RNAi therapy with givosiran significantly reduces attack rates in acute intermittent porphyria

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Acute hepatic porphyria (AHP) is a group of inherited metabolic disorders that affect hepatic heme biosynthesis. They are associated with attacks of neurovisceral manifestations that can be life threatening and constitute what is considered an acute porphyria attack. Until recently, the sole specific treatment for acute porphyria attacks consisted of the intravenous administration of hemin. Although attacks are often sporadic, some patients develop recurrent acute attacks, with devastating effects on quality of life. Liver transplantation has historically been the sole curative treatment option. The clinical manifestations of AHP are attributed to the accumulation of the heme precursor 5-aminolevulinic acid (ALA) and porphobilinogen (PBG). Advances in molecular engineering have provided new therapeutic possibilities for modifying the heme synthetic pathway. We reviewed the background and current status of AHP treatment using liver-directed small interfering RNA targeting ALAS1. The therapeutic aim was to normalize the levels of ALAS1, which is highly upregulated during acute porphyria attacks. Givosiran is now an approved drug for use in adults and adolescents aged 12 years and older. The results of clinical trials have shown that givosiran treatment leads to a rapid and sustained reduction of ALAS1 mRNA, decreased heme precursor levels, and a decreased rate of acute attacks compared with placebo. The clinical trials (phases I, II, and III) were all randomized and placebo controlled. Many patients enrolled in the initial clinical trials have continued treatment in open label extension and extended/compassionate-use programs in countries where givosiran is not yet commercially available.

Keywords: 5-aminolevulinic acid, porphobilinogen, acute hepatic porphyrias, ALAS1 (ubiquitous 5-aminolevulinic acid synthase), givosiran, heme precursors, siRNA-ALAS1 (small interfering RNA [siRNA] targeting hepatic ALAS1 mRNA)

Introduction

Porphyrias are rare inherited metabolic diseases caused by a deficiency in one of the eight enzymes involved in the heme biosynthetic pathway (Fig. 1). Deficient enzymes lead to the accumulation of neurotoxic heme precursors, causing neurovisceral symptoms in acute porphyrias, or phototoxic heme precursors causing photosensitivity in cutaneous porphyrias. The underlying genetic defect of each porphyria often has varying penetrance, and the phenotype is further modified by both genetic and environmental factors. The pattern of inheritance varies and includes autosomal dominant, recessive, and X-linked modes [1, 2].

Depending on whether the genetic defect mainly affects erythroid or hepatic heme biosynthesis, porphyrias can be classified as erythropoietic or hepatic.

Heme biosynthesis starts in the mitochondrial matrix with the condensation of succinyl-CoA and glycine under the enzymatic control of 5-aminolevulinic acid synthase (ALAS) (EC 2.3.1.37) to form 5-aminolevulinic acid (ALA) (Fig. 1). There are two different ALAS: one is expressed ubiquitously and encoded on chromosome 3 (3p21.1), and the other is expressed in erythroid cell lines and encoded on the X chromosome (Xp11.21). The synthase isoforms are referred to as ALAS1
Fig. 1 Heme biosynthesis. The first catalytic step of heme biosynthesis mediated by 5-aminolevulinic acid synthase (ALAS) (EC 2.3.1.37) takes place in the mitochondrial matrix with the condensation of succinyl CoA and glycine to build 5-aminolevulinic acid (ALA). The subsequent four intermediate steps take place in the cytosol. Two molecules of ALA are combined to form porphobilinogen (PBG), a reaction mediated by the 5-aminolevulinic acid dehydratase (ALAD) (EC 4.2.1.24), the second enzyme in the pathway. Four PBG molecules are then merged to form hydroxymethylbilane by the third enzyme in the pathway, hydroxymethylbilane synthase (HMBS) (EC 4.3.1.8), referred to in this paper as porphobilinogen deaminase (PBGD). Hydroxymethylbilane is enzymatically converted to a cyclic porphyrin ring, uroporphyrinogen III, which, after a series of decarboxylation, forms the tetrapyrrole coproporphyrinogen III. The next three synthetic steps take place inside the mitochondrion: the oxidative decarboxylation reaction mediated by coproporphyrinogen oxidase (CPOX) (EC 1.1.3.37), the removal of six hydrogen atoms by protoporphyrinogen oxidase (PPOX) (EC 1.3.3.4) to form protoporphyrin IX, and the incorporation of ferrous iron into protoporphyrin IX by ferrochelatase (FECH) (EC 4.99.1.1) resulting in the heme molecule, ferrous protoporphyrin IX. Heme biosynthesis involves several molecular transport mechanisms of heme intermediates and heme between the mitochondria membrane and the cytosol [2, 101]. The four acute hepatic porphyrias are shown in the figure.

and ALAS2 and have different regulatory mechanisms, although both forms require pyridoxal 5-phosphate (PLP) as a cofactor [2, 3].

Heme is the prosthetic group of a wide range of essential hemoproteins. Each cell synthesizes the amount of heme to supply its own metabolic requirement, including that of mitochondrial cytochromes. Erythropoietic cells account for 85% of the total body heme production, mainly for hemoglobin synthesis, and approximately 15% of heme production occurs in liver parenchymal cells for incorporation into heme-containing enzymes, the major one being the microsomal P-450 (66%), followed by catalase (16%), and tryptophan (Trp) 2,3-dioxygenase (TDO) (1.4%) [4, 5].

Acute hepatic porphyrias

The three acute hepatic porphyrias (AHPs)—acute intermittent porphyria (AIP, MIM 176000), hereditary coproporphyria (HCP, MIM 121300), and variegate porphyria (VP, MIM 176200)—are inherited in an autosomal dominant manner. Their clinical presentation is characterized by neurovisceral symptoms occurring in episodes referred to as acute attacks. AIP has solely neurovisceral manifestations, whereas VP and HCP are mixed forms of porphyria presenting with neurovisceral manifestations, cutaneous photosensitivity, or both. A fourth, extremely rare, recessive form of AHP called 5-aminolevulinic acid dehydratase (ALAD) deficiency porphyria (MIM 125270) is different from the other three in its clinical presentation and is not included in this review [6, 7].
There is great heterogeneity in the genetic variants underlying the three autosomal dominant AHP, the variants causing subnormal enzymatic activity of the enzymes involved: HMBS (porphobilinogen deaminase [PBGD]), CPOX, and PPOX. There are more than 527 variants reported in the HMBS gene (AIP): 93 variants have been reported in the CPOX gene (HCP) and 209 in the PPOX gene (VP) (HGMD v.2021.2: www.hgmd.org, 9 Sept 2021).

