nature medicine

ARTICLES

https://doi.org/10.1038/s41591-018-0199-z

Systemic messenger RNA as an etiological treatment for acute intermittent porphyria

Lei Jiang[1,10], Pedro Berraondo[2,3,4,10], Daniel Jericó[3,5,10], Lin T. Guey[1,10], Ana Sampedro[3,5], Andrea Frassetto[1], Kerry E. Benenato[1], Kristine Burke[1], Eva Santamaría[3,5,6], Manuel Alegre[7,8], Álvaro Pejenaute[9], Mayur Kalariya[1], William Butcher[1], Ji-Sun Park[1], Xuling Zhu[1], Staci Sabnis[1], E. Sathyajith Kumarasinghe[1], Timothy Salerno[1], Matthew Kenney[1], Christine M. Lukacs[1], Matías A. Ávila[3,5,6,11], Paolo G. V. Martini[1,11]* and Antonio Fontanellas[3,5,6,11]*

Acute intermittent porphyria (AIP) results from haploinsufficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway. Patients with AIP have neurovisceral attacks associated with increased hepatic heme demand. Phenobarbital-challenged mice with AIP recapitulate the biochemical and clinical characteristics of patients with AIP, including hepatic overproduction of the potentially neurotoxic porphyrin precursors. Here we show that intravenous administration of human PBGD (hPBGD) mRNA (encoded by the gene *HMBS*) encapsulated in lipid nanoparticles induces dose-dependent protein expression in mouse hepatocytes, rapidly normalizing urine porphyrin precursor excretion in ongoing attacks. Furthermore, hPBGD mRNA protected against mitochondrial dysfunction, hypertension, pain and motor impairment. Repeat dosing in AIP mice showed sustained efficacy and therapeutic improvement without evidence of hepatotoxicity. Finally, multiple administrations to nonhuman primates confirmed safety and translatability. These data provide proof-of-concept for systemic hPBGD mRNA as a potential therapy for AIP.

AIP (MIM: 176000) is an autosomal dominant metabolic disease caused by hepatic deficiency of PBGD (International Union of Biochemistry and Molecular Biology (IUBMB) nomenclature: EC 2.5.1.61), the third enzyme of the heme synthesis pathway[1,2]. In the liver, the first enzyme of this pathway, δ-aminolevulinate synthase 1 (ALAS1), controls the rate-limiting step of heme production in response to metabolic needs[3]. A genetic defect at the *PBGD* locus generates a significant reduction in PBGD activity, which in conjunction with impaired heme-mediated repression of hepatic ALAS1 leads to a marked overproduction and accumulation of the porphyrin precursors δ-aminolevulinic acid (ALA) and porphobilinogen (PBG).

The dominant clinical feature of AIP is acute neurovisceral attacks associated with high production of potentially neurotoxic porphyrin precursors. Symptoms of AIP are heterogeneous and include severe fatigue, abdominal pain, loss of appetite, nausea, vomiting and constipation[4,5]. Patients may also have trouble sleeping, anxiety, depression, confused states, hypertension and tachycardia. Severe neurological complications also occur, and death may result from respiratory and bulbar paralysis[5].

Current treatments include infusions of hemin (panhematin in the United States and normosang in the European Union, Recordati) and carbohydrate loading. Hemin replacement therapy, which restores the regulatory heme pool in the liver and suppresses ALAS1 induction[6], is more effective than glucose therapy, which antagonizes hepatic ALAS1 induction[7], in reducing ALA and PBG accumulation[5,8]. Reductions in the levels of ALA and PBG occur 3 d after intravenous (i.v.) hemin infusion (3–4 mg kg^{-1} day^{-1}), and pain and nausea typically resolve on day four[9]. Hemin treatment should therefore be started as soon as the diagnosis is established and monitored through assessments of urinary ALA and/or PBG levels, pain scoring and clinical improvement[10].

Approximately 5% of patients suffer recurrent attacks, which persist for many years[11]. Recurrent attacks have a marked impact on the quality of life of these patients[12–14]. Patients usually require hospitalization and strong analgesic treatment, and opiates are often necessary for pain control. Prophylactic hemin is increasingly used for treating chronic symptoms, as an off-label indication of the drug. Of note, repeated courses of this therapy have been shown to generate thromboembolic disease and increased serum ferritin levels, suggesting iron overload in the liver[15]. Thus, lifelong exposure to hemin replacement therapy for the control of AIP symptoms may cause adverse events[16].

Emerging alternatives to the use of chronic hemin infusions to prevent recurrence of acute attacks include the administration of a short interfering RNA (siRNA, Givosiran, Alnylam Pharmaceuticals) directed against hepatic *ALAS1*[17]. The reduction in ALA and PBG accumulation occurs 2–3 d post-treatment, and the effect is maintained over weeks in patients[18]. Another promising approach involves the use of gene therapy in which the

[1]Moderna Therapeutics, Cambridge, MA, USA. [2]Program of Immunology and Immunotherapy, Centre for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain. [3]Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain. [4]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain. [5]Hepatology Program, Centre for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain. [6]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Instituto de Salud Carlos III, Madrid, Spain. [7]Department of Clinical Neurophysiology, Clínica Universitaria, University of Navarra, Pamplona, Spain. [8]Neurophysiology Laboratory, Neuroscience Program, Centre for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain. [9]Department of Biochemistry and Genetics, University of Navarra, Pamplona, Spain. [10]These authors contributed equally: Lei Jiang, Pedro Berraondo, Daniel Jericó, Lin T. Guey. [11]These authors jointly supervised this work: Matías A. Ávila, Paolo G.V. Martini, Antonio Fontanellas. *e-mail: paolo.martini@modernatx.com; afontanellas@unav.es

wild-type *PBGD* gene is delivered to hepatocytes using viral vectors[19–21]. Preclinical gene therapy studies confirmed that overexpression of transgenic PBGD protein in 5% of hepatocytes provided full phenotypic protection against porphyric attacks. In a phase 1 open-label clinical trial, the administration of a recombinant adeno-associated vector expressing *PBGD* proved to be safe but failed to modify over-excretion of porphyrin precursors[22].

