like cartilage, leading to pathological pigmentation and destructive consequences “ochronosis”.<sup>87–89</sup> Ochronosis is a chronic process and clinical symptoms are typically observed from the second decade of life, including severe spondyloarthropathy, ruptures of the ligaments, tendons, and muscles; osteopenia, fractures; valvular heart disease; pigmentation of eyes and ears; central vein occlusion and elevated intraocular pressure; renal, prostatic, gallbladder, and salivary stones and renal insufficiency.<sup>83,90–98</sup> Early

degenerative ochronotic arthropathy is seen especially in weight-bearing joints, leading to severe pain in spine and large joints such as shoulder, hip, and knee.<sup>98</sup> Symptoms worsen from the fourth decade onward with progressive kyphoscoliosis and impaired spinal and thoracic mobility. Hearing loss (40%), cardiac arrhythmias (40%), and congestive cardiac failure (10%) also occur in AKU patients.<sup>86</sup> Acute metabolic decompensation, including oxidative hemolysis and/or methemoglobinemia, has also been reported.<sup>99,100</sup> There is currently no licensed pharmacological treatment for AKU, except palliative treatments.

Nitisinone inhibits 4-HPPD and has been demonstrated to significantly reduce the serum concentration and urinary excretion of HGA in both mouse models and humans.<sup>13,24,25,101–104</sup> The drop in HGA concentrations was rapid, with approximately 60% decrease in circulating HGA within 48 hours on 2 mg dose (unpublished data), and thus is an efficacious treatment.<sup>101</sup> Six clinical trials have been undertaken on the use of nitisinone to treat AKU.<sup>24,25,86,102–104</sup> The first study was an early dose ranging study in two older women with AKU.<sup>102</sup> An open-label, single-center study of 9 AKU patients showed that the dose required to decrease HGA significantly was up to 30-fold lower than that used in treating HT1.<sup>86</sup> Administering nitisinone (1.05 mg bid) daily with a regular diet decreased HGA by 95% and increased Tyr by 11-fold in 9 AKU adult patients over a 4-month period.<sup>86</sup> In another 3-year single-blind, controlled clinical trial of 30 patients revealed that the nitisinone group (2.1 mg/day) showed a sustained decrease in mean urinary HGA from 5.1 to 0.125 g/day and mean plasma HGA from 5.74 to 0.306 mg/L after treatment.<sup>24</sup> Urine and plasma HGA levels decreased by 98% and 95%, respectively. The increased plasma Tyr level did not cause corneal or any other toxicity. About 6 out of 7 patients who received nitisinone for more than 1 week noted decreased pain in their affected joints.

In an international, multicenter, randomized, open-label, nontreatment controlled, parallel-group, dose–response study of 40 patients, a clear dose–response relationship was observed between nitisinone and the urinary excretion of HGA.<sup>103</sup> The most efficacious dose was 8 mg daily, compared to 1, 2, and 4 mg daily with the mean reduction of urinary HGA being 98.8% with a daily 8 mg dose.<sup>103</sup> Olsson et al also found similar findings in 32 patients with AKU who received 1, 2, 4, or 8 mg nitisinone daily. Nitisinone decreased the urinary excretion of HGA in a concentration-dependent manner, up to serum nitisinone concentrations of about 3  $\mu\text{mol/L}$ .<sup>25</sup> This concentration was reached with a dose of 8 mg daily in seven of eight patients with that dose. These

patients had a reduction in urinary HGA of at least 99.4%. On the contrary, Gertsman et al revealed a significant decrease in urine HGA level in 7 AKU patients when the nitisinone dose was increased from 2 to 4 mg/day, with no significant changes at higher doses.<sup>104</sup>

Despite the dosage to treat AKU is lower than that to treat HT1, it still causes accumulation of Tyr over the recommended threshold of 400–500  $\mu\text{mol/L}$  with a large interindividual variability, and long-term monitoring of plasma Tyr levels is needed because the metabolic fate of this is largely unknown in AKU.<sup>13,24,25,102–104</sup> It is postulated that treatment with nitisinone should be started as early as possible before renal functions decline and other potentially fatal acute complications develop.<sup>99,100</sup>

## Nitisinone and neuroblastoma

Neuroblastoma, the most common solid neoplasm of childhood outside of the central nervous system, is a neural crest-derived neoplasm. The overall outcome is excellent for children with localized disease. Unfortunately, two-third of the children at the diagnosis have widespread disease with poor outcome, despite all treatment modalities.<sup>105,106</sup> As the tumor has a highly active Tyr pathway which produces large amounts of homovanilic acid, vanilmandelic acid (VMA), metanephrines, and other catecholamines, proximal blockade of this pathway is assumed to raise intracellular Tyr, alter the distribution of intracellular catecholamines, and facilitate the differentiation or cytotoxicity of neuroblastoma cells. Interestingly, nitisinone (0.8 mg/kg/day), when used as a single agent, demonstrated antitumor activity sustained for 10 weeks in a child with recurrent stage 4 metastatic neuroblastoma.<sup>107</sup> At recurrence, the drug induced a very good partial response when combined with low-dose cyclophosphamide and doxorubicin. This response was sustained for 18 months till he succumbed, during which he was pain-free, attended school, and had an excellent quality of life. The authors suggested that nitisinone may have a role to play in the treatment of other neural crest-derived tumors, which have highly active Tyr metabolic pathways.