AIP—the most common AHP—has an estimated prevalence of one in ~2000 Caucasians, but only a small fraction of the HMBS-heterozygote carrier population will experience an acute attack [1, 8, 9]. However, the penetrance of AIP is reported to be much higher in families of individuals with manifest AIP disease—~20% and up to 50% in some observations [10, 11]. A prospective incidence study of porphyrias in Europe [12] showed that the incidence of AIP was similar in all European countries (0.12 per year per million), except for Sweden, which has a fourfold increase in AIP incidence due to a genetic founder effect originating in northern Sweden [13]. This founder HMBS variant c.593G>A, present in 68% of Swedish AIP families, contributes to the high prevalence of AIP of 100 per million [13].

VP, which is less common worldwide than AIP, has a gene prevalence of approximately 3:1000 among the Caucasian South African population due to a genetic founder effect [14]. In Finland, VP has almost the same prevalence as AIP (1.9:100,000) [15]. HCP is the less common of the autosomal dominant AHP, and its prevalence has not yet been the subject of extensive studies [16].

**ALAS1 regulates hepatic heme biosynthesis**

The onset of AHP symptoms rarely occurs before puberty, and women of fertile age are more stricken than men—implying a special susceptibility of ALAS1 genomic regulation to female reproductive hormones [17]. AHP is characterized by acute neurovisceral and potentially life-threatening attacks precipitated by endogenous or environmental factors, such as reproductive hormones, fasting, stress, infections, or certain porphyrinogenic drugs and alcohol, which are factors that increase the requirements of hepatic heme biosynthesis [6, 18]. Hepatic heme demand is highly variable, and the ALAS1 gene is tightly regulated in a heme-dependent manner at the transcriptional, translational, and posttranslational levels [19].

In addition to heme-dependent regulation, the ALAS1 gene contains nuclear receptors such as the constitutive active receptor (CAR) and pregnane xenobiotic receptor (PXR), which can enhance ALAS1 transcription by interacting with xenobiotics (lipophilic drugs, other chemical substances) and steroid hormones [17, 20]. Nutritional factors regulate ALAS1 transcription through the peroxisomal proliferator-activated cofactor 1α (PGC-1α), which constitutes an important link between nutritional status and heme biosynthesis and AHP [21]. Several proteases and peptidases have been reported to play important roles in ALAS1 protein quality control within the mitochondrial matrix. To date, two proteases, LONP1 and ClpXP, have been reported to function as ATP-dependent proteases in the mitochondrial matrix of human cells [22].

**AHP pathogenesis**

Efforts to identify the neurotoxic agents in AHP have resulted in several hypotheses [23]. One explanatory model postulated that the acute attack is caused by a state of neuronal heme deficiency; this hypothesis was abandoned after non-AHP individuals developed acute porphyria attacks shortly after receiving liver transplants from AHP patients [24]. ALA has long been recognized as a putative neurotoxic factor [25] and is the common metabolite/heme precursor increased in all AHP—including the rare, recessive ALAD-deficient porphyria, which is characterized by severe neurological manifestations [26]. Inhibition of ALAD enzyme activity causes an increase in ALA in the rare metabolic disorder tyrosinemia type 1 and in lead poisoning, both of which have neurological manifestations that can resemble an acute porphyria attack [27, 28]. The position that ALA is the sole neurotoxic agent is somewhat contradictory to the fact that individuals with chronically elevated excretion of ALA do not always exhibit signs of neurotoxicity and that the concentration of ALA during acute attacks does not always correlate with the degree of neurological disability. Furthermore, previous studies by Mustajoki et al. showed no direct harm from increased ALA levels in the blood [29]. Assuming that ALA, like other amino acids, can cross the placental barrier, children of pregnant AHP women with acute attacks would show signs of neurological affectation, which has not been the case. Thus, the case for ALA being the neurotoxic agent in acute porphyria attack requires further investigation.
**Acute porphyria attacks**

Despite their autosomal dominant inheritance pattern, AHP have low phenotype penetrance. For AIP, there is a significant prevalence of deleterious AIP variants; however, the disease penetrance is comparatively very low, affecting 1–2 individuals in 100,000 [8, 12, 30]. In AIP, the residual enzyme activity of PBGD is typically reduced to approximately 50% of normal levels [31]. Residual activity is usually sufficient to meet physiological heme demands, but exogenous and/or endogenous factors can accelerate hepatic heme biosynthesis by inducing ALAS1. Under conditions of strong hepatic ALAS1 induction, the downstream deficient catalytic step becomes overloaded by its substrate, porphobilinogen (PBG). Consequently, PBG and the substrate of the previous enzyme, ALA, accumulate [32]. These metabolites are the biochemical markers of disease activity.

**Acute porphyria attacks: Clinical presentation**

The clinical manifestations of AHP are mainly neurological, with symptoms deriving from the autonomic, peripheral, and central nervous systems. Variations in clinical manifestations can cause diagnostic delay, and AHP can remain unrecognized or misdiagnosed as other medical or surgical conditions. The most common symptoms are severe abdominal pain without signs of peritoneal irritation (seen in 90% of patients), nausea, vomiting, abdominal distention, and constipation. Non-specific symptoms such as fatigue, malaise, and headaches often accompany acute attacks.

Sensory neuropathy with muscle pain is usually present, as is motor neuropathy, which can lead to paralysis. Dysregulation of the autonomous nervous system during an attack can lead to hypertension, tachycardia, respiratory dysfunction, bladder paresis, and severe arrhythmias, which in the untreated attack cause significant morbidity and mortality [7, 33, 34].

Hyponatremia is often present due to sodium loss, overhydration, hypothalamic involvement, or a combination of these conditions. Seizures are manifestations of severe attacks caused by electrolyte disturbances or central nervous system affection by neurotoxic heme precursors. Autonomous and central nervous system involvement can lead to posterior reversible encephalopathy syndrome. Psychiatric manifestations are common—ranging from depression, insomnia, and agitation to confusion, delirium, and hallucinosis [35].