In our study, we evaluated the potential of mRNA encoding *PBGD* delivered in lipid nanoparticles (LNPs) to address the disease in two models of acute hepatic porphyria. As described previously, LNPs encapsulating mRNA are taken up by hepatocytes through receptor-mediated endocytosis[23,24]. Once the mRNA reaches the cytoplasm, the encoded protein is rapidly produced[25]. Here, we report acute intervention and chronic repeat dosing in AIP mice and in rabbits with chemically induced porphyrin precursors. In addition, we demonstrate the translatability of this approach over multiple doses in nonhuman primates (NHPs).

Results

The administration of hPBGD mRNA rapidly counteracts an acute porphyria attack in AIP mice. We performed pharmacokinetic and pharmacodynamic studies in relevant experimental models of acute porphyria with a codon-optimized mRNA that encodes hPBGD (Supplementary Table 1). More than 50 different *PBGD* mRNA constructs were screened in vitro (Supplementary Fig. 1). The mRNA with the highest *PBGD* expression was selected for further studies (see Methods).

Administration of a single dose of 0.5 mg kg^{-1} (intravenously (i.v.)) of hPBGD mRNA induced high PBGD protein expression in wild-type mouse livers as early as 2 h post-injection (Fig. 1a). Expression of hPBGD protein peaked at 10 h post-injection and thereafter decayed over time. At 10 d post-injection, the residual hPBGD protein was still detectable by liquid chromatography–tandem mass spectrometry (LC–MS/MS), indicating a long residence time in the liver. Administration (i.v.) of the hPBGD mRNA in mice showed a wide intrahepatic distribution of mRNA-encoded protein as measured by immunohistochemistry (Fig. 1b). hPBGD protein expression was detected in more than 90% of mouse hepatocytes (percentage of hPBGD$^+$ area of the total area was 91% ± 1.9%).

Therapeutic efficacy was assessed in AIP mice by administering hPBGD mRNA during a porphyric attack, which can be induced by challenging the mice with multiple increasing doses of phenobarbital[26]. Administration of the mRNA after the third injection of phenobarbital, at which point accumulation of PBG and ALA reach their peak, was used to test the ability of the mRNA to counteract an ongoing acute attack. Consistent with previous studies in AIP mice, phenobarbital challenges increase PBG (Fig. 1c,d) and ALA (Fig. 1e,f) accumulation in the urine. On day 3, 0.5 mg kg^{-1} hPBGD or vehicle control luciferase mRNAs were administered as a single i.v. bolus. Measured urinary porphyrin precursor excretion over time (PBG (Fig. 1c) and ALA (Fig. 1e)) or the excretion expressed as the total peak area before and after i.v. administration of hPBGD mRNA (PBG (Fig. 1d) and ALA (Fig. 1f)) reflects the quick and efficient therapeutic effect of a single dose of the hPBGD mRNA. These results demonstrate that the rapid restoration of hepatic PBGD activity ensured a quick normalization of porphyrin precursor levels within hours of phenobarbital administration to AIP mice (Fig. 1c–f).

The high porphyrin precursor accumulation associated with the phenobarbital-induced attack did not alter hepatic mRNA delivery and the rapid onset of the expression of the therapeutic or control protein, as confirmed by administration of the luciferase mRNA into AIP mice (Supplementary Fig. 2).

The residence time of hPBGD in tissue was at least 10 d at the tested doses (0.2 and 0.5 mg kg^{-1}), which was confirmed by analyzing hepatic PBGD activity in AIP mice (Fig. 2a). Because acute attacks in patients last between 5 and 7 d[27], a single dose of hPBGD mRNA could offer protection throughout an entire episode. The dose-dependent efficacy of a single administration of hPBGD mRNA prior to the increase in PBG and ALA levels was assessed to test the ability of the mRNA to prevent acute attacks. In a prevention study against recurrent attacks, the protective effect of increasing doses of the mRNA was assayed in AIP mice by administering mRNAs 2 h before the second phenobarbital dose (Fig. 2b–f) to mimic what happens in patients who receive therapy when they start to feel prodromal attack symptoms. As expected, urinary PBG (Fig. 2b) and ALA (Supplementary Fig. 3a) increased by sixtyfold and twentyfold, respectively, throughout the phenobarbital challenge in AIP mice that were treated with control luciferase mRNA or saline. The group of AIP mice that received the lower doses of the hPBGD mRNA (0.05 mg kg^{-1} or 0.1 mg kg^{-1}) showed partial protection against PBG accumulation (Fig. 2b). By contrast, we observed full protection against PBG overexcretion in mice injected with 0.2 mg kg^{-1} or 0.5 mg kg^{-1} hPBGD mRNA (Fig. 2b). The same pattern was observed for ALA accumulation (Supplementary Fig. 3a). Quantification of the total peak area of the urinary PBG excretion and ALA excretion statistically confirmed these observations (PBG (Fig. 2c) and ALA (Supplementary Fig. 3b)).