## Adverse effects of nitisinone

Nitisinone is a well-tolerated drug and the first adverse effect reported was the observation of development of corneal lesions in rats treated with nitisinone, which also resulted in discovery of the drug for treatment of HT1. Hypertyrosinemia is likely to be one of the causes of these deposits and can be prevented by the restriction of Tyr in the diet.<sup>3</sup> Nitisinone biochemically switches the enzymatic defect from HT1 to

tyrosinemia type 3, inducing elevated Tyr concentrations up to 1,500 mmol/L (normal, 40–90 mmol/L) when dietary treatment is not provided.<sup>9</sup> Transient ocular symptoms such as irritation, corneal erosion, and photophobia have been reported in patients who are poorly compliant with the diet.<sup>108,109</sup> However, a longitudinal study demonstrated no development of these opacities despite high plasma Tyr concentrations in some cases.<sup>110</sup> There seems to be no exact relationship between the plasma Tyr levels and occurrence of the ocular symptoms, and it is difficult to set up a level for Tyr concentration that might be related to corneal lesions.<sup>110,111</sup> Some researchers consider predisposition to ocular toxicity existing independent of the peak plasma Tyr concentration and the differences in Tyr concentrations in different body pools.<sup>18,24</sup> In the treatment of HT1 with nitisinone, it is recommended that the serum Tyr be kept below 400  $\mu\text{mol/L}$ , by using a Tyr-restricted low protein diet.<sup>45,46,48</sup> Nitisinone, even with the lowest dose, 1 mg daily, led to increase in Tyr levels above this limit in AKU patients.<sup>24</sup> Two AKU patients have been reported to develop reversible keratopathy due to nitisinone therapy.<sup>24,112</sup>

With regard to the effects on the central nervous system, nitisinone is reported to have low blood-to-brain permeability in rats, and based on the results of the studies on the cerebrospinal fluid concentrations of nitisinone after its administration to patients with HT1, its blood-to-brain permeability is considered low in humans too.<sup>112</sup> In the NTBC clinical study in patients with HT1, the following events were reported: convulsions (3 of 291 subjects), hyperkinesia (2 of 291 subjects), headache (1 of 291 subjects), hypokinesia (1 of 291 subjects), and somnolence (1 of 291 subjects).<sup>113</sup> However, it is not known if nitisinone has direct effects on these events. HT1 patients treated with nitisinone are found to be at risk of developing impaired cognitive function and a total intelligence quotient (IQ) score below the average despite a protein-restricted diet.<sup>114,115</sup> They were found to have 20 IQ points lower when compared to their unaffected siblings.<sup>114</sup> The lower IQ may be an unwanted side effect of treatment. In support, a significant decrease in IQ was noted in a subset of patients in whom IQ was regularly tested.<sup>114</sup> Most likely, nitisinone affects cognitive function indirectly, by inducing profoundly elevated plasma Tyr levels. In addition, attention deficit; decreased ability to verbal reasoning, comprehension, and verbal expression; and school difficulties are described in HT1 patients treated with nitisinone.<sup>116</sup> This finding is thought to be connected with the variation of the plasma Tyr level, and therapeutic trials to stabilize the Tyr level could alleviate the difficulties in focusing attention. Despite these

neurological findings in HT1 patients, none of the patients with AKU developed neurological complications after being treated with nitisinone.<sup>13,24,25,102–104</sup>

Still, all patients taking nitisinone should adhere to a phenylalanine- and Tyr-restricted diet to minimize the tyrosinemia produced by the compound.

Mild gastrointestinal system complaints (diarrhea, enanthema, gastroenteritis), transient thrombocytopenia, and leucopenia have also been reported but this has rarely necessitated discontinuation of therapy.<sup>56</sup> There was no bleeding or infection because of the low platelet and leucocyte counts.

The potential of nitisinone for undesired pharmacological effects and pharmacodynamic interactions with other medicines is still unknown, as the general receptor binding profile of nitisinone has not yet been established.<sup>9</sup>

Three successful pregnancies carried to term by three unrelated HT1 patients maintained with nitisinone have been reported till now. All pregnancies were uneventful and there was no evidence of NTBC-induced harm to the developing fetus.<sup>118–120</sup>

## Conclusion

Nitisinone is the licensed, well-tolerated treatment for HT1 based on the inhibition of 4-HPPD in the Tyr degradation pathway. The drug effectively prevents acute hepatic insufficiency, neurologic crises and cardiomyopathy, improves liver functions, but cirrhosis with high grade dysplasia or hepatocarcinoma still develops, especially in some late-treated patients. Furthermore, normal liver function can be achieved in children with HT1 detected by newborn screening and early treated within a month of birth. The combination of neonatal screening plus early treatment is believed to be the best medical treatment of choice for HT1. Inhibition of the Tyr degradation pathway at 4-HPPD level also strongly increases the hope for applying the treatment of other diseases in this pathway like AKU.

## Disclosure

The authors report no conflicts of interest in this work.

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