It is imperative that each patient be evaluated clinically to exclude other differential diagnoses and conditions that are unrelated to, but could aggrivate, AHP. Measuring the excretion of heme precursors (ALA, PBG, and, in VP and HCP, porphyrins) is necessary for the assessment of an acute attack. ALA and PBG increase are best detected at the early stages of an attack [36].

**Acute porphyria attacks: Treatment**

The identification and elimination of triggering factors, such as porphyrinogenic medications (https://drugs-porphyria.org), are the first steps in treating acute attacks. Hospitalization is usually necessary, as is the administration of symptomatic treatment with adequate analgesia, laxatives, hypnotics, and anxiolytics when necessary. Specimens should be collected as soon as possible for measuring heme precursor excretion. Carbohydrate administration, aimed at increasing intracellular energy availability, can be initiated early in acute attacks. If intravenous carbohydrate solutions are administered, sodium levels must be carefully monitored, as glucose infusion may aggravate hyponatremia. Carbohydrate loading can ameliorate symptoms and may suffice as a treatment for mild attacks [36, 37].

Intravenous administration of hemin should be considered for severe acute porphyria attacks. Hemin restores heme deficiency and downregulates heme biosynthesis in hepatocytes [7, 33]. There are two human hemin therapeutics: one is a lyophilized human hemin (Panhematin®), mainly used in the USA [38, 39], and the other is a stabilized complex with arginine (Normosang®) [40], which is used in Europe and several other continents [41]. Consensus favors doses of 3–4 mg/kg body weight/day (a maximum of 250 mg heme arginate or 313 mg Panhematin®), administered as a single dose into a large vein. The treatment is given for 2–4 consecutive days depending on the clinical response and severity of the attack. The use of hemin has not shown any negative effect on pregnancy and no adverse effects on the fetus and/or neonate [42].

**AHPs: Clinical course and disease patterns**

Individuals with pathogenic variants in AHP genes can have different clinical disease patterns. A
majority can be described as latent, in that the individuals never experience any AHP-related symptoms and have normal excretion of heme precursors. Some individuals present with a pattern of elevated excretion of heme precursors while lacking clinical manifestations of AHP. These individuals have been described using the term asymptomatic high excreter (ASHE), and they may or may not have experienced AHP attacks or symptoms [43]. Individuals with persistently high excretion of heme precursors and AHP-related symptoms, not necessarily requiring specific treatment, are referred to as chronic high excreters (CHE). It should be noted that the levels of ALA and PBG in urine after an acute attack can remain elevated for a longer period of time [43, 44].

Notably, the urine PBG/ALA excretion ratio has been shown to be approximately 2 in patients with a high excretion pattern (ASHE/CHE) compared to 0.3 in biochemically latent patients and healthy controls [44, 45].

AHP patient phenotypes can also be categorized by the frequency of AHP attacks. A sporadic pattern of attacks characterizes some individuals who may have fewer than four attacks during a year or have experienced only a handful of attacks during their lifetime. A small proportion of AHP patients present with what can be described as a pattern of recurrent acute attacks [43], defined as more than four acute attacks per year.

In mixed porphyrias, cutaneous symptoms may be the predominant or sole manifestation, particularly in the case of VP [12, 14, 15, 46]. Cutaneous manifestations are not part of the AIP phenotype, except in cases with end-stage renal disease, when the inability to excrete excess PBG causes accumulation of photoreactive uroporphyrinogen I [47].

**AHPs: Late complications**

Chronic neuropathy: Neuronal motor involvement during acute attacks may become chronic, with paresis or decreased motor strength. Permanent sensory damage, such as chronic paresthesia and sensory loss, is also a possible complication in patients with AHP and chronic disease. The neuropathy symptoms are largely irreversible, and the treatment options are limited [35, 48].

Chronic kidney disease: Patients with AHP have an increased risk of developing chronic kidney disease [47, 49, 50]. The pathophysiology resembles chronic tubulointerstitial nephropathy or focal cortical atrophy with no glomerular lesions [50, 51]. A study including a large cohort of AIP patients showed that approximately 60% of symptomatic AIP patients developed chronic kidney disease, with a decline in the glomerular filtration rate of ∼1 ml/min per 1.73 m² annually [51]. A retrospective French study was done on 10 AIP patients with acute porphyria symptoms and end stage renal disease (ESRD) who were submitted to kidney transplantation. Following the procedure, disease activity was significantly reduced, including secondary skin lesions. This study provides evidence that kidney transplantation should be considered as a treatment option in afflicted AIP patients with ESRD [52].

Primary liver cancer: There is an increased risk of primary liver cancer (PLC) in patients with AHP, as has been shown in several published reports mainly from Sweden [12, 54–57]. These studies, predominantly including Swedish individuals with AIP, have provided an evidence base for recommending liver surveillance in AHP. The Swedish cohort includes patients with several pathogenic AIP variants, as well as HCP and VP variants, and thus the high incidence of PLC cannot be solely attributed to the founder mutation c.593G>A (occurring in ∼60% of the Swedish AIP population) [56]. Guidelines for annual radiological liver surveillance over the age of 50 years have been established and implemented in many parts of the world. Early tumor detection is of paramount importance for the outcomes of PLC treatment. Surgical resection is the treatment of choice whenever possible, with radiofrequency ablation, systemic chemotherapy, and arterial chemoembolization constituting secondary treatment options.

**AHPs: Recurrent acute attacks**

The manifestations of the acute attack are, as a rule, resolved after the administration of hemin.
However, sporadic hemin treatment during acute attacks does not prevent relapse in a minority of patients who develop a pattern of recurrent acute attacks. It is estimated that 2.8% of men and 5.3% of women among AIP patients [12] develop recurrent attacks after experiencing their first attack [31, 46]. Recent studies [58, 59] have confirmed female dominance among recurrent patients to be over 80%. In most patients, no specific triggering factor can be identified and there is currently no evidence-based consensus on the treatment of recurrent attacks. Recurrent porphyria attacks have a significant effect on quality of life [60] and there is an unmet need for efficacious, long-acting, and safer therapies to prevent attacks and improve chronic disease manifestations [59].