The phenobarbital challenge induced pain in mice with AIP as a high score was measured on the pain scale, which quantifies the following parameters: orbital tightening, nose bulge, cheek bulge, ear position and whisker change[28,29] as well as disorder in motor coordination and forced breathing (Fig. 2d). Notably, all of the animals injected with a dose of 0.2 mg kg^{-1} or 0.5 mg kg^{-1} hPBGD mRNA showed a marked reduction in pain scores. Lower doses of therapeutic mRNA conferred only partial protection against pain (Fig. 2d). Motor neuropathy was analyzed using a rotarod device (Fig. 2e) and by measuring stride length (Fig. 2f). The highest degree of protection was obtained after a single administration of 0.5 mg kg^{-1} of hPBGD mRNA. These data demonstrate the preclinical efficacy of the hPBGD mRNA treatment and the existence of a dose–response effect.

Heme is an essential cofactor of complex components of the mitochondrial respiratory chain. Experimental reports have shown that a decrease in heme availability reduces the activity, transcription and translation of the mitochondrial complex IV in mouse livers[30] and in cell lines[31–34]. In this study, the maximal activity of complex II–IV was reduced in AIP mice during the phenobarbital challenge as measured by the oxygen consumption rate (OCR) of isolated hepatic mitochondria (Fig. 3a). The OCR was restored to normal after the administration of a single dose of hPBGD mRNA (0.5 mg kg^{-1}) or three consecutive doses of hemin (Fig. 3a).

Hypertension is a common feature in patients with AIP[2], but AIP mice have blood pressure readings in the range of wild-type mice. Phenobarbital challenge significantly increases both systolic (Fig. 3b) and diastolic (Supplementary Fig. 4a) blood pressure values. Protection against hypertension induced by phenobarbital challenge was achieved in AIP mice that were injected with hPBGD mRNA (0.5 mg kg^{-1}). By contrast, up to three doses of hemin were needed to reduce hypertension in AIP mice (Fig. 3b and Supplementary Fig. 4a). After the last blood pressure measurement, a new dose of phenobarbital was administered, and urine samples from individual mice were collected over the subsequent 20 h. As expected, urinary ALA levels were high in the group of mice that were challenged with phenobarbital, whereas those mice that were treated with hPBGD mRNA showed levels similar to those measured in AIP mice that were not exposed to the drug (Supplementary Fig. 4b). Notably, three consecutive doses of hemin only partially protected against the accumulation of porphyrin precursors (Supplementary Fig. 4b), although all these cases involved normotensive mice.

Next, we evaluated the duration of the protection of a single administration of the hPBGD mRNA. A phenobarbital challenge