Prophylactic hemin: Due to the lack of other treatment options, prophylactic treatment with hemin at regular intervals has been the treatment of choice to enable a state of subchronic disease. In prophylactic treatment with hemin, the intervals and doses are individually adjusted. Weekly, biweekly, or monthly intravenous hemin infusions have been used to reduce the frequency of recurrent attacks in severe cases [46, 58]. Yarra et al. in 2019 [61] presented a report of two patients with frequent attacks of AIP, in which weekly prophylactic hemin administration had a beneficial effect on symptoms, heme precursor excretion, health care resources, and quality of life. During the 11-month observation period, no attacks were registered for one patient, while the other experienced a 75% decrease in attack frequency. A UK study of 22 patients with severe recurrent attacks reported hemin infusions 1–8 times monthly. Reduction in pain severity was achieved in 67% of patients; nevertheless, 55% continued to be repeatedly admitted to hospital due to chronic pain, acute attacks despite hemin, and tachyphylaxis [62].

The greatest challenge with prophylactic hemin is the tapering off or withdrawing of treatment [62]. There are significant, well-documented long-term complications with repeated hemin treatment: damage of peripheral vessels, infection of the required indwelling venous ports, and hepatic iron overload [34, 37]. Studies performed on explanted livers from AIP patients who had received hemin prophylactic treatment revealed inflammatory hepatic disease, upregulated ALAS1, and heme oxygenase activity. Iron overload secondary to hemin occurs equally in all patients and is probably regulated by other factors [63, 64].

GnRH analogues: The consensus that there is a connection between hormonal factors and AHP relies on the fact that symptoms rarely appear before puberty and are unusual in elderly AHP individuals. When comparing premenopausal women with men, the reported frequency of acute attacks is significantly higher, ranging from 2:1 [65] to 5:1 [12] to 6:1 [66]. These observations, and the fact that in premenopausal women acute attacks are often correlated with menstruation and ovulation, highlight the impact of sex hormones on heme biosynthesis. The decision to treat with GnRH agonists is difficult and should be balanced against the negative effects of the treatment. Studies in selected patient cohorts show varying results and rarely show a clear reduction in attacks without negative effects on women’s health [67, 68].

Liver transplantation: Liver transplantation is a curative treatment option for patients with recurrent attacks of AHP. A recent report [69] reviewed the outcome—defined as survival, retransplantation, and neurological and renal impairment—of 38 European AIP cases (89% were female) who underwent liver transplantation between 2002 and 2019. The median age at transplantation was 37 years, and the 1-year and 5-year overall survival rates were 92% and 82%, respectively, an outcome comparable to transplantation results due to other metabolic diseases. All patients were rendered attack free after transplantation, apart from one who received an auxiliary graft, with native liver tissue still in place. This study concluded that when other treatment options have been exhausted, patients with long-standing AHP and recurrent attacks should be evaluated for liver transplantation [69]. Patients with end-stage renal disease should undergo combined liver and kidney transplantation [70].

Small interfering ribonucleic acid: A new therapeutic strategy

The 2006 Nobel Prize in Physiology or Medicine was awarded to Andrew Fire and Craig Mello for their discovery of RNA interference—gene silencing by double-stranded RNA [71]. RNA interference is a fundamental mechanism for controlling the flow of genetic information in cells [71]. This endogenous mechanism, enabling degradation of a specific mRNA, has enabled novel pharmacological approaches to treat diseases and target specific cellular functions (Fig. 2) [72, 73].
Fig. 2 Liver-directed delivery. Givosiran is a double-stranded small interfering ribonucleic acid (siRNA) comprised of a 21-nucleotide sense strand and a 23-nucleotide antisense strand, with the sense strand conjugated to a triantennary N-acetylgalactosamine (GalNAc), which enables targeted delivery to the liver via uptake by the asialoglycoprotein receptors (ASGPR) [74, 76] (Alnylam Pharmaceuticals Inc). ASGPR, primarily expressed on the surface of hepatocytes, specifically bind to the GalNAc ligand triggering receptor-mediated endocytosis of the ligand-receptor complex followed by the release of the givosiran siRNA into the hepatocyte. Upon delivery to the liver, givosiran is incorporated into the RNA-induced silencing complex and silence ALAS1 mRNA, thereby preventing the synthesis of the corresponding ALAS1 protein [80]. RNA interference (RNAi) mechanism: RNA interference (RNAi) involves the pairing of a short RNA sequence with a 21-nucleotide endogenous mRNA target. The short, double-stranded RNAs are cut into fragments of about 20 bp and are loaded into a complex called the RNA-induced silencing complex (RISC), which comprises several proteins, including ribonucleases from the Argonaute protein family. One strand, the passenger strand, is discarded, and the guide strand is paired to a complementary mRNA sequence via the RISC complex. Upon binding, silencing of the mRNA is induced via RNase-mediated degradation or translational repression [73].

Because RNAs are relatively unstable, the delivery technology best suited to the target is a significant part of the procedure for developing a new RNAi therapeutic.

Proof of concept: Small interfering ribonucleic acid silencing of hepatic ALAS1 for the treatment of AHP

Givosiran is an ALAS1 directed small interfering ribonucleic acid (siRNA) covalently linked to a ligand containing three N-acetylgalactosamine (GalNAc) residues to enable delivery of the siRNA to hepatocytes, aimed at downregulating ALAS1 and reducing the toxic heme intermediates, mainly ALA and PBG. The first experimental trial by Yasuda et al. in 2014 [74] showed that a single prophylactic intravenous dose of ALAS1 siRNA formulated into lipid nanoparticles prevented phenobarbital-induced acute attack in the AIP mouse model for a period of 2 weeks [75]. Injection of ALAS1 siRNA during an induced acute attack significantly decreased plasma ALA and PBG levels within 8 h, more rapidly and effectively than a single hemin infusion. ALAS1 siRNA was well tolerated, and a therapeutic dose did not cause hepatic heme deficiency. Developmental studies by Chan et al. from 2015 [76] investigated the effect of the modified therapeutic drug ALAS1-RNAi (ALN-AS1) conjugated to GalNAc (GalNAc–siRNA) in animals; the conjugation enabled subcutaneous administration and liver-specific uptake (Fig. 2). Studies in these animal models confirmed the efficacy of givosiran in preventing porphyria attacks induced by xenobiotics by avoiding ALAS1 upregulation. Givosiran treatment was shown to be more efficient than hemin treatment. These studies provided the proof-of-concept for the clinical development of givosiran for the prevention and treatment of acute attacks in AHP [63, 74, 76] and increased our understanding of the disease pathophysiology.