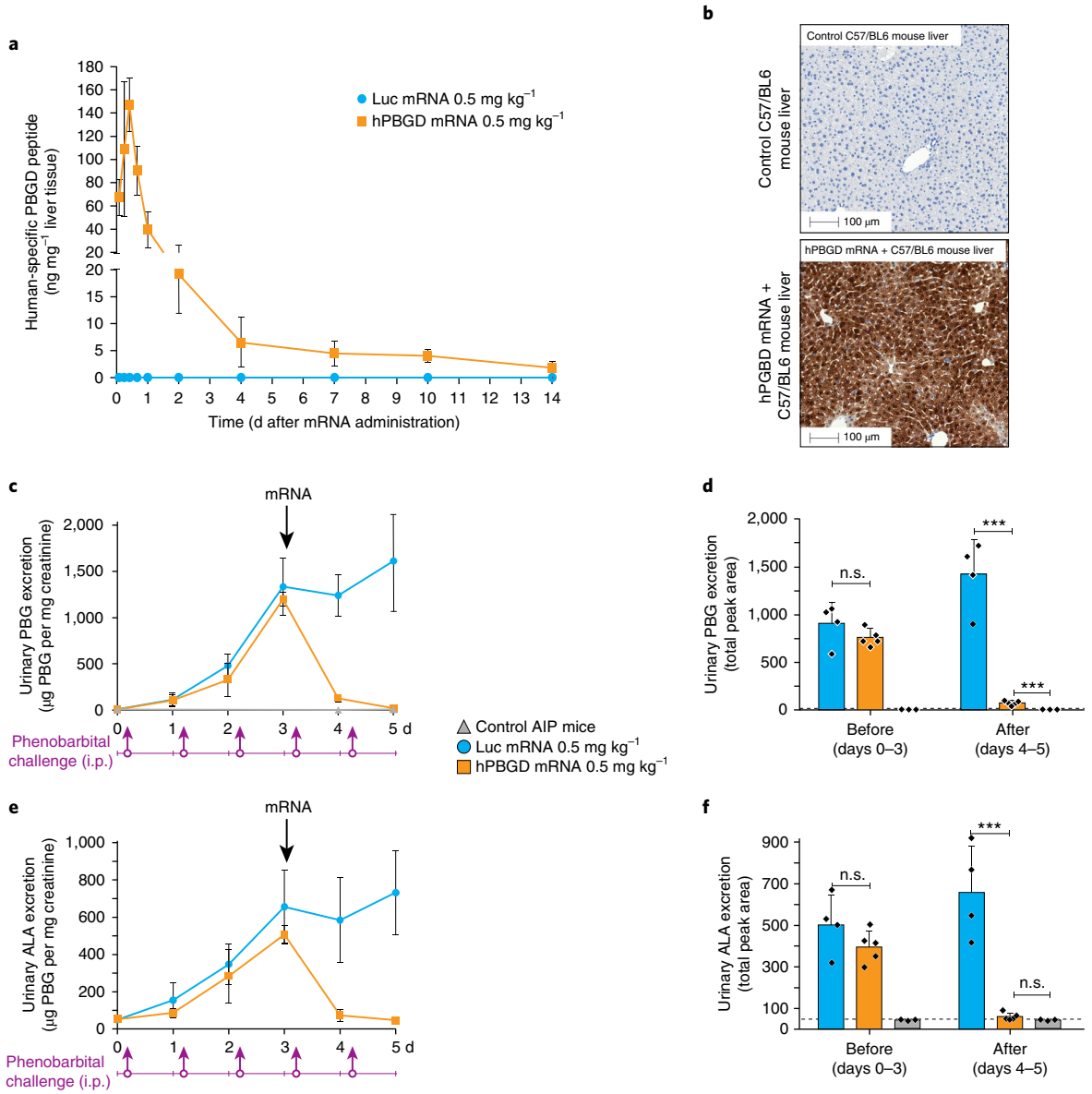


Fig. 1 | Kinetics of PBGD expression and therapeutic efficacy of a single i.v. injection of hPBGD mRNA administered at the peak of a phenobarbital-induced acute attack in AIP mice. hPBGD mRNA (0.5 mg kg^{-1}) was i.v. injected into male wild-type mice and AIP mice. **a**, Kinetics of hPBGD were measured by LC–MS/MS in livers from wild-type CD-1 mice ($n=4$) at 2, 6, 10, 16 h and 1, 2, 4, 7, 10 and 14 d post-injection. Luciferase (Luc) mRNA at 0.5 mg kg^{-1} was administered as a vehicle control ($n=4$). Data are mean ± s.d. of $n=4$ mice per timepoint. **b**, Representative images of immunohistochemistry staining of hPBGD in liver samples from a mouse injected with 0.5 mg kg^{-1} hPBGD mRNA (bottom) and an uninjected mouse (top). Immunohistochemistry was performed in $n=4$ independent mice per group. The experiment was repeated independently two more times with similar results. Scale bar, 100 µm. Efficacy of a single i.v. injection of hPBGD mRNA against a phenobarbital-induced acute attack in AIP mice. For the acute treatment study, an attack was induced in male AIP mice by intraperitoneal (i.p.) injection of increasing doses of phenobarbital (single doses of 75, 80 and 85 mg kg^{-1} and two doses of 90 mg kg^{-1}) every 24 h. Two hours before the fourth phenobarbital injection, mice were treated with a single dose of 0.5 mg kg^{-1} hPBGD ($n=5$) or luciferase mRNA ($n=4$). AIP mice without phenobarbital challenge were used as control ($n=3$). Mice were housed in metabolic cages, and urine samples were collected after 24 h. **c**,**e**, Urinary PBG (**c**) and ALA (**e**) excretion over time. **d**,**f**, Quantification of the total peak area of the urinary PBG (**d**) and ALA (**f**) excretion. Data are mean ± s.d. Pharmacodynamic studies were performed with male mice because they have increased sensitivity to phenobarbital as shown by a greater accumulation of precursors after the challenge. Dotted lines represent baseline values for total peak area of ALA and PBG. n.s., not significant. ***$P<0.001$, one-way ANOVA followed by Bonferroni post-test.

was performed every 10 d after mRNA administration. Consistent with previous studies, full protection against porphyrin precursor overexcretion was obtained in mice injected with 0.5 mg kg^{-1} hPBGD mRNA after the first phenobarbital challenge, as measured by total peak area during each challenge (PBG (Fig. 3c) and ALA (Supplementary Fig. 5a,b)). Notably, this group of mice was still markedly protected against ALA and PBG accumulation after the second phenobarbital challenge on D10 (PBG (Fig. 3c) and ALA (Supplementary Fig. 5a,b)). No effect on ALA or PBG accumulation was observed after the third challenge (D22), which was induced 21 d after the administration of the hPBGD mRNA. Pain measurements were taken at the end of each phenobarbital challenge (Fig. 3d). Extended protection against pain during the second phenobarbital challenge (D10) was observed in mice treated with

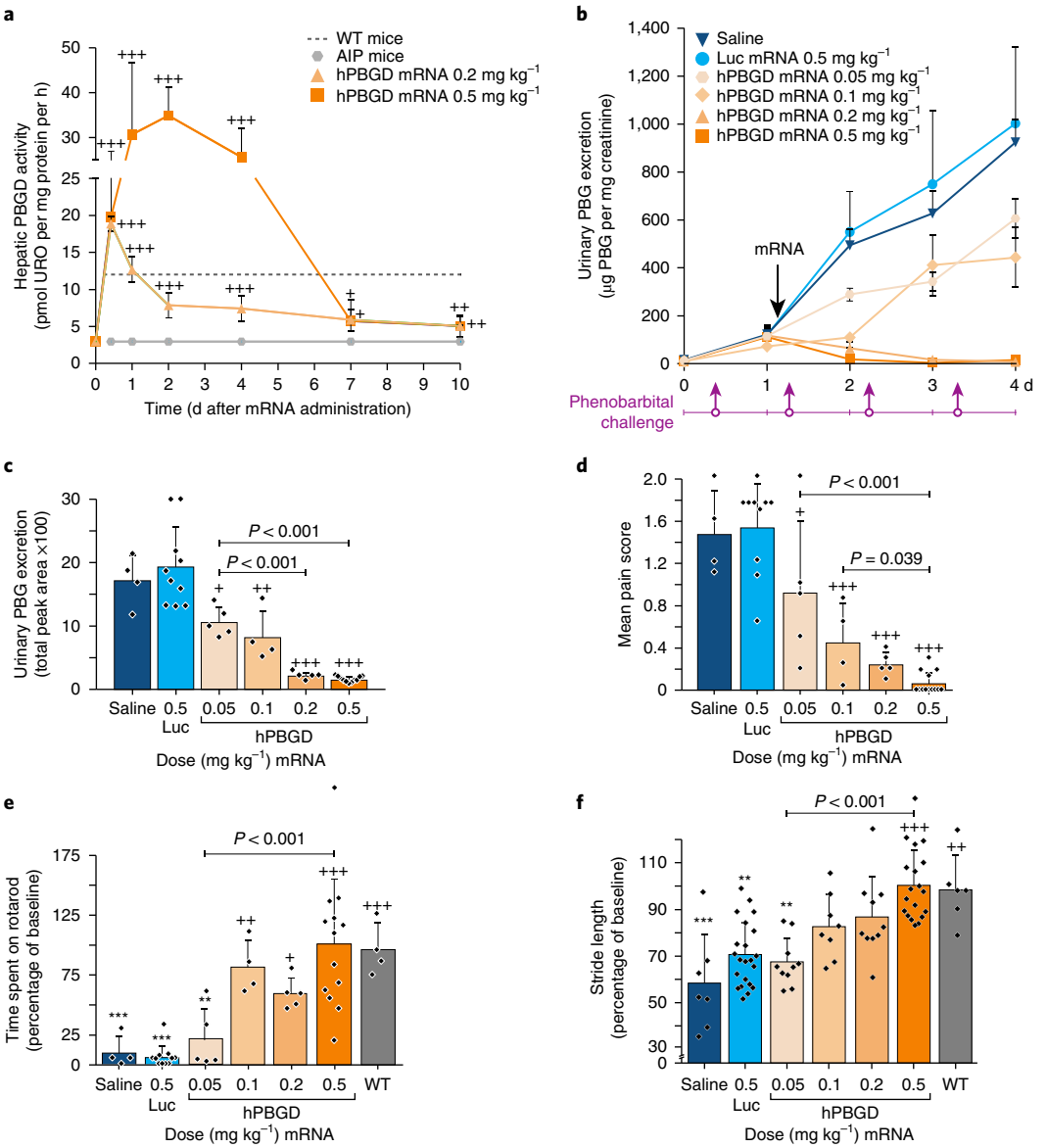


Fig. 2 | Therapeutic efficacy of a single i.v. administration of hPBGD mRNA to prevent a phenobarbital-induced acute attack in AIP mice. a, Kinetics of PBGD activity in livers from female AIP mice injected with 0.2 and 0.5 mg kg^{-1} hPBGD mRNA. The dotted and grey lines represent PBGD activity in wild-type and AIP mice, respectively. Five animals were euthanized at 8 h, and 1, 2, 4, 7 and 10 d after mRNA administration, and the hepatic PBGD activity was determined in fresh samples. Data are mean ± s.d. from two independent experiments. Day 7, $^{+}P = 0.021$ AIP mice versus treatment with 0.2 mg kg^{-1}; $^{+}P = 0.044$ AIP mice versus treatment with 0.5 mg kg^{-1}. Day 10 $^{++}P = 0.009$ AIP mice versus treatment with 0.2 mg kg^{-1}; $^{++}P = 0.008$ AIP mice versus treatment with 0.5 mg kg^{-1}; $^{+++}P < 0.001$ AIP mice versus treatment with 0.2 mg kg^{-1} or 0.5 mg kg^{-1}. URO, uroporphyrin. **b**, Protective efficacy of hPBGD mRNA against acute attacks was assayed in phenobarbital-challenged male AIP mice. Two hours before the second phenobarbital injection, mice were treated with a single i.v. injection of different doses (0.05 ($n = 5$), 0.1 ($n = 4$), 0.2 ($n = 5$), and 0.5 mg kg^{-1} ($n = 11$)) of hPBGD mRNA or 0.5 mg kg^{-1} luciferase mRNA ($n = 10$). Four AIP mice were injected with saline. Urinary PBG excretions during four consecutive days are shown. **c**, Quantification of the total peak area of the urinary PBG excretion. $^{+}P = 0.02$, $^{++}P = 0.01$, $^{+++}P < 0.001$ versus AIP mice treated with luciferase mRNA. **d**–**f**, Four hours after the last phenobarbital challenge, several functional assays were performed. **d**, Pain measurement using the mouse grim scale (MGS)[28,29]. $^{+}P = 0.014$, $^{+++}P < 0.001$ versus AIP mice treated with luciferase mRNA. **e**, Motor coordination measured using the rotarod test. $^{**}P = 0.008$, $^{***}P < 0.001$ versus wild-type (WT) mice. $^{+}P = 0.025$, $^{++}P = 0.002$, $^{+++}P < 0.001$ versus AIP mice treated with luciferase mRNA. **f**, Motor coordination according to the stride length. $^{**}P = 0.003$, $^{***}P < 0.001$ versus wild-type (WT) mice. $^{++}P = 0.003$, $^{+++}P < 0.001$ versus AIP mice treated with luciferase mRNA. **b**–**f**, Data are mean ± s.d. from two independent experiments. All comparisons were performed by one-way ANOVA followed by Bonferroni post-test.