Technology to isolate multivesicular bodies, for example, exosomes containing released RNAs, and therapeutic siRNA from plasma and urine [77], made it possible to study changes in hepatic ALAS1 activity in the AIP mouse model, nonhuman primates, healthy volunteers, and AHP patients by measuring ALAS1 mRNA in exosomes isolated from serum or urine. Across these studies, there was a strong correlation between serum, urine, and liver ALAS1 mRNA levels following subcutaneous dosing with givosiran. A study in human serum and urine samples obtained from healthy volunteers and patients with AIP demonstrated differences in
the habitual state of ALAS1 mRNA levels between these two groups (Fig. 3) [76, 78].

Givosiran clinical trials

Before the clinical trials, a prospective observational study of patients with recurrent AHP attacks was initiated with the aim of characterizing the natural history and clinical management of this rare population. A total of 112 patients were enrolled from the USA (44%) and Europe (56%), 104 of whom had AIP, three of whom had HCP, and five of whom had VP [59]. The patients were predominantly female, with a median age of 38 years (range, 19–70 years). Recurrency was determined as either having more than three attacks 12 months prior to inclusion or prophylactic treatment with hemin. Several important data and characteristics of the recurrent AHP population were uncovered by this study, revealing the need for an efficacious and safe therapy to prevent attacks and improve chronic disease manifestations in patients with recurrent AHP [59].

Phase 1 trial of an RNA interference therapy for AIP and open label extension study

This was the first clinical study of givosiran and included two different patient cohorts of CHE (aged 18–65 years) (part A and B, n = 23), defined as having no acute attack in the past 6 months and having a stable urinary excretion level of PBG >4 mmol/mol creatinine, and recurrent AIP (part C, n = 17), defined as having had more than two attacks within 6 months prior to study run-in or receiving prophylactic hemin treatment [78]. Parts A and B were single blind, and patients were randomly assigned in a 3:1 ratio to either givosiran or a placebo. Patients in part A had a single dose of givosiran in one of five ascending dose cohorts, and in part B, patients received once-monthly injections in one of two different dose cohorts. The study protocol was initially designed to follow subjects 42 days (part A) or 70 days (part B) postdose but the follow-up had to be continued for 1.5 years until either ALA and/or PBG levels returned to 80% of baseline. In part C, patients were followed during a run-in period (4–24 weeks) and were required to have had at least one attack before randomization. The patients were then assigned, in a double-blind 3:1 ratio, to receive injections of one of two doses of givosiran (2.5 or 5.0 mg/kg) or placebo once monthly (total of four injections) or once quarterly (total of two injections) during a 12-week period. The patients were followed up for an additional 12 weeks after the last injection. The primary endpoints were safety and tolerability, pharmacokinetics (PK), and the pharmacodynamics (PD) of givosiran. Exploratory endpoints were the rates of porphyria attacks and hemin use. After the treatment period, patients continued in the open label extension (OLE) part of the study, initially and for a short period, at the same dose cohort as in part C, but after a protocol amendment, all patients were dosed at 2.5 mg/kg once monthly [79]. The total study period of 48 months has recently been finalized.

Safety and tolerability

This clinical study was published by Sardh et al. [78]. The most common adverse events (AEs) across trial parts A through C were abdominal pain, nausea, diarrhea, and nasopharyngitis. In parts A and B, all AEs were mild to moderate in severity. None of the patients discontinued the trial regimen. In part A, a serious AE of abdominal pain occurred in two patients who received givosiran. In part B, there was a serious AE of spontaneous abortion in a patient at 7 weeks after conception (90 days after the last givosiran dose). The safety data for Part C are presented in Table 1. Three patients treated with givosiran reported three serious AEs, none of which were assessed as study drug related; in two patients, the AEs resolved with treatment. Fatal hemorrhagic pancreatitis occurred in a single patient. This patient had a complex medical history and a clinical course complicated by an acute pulmonary thromboembolism that resulted in right heart failure [78].
Table 1. Safety outcome of the givosiran trials

| Treatment period | Part C phase 1/2 | Open label extension (OLE) | Phase 3 (ENVISION) | OLE |
|------------------|------------------|---------------------------|--------------------|-----|
|                   | 6 months\(^a\) [78] | Median 24.7 months\(^b\) [81] | 6 months\(^b\) [82] | Median 11.22 months\(^d\) [97] |
| Placebo          | Givosiran        | Placebo                   | Givosiran          | Givosiran |
| (N = 4)          | (N = 13)         | (N = 15)                  | (N = 46)           | (N = 94) |
| Any adverse event | 4 (100%)         | ND                        | 37 (80%)           | 88 (94%) |
|                  | 13 (100%)        | ND                        | 43 (90%)           | ND |
| Any severe adverse event | ND | ND | 5 (11%) | 8 (17%) |
| Any serious adverse event | 0 | 3 (23%) | 4 (9%) | 10 (21%) |
| Nausea           | 1 (25%)          | >3 patients               | 5 (11%)            | >10% |
| Fatigue          | ND               | >3 patients               | 2 (4%)             | >10% |
| Injection site reaction | 0 | 3 (23%) | 7 (47%) | 0 |
| Elevated aminotransferases (ALT) | ND | ND | ND | 1 (2%) |
| Increased serum creatinine or decreased eGFR | ND | ND | ND | 2 (4%) |
| Increased international normalized ratio | ND | ND | >3 patients | 7 (15%) |

\(^a\)Monthly dosing four injections of either 2.5 mg/kg or 5 mg/kg, quarterly dosing two injections of either 2.5 mg/kg or 5 mg/kg.
\(^b\)Monthly dosing at 2.5 mg/kg, seven injections.
\(^c\)Range not reported in abstract.
\(^d\)Range 1.8–19.5 months
\(^e\)Reported in six patients; one case of anaphylaxis; one case of upper extremity venous thrombosis.
\(^f\)Alanine aminotransferase (ALT) elevation >3 × ULN occurred in 10 patients (10.6%), of whom three (3.2%) had ALT >5 × ULN. Two patients had ALT >5 × ULN—one on 2.5 mg/kg had dose interruption with resumption at 1.25 mg/kg and one on 1.25 mg/kg had resolution during ongoing dosing. Seven patients had ALT >3 × ULN; six resolved during ongoing dosing and one with transient interruption.

Continuing safety monitoring in the OLE study (Table 1) has reported an additional 10 serious AEs, with one case of anaphylaxis assessed as definitely related to the study drug. One patient had an upper extremity deep venous thrombosis, assessed as unlikely related to the study drug due to prior indwelling central venous catheter and venous damage from chronic hemin usage [79].

**PK and PD**

The PK and PD of givosiran and its active metabolite AS\(\text{N-1}\)\(^3\) givosiran, based on the AIP patients from the phase 1 trial [78], were described in 2020 [80]. In summary, givosiran was rapidly absorbed after subcutaneous administration with peak plasma concentrations after 0.5–5 h followed by elimination with a short half-life of 4–10 h. Givosiran showed a rapid and dose-dependent reduction in urinary ALA and PBG, and a maximum reduction from baseline of approximately 95% was achieved in the recurrent patients with a 2.5 mg/kg once-monthly dose. Increasing the dose to 5.0 mg/kg did not result in any additional reduction over the 2.5 mg/kg dose. The PD effect of givosiran was long lasting in CHE subjects, with substantial reductions in urinary ALA and PBG observed for weeks to months after a single SC dose. This contrasts with the short-lived plasma PK, in which the rapid liver uptake of givosiran and AS\(\text{N-1}\)\(^3\) givosiran results in a short half-life of <10 h. This indicates that the driver of PD is exposure to givosiran and the active metabolite in the liver [80].
The therapeutic effect on hepatic heme biosynthesis

Baseline urinary ALAS1 mRNA levels were higher than normal by a factor of two in patients with chronic high excretion and by a factor of 3–4 in those with recurrent attacks (Fig. 3) [78]. A single dose of givosiran led to rapid and dose-dependent reduction of ALAS1 mRNA with corresponding reductions in urinary ALA and PBG, and the remaining ALAS1 mRNA levels after the highest dose in CHE patients were similar to those in healthy individuals. In part C, among the patients who had recurrent attacks, two once-quarterly injections of givosiran resulted in maximum reductions in ALAS1 mRNA levels of 49% ± 3% in the 2.5 mg/kg cohort and 53% ± 7% in the 5.0 mg/kg cohort. Among the patients who received four once-monthly injections of givosiran, the maximum reductions in ALAS1 mRNA level from baseline were 67% ± 3% in the 2.5 mg/kg cohort and 74% ± 6% in the 5.0 mg/kg cohort. Residual ALAS1 mRNA levels after once-monthly injections of givosiran were at or above the levels observed in healthy individuals. The reduction in ALAS1 mRNA levels with givosiran was sustained until the end of the trial, and there was no evidence of ALAS1 mRNA returning to baseline levels in the two cohorts that received once-monthly injections [78].

Clinical efficacy

Clinical effect on recurrency and need for rescue treatment (hemin) is summarized in Fig. 4. Once-monthly injections led to greater, sustained reductions in ALA and PBG levels compared to once-quarterly injections, which showed more fluctuations. Normalization of ALA and PBG levels occurred in both once-monthly dose cohorts [78]. Continuing dosing at 2.5 mg/kg monthly in the OLE has been reported up to a median time of 24.7 months [81], showing a robust and sustained lowering of ALA and PBG from baseline by >85% at 18 months. The clinical effect is potentially enhanced, showing mean reductions of >90% in annualized attack rate (AAR) and annualized hemin doses, relative to the phase 1 run-in period [81].

The results from part C defined the dose of 2.5 mg/kg monthly to move forward to the pivotal phase 3 study of givosiran.

Phase 3 trial of RNAi therapeutic givosiran for AIP (ENVISION) and OLE

This was a double-blind, placebo-controlled phase 3 trial, in which 94 patients (89 AIP, 1 HCP, 2 VP, 2 AHP without identified mutations) with recurrent acute attacks were randomized 1:1 to receive either givosiran (2.5 mg/kg of body weight, 48 patients) or placebo (46 patients) monthly for 6 months [82]. The inclusion criteria were an age of at least 12 years, a diagnosis of AHP, an elevated level of urinary ALA or PBG (≥4 times the upper limit of the normal range), and either a confirmed pathogenic mutation associated with AHP or biochemical and clinical criteria consistent with a diagnosis of AHP if such a mutation was not identified in genetic testing. Patients were required to have documentation of at least two composite porphyria attacks—defined as attacks requiring hospitalization, urgent health care, or hemin administration—within 6 months before randomization. Patients were also required to discontinue or not initiate prophylactic
hemin during the trial. The primary endpoint was the AAR of composite porphyria attacks. Secondary endpoints were composite AAR in AHP, effect on ALA and PBG levels, annualized number of days of hemin use, daily worst pain, fatigue, nausea, and change from baseline in the Physical Component Summary of the 12-Item Short-Form Health Survey (SF-12). Exploratory endpoints included changes in analgesic use during the intervention and patient experience, as assessed by quality-of-life measures [82]. After the 6-month placebo-controlled study period, patients continued in the OLE with a total study time of 36 months. During the first OLE period, a lower dose of givosiran was introduced to assess safety and efficacy, and patients were randomized to either givosiran 2.5 or 1.25 mg/kg monthly. After the first 12 months, all patients were continued at 2.5 mg/kg. Outcome data were not reported.

Safety and tolerability

Key safety data are summarized in Table 1. The most common givosiran-related AEs were injection site reactions, nausea, and fatigue.

Three serious adverse events (SAE) in givosiran patients were reported as study drug related—one pyrexia, one chronic kidney disease, and one abnormal liver function test of ALT 9.9 times the upper level of normal (ULN). This patient discontinued the study according to the protocol, and the elevation resolved with normal ALT values at 6 months. No deaths occurred [82]. Two chronic kidney disease AEs were considered serious due to elective hospitalization for diagnostic evaluation; renal biopsies were performed and were consistent with the underlying disease of hypertension and porphyria-related nephropathy. There was no indication of immune complex or other primary glomerular renal disorders [82]. Hepatic AEs with ongoing dosing were reported in 17% of patients (Table 1). The majority were serum aminotransferase elevations, all of which were mild or moderate in severity (Table 1) [83] and occurred primarily 3–5 months after the initiation of givosiran.

Ten patients (11%) had renal AEs (Table 1), characterized by increased serum creatinine and/or decreased estimated glomerular filtration rate (eGFR). The majority were mild or moderate in severity, and none led to study discontinuation. Small increases in serum creatinine were observed at months 6 and 12, with mean eGFR being stable over time. A decrease in eGFR has been observed in some patients with pre-existing renal disease [79, 83].