0.5 mg kg^{-1} of the hPBGD mRNA. In the group of mice treated with 0.1 mg kg^{-1} hPBGD mRNA, partial protection against pain (Fig. 3d) was observed after the first challenge (D0), but this effect was lost by the time of the second (D10) and the third challenges (D22).

Therapeutic effect of repeat administration of hPBGD mRNA in AIP mice during recurrent acute attacks. We also studied whether repeat administration of hPBGD mRNA could protect against recurrent phenobarbital challenges (D0, D13 and D27). Full protection against accumulation of the precursor PBG was maintained over the three consecutive administrations in AIP mice that were injected with doses of 0.2 and 0.5 mg kg^{-1} of hPBGD mRNA (Fig. 4a,b). The same accumulation pattern was observed for ALA (Supplementary Fig. 6). Those mice injected with the highest dose

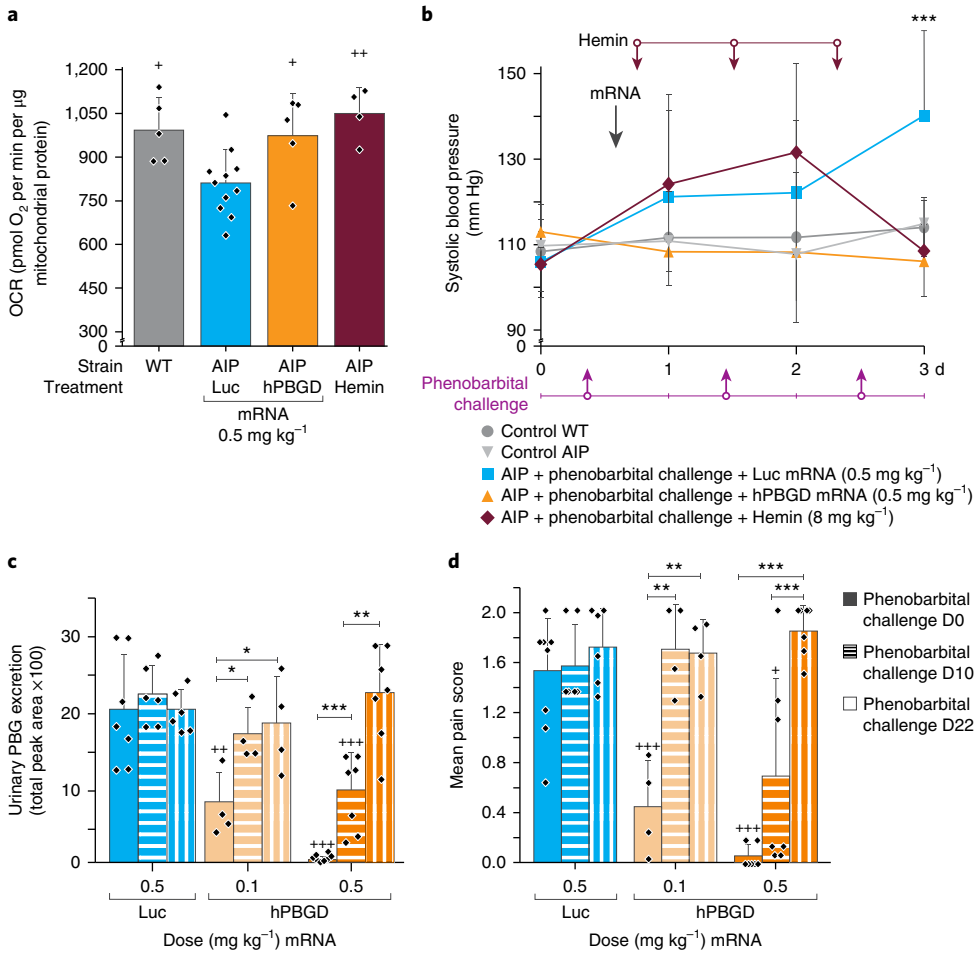


Fig. 3 | Therapeutic efficacy of a single i.v. administration of 0.5 mg kg^{-1} hPBGD mRNA against one or three consecutive phenobarbital-induced acute attacks in AIP mice. a, OCR in isolated hepatic mitochondria showing the maximum activity of complex II–IV in a manner independent of the TCA cycle. The OCR study was performed twice in the following groups of mice: wild-type mice ($n=6$), AIP mice treated with luciferase mRNA ($n=11$), AIP mice treated with hPBGD mRNA ($n=5$) and AIP mice treated with hemin ($n=4$). Data are mean ± s.d. from two independent experiments. $^{+}P=0.019$, $^{++}P=0.008$ versus AIP mice treated with luciferase mRNA. Analyses were performed using one-way ANOVA followed by Holm–Sidak post-test. **b**, Systolic blood pressures were measured before and during the single phenobarbital challenge. Up to four rounds of ten blood pressure measurements per day were taken for each mouse. Data are the mean of the means from all rounds for each parameter determined in the following groups of mice: control wild-type mice ($n=9$), control AIP mice ($n=11$), AIP mice treated with luciferase mRNA ($n=9$), AIP mice treated with hPBGD mRNA ($n=11$) and AIP mice treated with hemin ($n=5$). Data are mean ± s.d. from three independent experiments. $^{***}P<0.001$ versus control AIP mice; analyzed using a two-tailed Student's *t*-test. Protective efficacy of the hPBGD mRNA against acute attacks was analyzed in AIP mice after repeated phenobarbital challenges. Three acute attacks were induced in male AIP mice by i.p. injection of increasing doses of phenobarbital (75, 80, 85 and 90 mg kg^{-1}) every 24 h starting at days 0, 10 and 22 (challenges D0, D10 and D22, respectively). One day after the first phenobarbital injection, mice were treated with a single administration of 0.1 mg kg^{-1} ($n=4$) or 0.5 mg kg^{-1} ($n=8$) hPBGD mRNA or 0.5 mg kg^{-1} luciferase mRNA ($n=7$) injected as a systemic bolus. Mice were housed in metabolic cages, and urine samples were collected after 24 h during each cycle of phenobarbital administration. **c**, Quantification of the total peak area of the urinary excretion of PBG in each acute attack. $^{++}P=0.002$, $^{+++}P<0.001$ versus AIP mice treated with luciferase mRNA on D0. $^{+++}P<0.001$ versus AIP mice treated with luciferase mRNA on D10. Dose of 0.1 mg kg^{-1} hPBGD mRNA: $^{*}P=0.035$ D0 versus D10; $^{*}P=0.026$ D0 versus D22. Dose of 0.5 mg kg^{-1} hPBGD mRNA: $^{***}P<0.001$ D0 versus D10; $^{**}P=0.003$ D10 versus D22. All comparisons were performed using one-way ANOVA followed by Bonferroni post-test. **d**, Four hours after the last phenobarbital injection of each challenge, pain measurements were performed using the MGS. Data are mean ± s.d. from two independent experiments. $^{+++}P<0.001$ versus AIP mice treated with luciferase mRNA on D0. $^{+}P=0.015$ versus AIP mice treated with luciferase on D10. Dose of 0.1 mg kg^{-1} hPBGD mRNA: $^{**}P=0.002$. Dose of 0.5 mg kg^{-1} hPBGD mRNA: $^{***}P<0.001$. All comparisons were performed using one-way ANOVA followed by Bonferroni post-test.