Clinical efficacy

Clinical outcome on primary endpoint and key secondary endpoints is summarized in Table 2. Among the AIP patients, levels of urinary ALA and PBG were significantly lower in the givosiran ALA group than in the placebo group. Reductions were sustained throughout the trial period and the median percent reduction from baseline at 6 months was 86% for urinary ALA levels and 91% for PBG levels [82]. Continued treatment in givosiran/givosiran patients led to a median AAR of 0.58 through month 18. Sustained ALA/PBG lowering was accompanied by sustained reductions in hemin use, and more than half of the placebo/givosiran patients experienced zero days of hemin use [83].

Givosiran effect on hemoproteins

There is little evidence of hepatic heme deficiency in patients with AIP, as exemplified by normal microsomal heme content and cytochrome P450 isozyme activity in a severely affected patient who underwent liver transplantation [63]. Treating AHP patients with ALAS1 siRNA introduces an additional block in the pathway that may further reduce heme cell content and enhance the deficiency of hepatic hemoproteins. In the first preclinical trials by Yasuda et al. in 2014 [74], it was shown in AIP mice that a single intravenous injection of ALAS1 siRNA at a dose of 1 mg/kg did not reduce the heme saturation of TDO nor diminish CYP2E1 activity in liver cells [74]. Recently, it was demonstrated in givosiran-treated AIP patients that the kynunerine–Trp ratio did not change, indicating that the enzyme activities of both TDO and indoleamine 2,3-dioxygenase were uninfluenced by givosiran treatment [84]. In the first clinical trial of AIP patients using repeated doses of givosiran at 2.5 or 5 mg/kg, dosed monthly or quarterly, ALAS1 mRNA levels were not below those found in healthy individuals, although one patient in the OLE undergoing warfarin treatment had an important increase in international normalized ratio (INR) after givosiran was introduced, indicating a possible effect on hepatic drug metabolism [81].

Polypharmacy is common in patients with AHP due to comorbidities such as chronic pain, renal impairment, and hypertension. To ensure that
Table 2. Clinical outcome on primary endpoint and key secondary endpoints compared to placebo of the phase 3 trial

| Phase 3 (ENVISION) | Placebo (N = 46) | Givosiran (N = 48) (%) |
|--------------------|------------------|------------------------|
| Annualized rate of composite porphyria attacksc (mean) | 12.5 | 3.2 (74) |
| Annualized rate of porphyria attacks (median) | 10.7 | 1.0 (90) |
| Annualized no. of days of hemin use (mean) | 29.7 | 6.8 (77) |
| Annualized no. of days of hemin use (median) | 27.6 | 0.0 |
| Percentage of patients reporting no acute attacks | 17.4 | 50.0 |
| Daily worst score for pain in AIPd | | |
| Median change in AUC from baseline | −5.3 | −11.5f |
| Least-squares mean of change in AUC from baseline | −4.2 | −11.1f |
| Daily worst fatigue in AIPd | | |
| Least-squares mean of change in AUC from baseline | −4.0 | 1.5f |
| Least-squares mean of change from baseline at 6 months | 1.4 | 5.4f |

Abbreviations: AIP, acute intermittent porphyria; AUC, area under the curve.

aMonthly dosing at 2.5 mg/kg, seven injections.

bReduction in percentage compared to placebo.

cDefined as hospitalization, urgent health care visits, or intravenous hemin administration at home. For each of the three components, there was a greater reduction in the givosiran group compared to placebo [82].

dScores for pain, fatigue, and nausea were measured on a numerical rating scale from 0 to 10, with a higher score indicating more severe symptoms.

eScores on the Physical Component Summary of the Short-Form Health Survey, version 2 (SF-12), range from 0 to 100 (worst to best functioning). A change from 2 to 5 points represents a clinical meaningful difference.

fStatistical significance in hierarchical testing not met.

givosiran treatment would still provide enough heme to the site of utilization—mainly CYP450—a specific trial was designed to investigate the drug–drug interaction of five specific CYP enzymes that metabolize ∼80% of clinically used drugs [85]. This was an open-label, phase 1 study that investigated the effect of givosiran on the PK of standard CYP enzyme substrates in 10 AIP patients selected from a cohort of CHEs. The mean age was 49 years (range, 39–59 years) with seven women and three men, all Caucasians. Mean baseline urinary ALA was 7.14 mmol/mol Cr (ULN 3.9) and urinary PBG 14.6 mmol/mol creatinine (ULN 1.6). Mean ALAS1 mRNA, as measured in urine, was 2.13 (ratio relative to healthy subjects), consistent with previous results in the CHE population [78, 86].

All participants had normal or intermediate metabolizer phenotypes for CYP2C9, CYP2C19, and CYP2D6. The PK of probe substrates for five major CYP enzymes, midazolam (CYP3A4), caffeine (CYP1A2), losartan (CYP2C9), omeprazole (CYP2C19), and dextromethorphan (CYP2D6) were evaluated before and after a single 2.5 mg/kg dose of givosiran in trial subjects. A 28-day window post givosiran administration enabled the evaluation of maximum ALAS1 mRNA and ALA reduction. The results showed a moderate reduction in CYP1A2 and CYP2D6 activity (≤3.2-fold), a weak reduction in CYP2C19 and CYP3A4 activity (≤1.59-fold), and no effect on CYP2C9 activity. In conclusion, patients on drugs with narrow therapeutic indexes primarily metabolized by CYP2D6 or CYP1A2 may need to be monitored more frequently to determine whether dose adjustment of a concomitant medication is required [86]. The OLE of the phase 1/2 study reported increased INR in more than three patients [81], implying the importance of monitoring when treated with anticoagulant medication.

Cystathionine beta-synthase (CBS) is a heme-dependent enzyme that catalyzes the pyridoxal phosphate (PLP)-dependent condensation of serine and homocysteine to cystathionine. PLP-1 is
also a cofactor for cystathionine $\gamma$-lyase (CGL), an enzyme downstream of CBS, and a cofactor for ALAS1 in hepatic heme synthesis [87–89]. Elevated homocysteine levels in clinically active AIP patients were first described in 2010, implicating heme deficiency or possibly pyridoxine deficiency as a possible cause of hyperhomocysteinemia [90]. AHP patients with recurrent acute attacks have a 3- to 4-fold increase in their ALAS1 activity compared with healthy individuals [78]; in these patients, pyridoxine availability could affect both heme biosynthesis and homocysteine metabolism, resulting in hyperhomocysteinemia. Reports on hyperhomocysteinemia in givosiran-treated patients have recently emerged [90–92]. The etiology behind the observed homocysteine elevation in active/recurrent AHP patients is not fully elucidated but indicates a causative connection between ALAS1 inhibition and hyperhomocysteinemia, suggesting that all patients considered for treatment with givosiran should be closely monitored for homocysteine levels [91].