showed symptomatic protection as measured by parameters such as normal motor coordination (Fig. 4c) and reduced pain (Fig. 4d).

The group of AIP mice that received repeat administration of hemin (three doses of 8 mg kg^{-1} per challenge) also showed reduced excretion of PBG (Fig. 4a,b) and ALA (Supplementary Fig. 6), improving further with each treatment. However, the administration of 0.5 mg kg^{-1} of hPBGD mRNA was more efficient in controlling the accumulation of porphyrin precursors, especially during the first two challenges (D0 and D13). Animals injected with hemin or the highest doses of hPBGD mRNA exhibited a similar protection against motor coordination dysfunction (Fig. 4c) and pain (Fig. 4d).

Abnormalities in nerve conduction velocities occur in AIP mice after recurrent acute attacks induced by repeated phenobarbital challenges (Fig. 4e). Preclinical efficacy of the therapeutic mRNA was tested by measuring the compound muscle action potentials,

which were evoked by proximal stimulation of the sciatic nerve after three recurrent attacks. The administration of recurrent doses of 0.5 mg kg^{-1} of hPBGD mRNA fully protected against the loss of amplitude (Fig. 4e) in AIP mice.

Finally, repeat i.v. administration of hPBGD mRNA was well-tolerated in all AIP mice, and no adverse events related to the experimental agent occurred during either treatment administration or follow-up. The intrahepatic overexpression of the hPBGD protein obtained after the administration of hPBGD mRNA was not hepatotoxic, as indicated by normal levels of serum alanine aminotransferase (ALT), alkaline phosphatase (ALP) and albumin (Supplementary Table 2). No antibodies against mRNA-encoded hPBGD protein (anti-drug antibodies (ADAs)) were detected using a sensitive enzyme-linked immunosorbent assay (ELISA analysis) (data not shown).

On day 31, animals received a supplementary dose of phenobarbital (90 mg kg^{-1}) and were euthanized 20 min later to study the hepatic expression of important genes involved in the acute attack. Phenobarbital induces rapid expression of the *Alas1* gene (Fig. 4f). *Alas1* expression is low in wild-type mice and high in AIP mice. Mice treated with three doses of hemin or hPBGD mRNA showed mild or high protection, respectively, against the induction of *Alas1* in the liver. Presumably, the lack of *Alas1* induction in AIP mice that were treated with hPBGD mRNA indicates normal regulatory heme levels, and therefore strong induction of *Alas1* does not occur (Fig. 4f). Hepatic expression of heme oxygenase-1 (HMOX1, EC 1.14.14.18) was not modified in any of the groups (Supplementary Fig. 7a). Previous studies[21] have reported disturbances in the protein-folding machinery in the liver of AIP mice and that this machinery was further impaired during phenobarbital challenge. In a separate study, we found that the administration of a single dose of hPBGD mRNA fully protected against endoplasmic reticulum and cytoplasmic protein-folding stress as measured by the XBP1s to XBP1t ratio (Supplementary Fig. 7b) and HSP70 levels (Supplementary Fig. 7c), respectively.