The therapeutic use of givosiran

Givosiran OLE studies

The collection of safety, tolerability, and efficacy data is ongoing. The OLE studies have confirmed a continued, and even enhanced, efficacy up to 18 months in ENVISION [83] and 24 months in phase 1/2 OLE [81]. The main observed AEs in the long term have been changes in liver function tests that often have recovered after a transient nontreatment period, and in a few cases, withdrawal of treatment. In addition, some patients have shown a decreased glomerular filtration rate but within an acceptable range, as many chronically affected AHP patients have pre-existing renal disease [93]. Injection site reaction has been a more frequent AE, as well as nausea and fatigue. No additional safety issues have been raised during continued dosing. A possible effect on hepatic hemoproteins has been reported by several investigators, primarily the effects on homocysteine metabolism [84, 91, 92]. Hyperhomocysteinemia has been shown to be reversible following pyridoxine supplementation [91]. These data confirm that patients must be carefully monitored for possible effects on hemoproteins and drug metabolism.

Givosiran in clinical practice

The indications from regulators are broad, requiring only a confirmed diagnosis and age. This implies a responsibility for the porphyria community to develop treatment guidelines and define who to treat and for how long. Givosiran entails a high cost, and clear start and stop criteria are important for the respective national healthcare system to ensure that the treatment is provided cost effectively. Administration of givosiran is feasible and only requires subcutaneous injection, and the treatment has important clinical benefits. However, monitoring is demanding, involving several healthcare disciplines to evaluate treatment outcomes and side effects. Givosiran can be administered at home or at an infusion clinic, but due to the risk of anaphylaxis the first doses should be administered at a hospital with intensive care capacity.

Givosiran has not been studied during sporadic attacks, and little is known about its effect on dosage or dosing intervals. From our experience, for patients suffering from a longstanding period of recurrence, chronic pain, and neurological complications, treatment should continue for at least some years before discontinuation. A few recurrent patients who suspended givosiran after a short treatment period were symptom free for approximately 1 year before recurrence resumed.

Porphyrin precursors have been implicated in the development of renal damage [51] and PLC [55, 56]. A large registry study of Swedish AHP patients confirmed a high risk of PLC in AIP patients and identified a strong association with clinical and biochemical disease activity [57]. Charting the effect of givosiran on renal damage and PLC development requires long-term studies.

Hemin is the standard of care for sporadic attacks. An emerging therapy in AIP is the systemic administration of human $HMBS$ mRNA aimed at restoring enzymatic deficiency in hepatocytes. Preclinical trials have shown that human $HMBS$ mRNA may be a promising treatment for sporadic and recurrent diseases [94].

Summary

This review includes a thorough description of the AHP condition and its clinical manifestations, treatment, disease burden, and the impact of givosiran—a new treatment modality. We highlight its benefits in AHP patients suffering from recurrent acute attacks, alleviating their chronic pain, their health care dependency, and the
longstanding undermining of their quality of life [60, 82, 95–98].

We refer to the trajectory of the development of RNA interference from the reports by Nobel Laureates in 1998 [71] to the pharmacological development of ALAS1 siRNA achieved by Yasuda et al. in 2014 [74], Chan et al. in 2015 [76], and Alnylam Pharmaceuticals Inc.

These successfully conducted preclinical studies made it possible to start the first clinical trials in 2015 aiming to evaluate the safety and tolerability of givosiran and determine the PK and PD profiles of asymptomatic AIP patients with chronic high excretion of porphyrin precursors. This patient cohort was defined during enzyme replacement therapy studies in which recombinant human PBGD was investigated as a possible therapy for acute AIP attacks [99]. The validity of this high excroter cohort was maintained by the Porphyria Centre Sweden national registry (Dnr 647–88) for decades through a systematic outpatient clinic and regular biochemical monitoring, allowing for the selection of well-characterized patients for the first clinical trial.

CHE patients were used in the phase 1/2 trial, parts A and B, to evaluate the safety, tolerability, PK, and PD of givosiran. In part C, 17 recurrent AIP patients were included to evaluate clinical efficacy and establish the dose to move forward into the pivotal phase 3 study ENVISION [78, 82]. The PK and PD parameters from the phase 1/2 trial have been reported separately [80].

A multinational prospective study (EXPLORE) was initiated shortly before the start of the phase 1/2 study, including 112 severely afflicted AHP patients from 21 centers in Europe and the US. The aim was to characterize the disease's natural history and current treatment practices to provide updated clinical and biochemical knowledge in a large cohort [59] before the start of the phase 3 trial.

The phase 3 study (ENVISION) was a randomized, double-blind, placebo-controlled study of 94 recurrent AHP patients, mainly AIP, from 36 sites in 18 countries [82]. This study confirmed a significant reduction in the annualized rate of porphyria attacks and better results regarding multiple other disease manifestations compared to a placebo under an acceptable safety profile.

Based on the results from ENVISION, givosiran has recently been approved for the treatment of AHP in adults (US, Brazil, and Canada) and in adults and adolescents aged 12 years and older (European Economic Area, UK, Switzerland, and Japan) (www.fda.gov; www.ema.europa.eu). For many countries, givosiran is not yet commercially available because of different national health care insurance systems. For those countries, Alnylam Pharmaceuticals provides givosiran free of charge through an early access program (https://www.alnylam.com/medical-professional-resources/early-access-program/).

The successful development of givosiran from a scientific hypothesis to a medically approved drug in a relatively short time has been possible through committed collaboration between investigators, research nurses, patient organizations, patients, and a dedicated team at Alnylam Pharmaceuticals Inc. The international porphyria community represented by the European Porphyría Network and the American Porphyrias Consortium has had an important impact on these achievements.

Conflict of interest
The authors report receiving grant support and personal fees, paid to Karolinska Institutet, from Alnylam Pharmaceuticals.

Author contributions
Eliane Sardh: conceptualization; data curation; formal analysis; writing – original draft; writing – review and editing. Pauline Harper: conceptualization; formal analysis; writing – original draft; writing – review and editing.

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