Hepatic hPBGD expression after hPBGD mRNA administration in large animals. In the case of therapeutic products that require transfer to the liver, the efficacy depends on multiple factors, including an immunological response against the therapies and metabolic status and the size of the target organ, among others. Therefore, it is important to explore the performance of these therapies in larger animal species with the potential to inform dosing levels for clinical trials. The preclinical proof-of-concept studies performed in AIP mice demonstrated that hPBGD mRNA had a rapid and high therapeutic efficacy at a dose of 0.5 mg kg^{-1}. Notably, the administration of this same dose to rats, rabbits and NHPs resulted in a gain of approximately 75–80% of endogenous PBGD activity 24 h after the administration (Fig. 5a). In healthy humans, hepatic PBGD activity is 5.2 ± 0.13 U, whereas explants from two patients with recurrent active chronic AIP showed activity of 3.3 ± 0.26 U. Thus, the gain of 2 U in the activity of PBGD that was observed 7 d after mRNA administration (Fig. 2a) may be sufficient to transiently normalize hepatic PBGD activity and prevent overproduction of ALA and PBG.

Finally, the administration of a single i.v. dose of *PBGD* mRNA did not increase markers of liver toxicity in rabbits (Supplementary Table 3) or in NHPs (Supplementary Table 4) compared to animals that received Tris-sucrose buffer or to baseline levels.

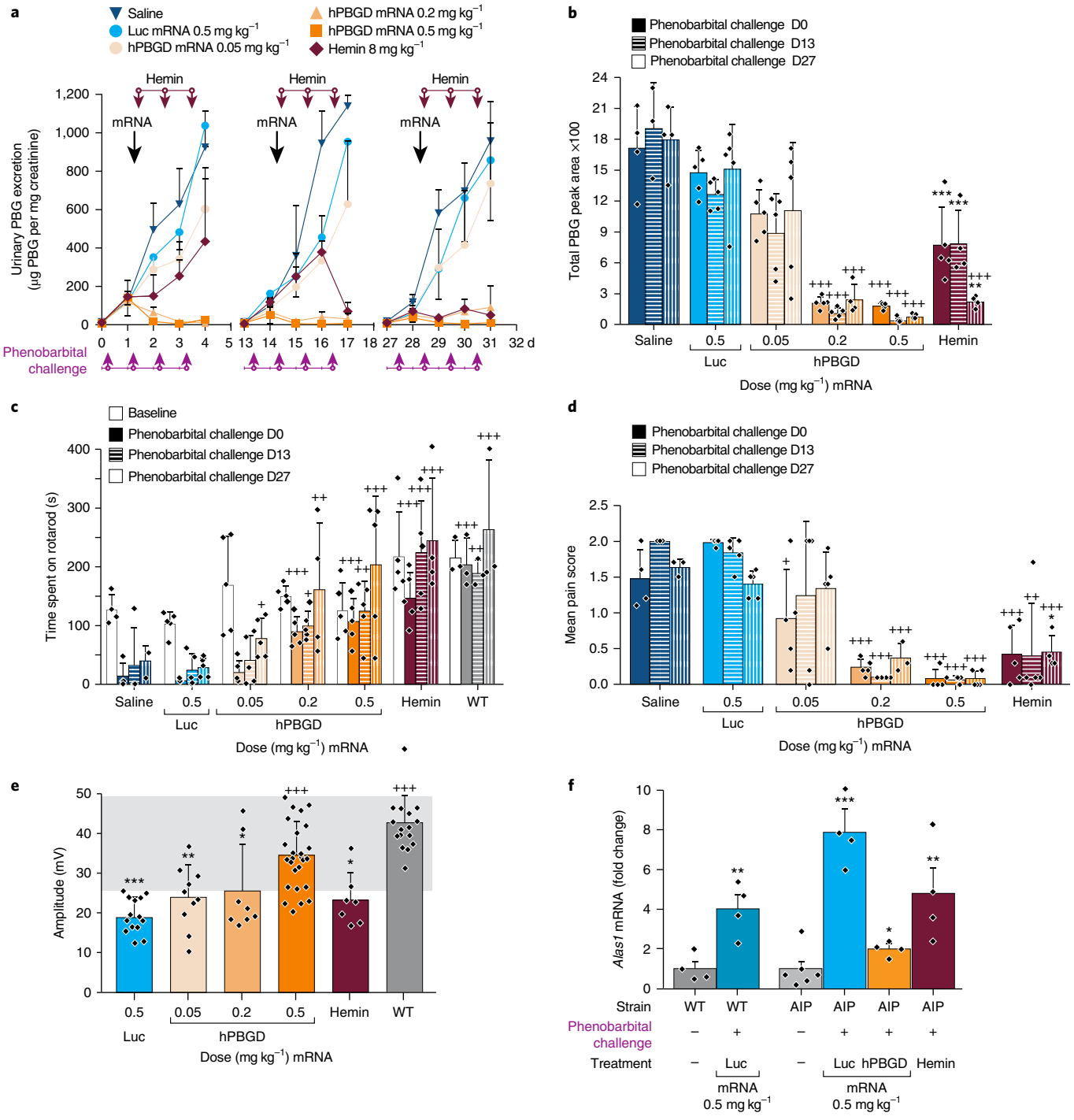
Therapeutic efficacy of hPBGD mRNA administration in a model of chemically induced porphyrin precursors in rabbits. The effectiveness of the hPBGD mRNA therapy to metabolize high amounts of porphyrin precursors was evaluated in rabbits. Recurrent administration of allyl isopropyl acetamide (AIA) and rifampicin for five consecutive days induced a significant accumulation of PBG (Fig. 5b,c) and ALA (Supplementary Fig. 8a,b) in the urine of female rabbits. The excretion of PBG per mg of creatinine that was chemically induced in rabbits was fivefold higher than that found in human porphyria during an acute attack[22]. The efficacy of the hPBGD mRNA was tested at the onset of the chemically induced increase in porphyrin precursors in rabbits. On day 3 of the challenge with AIA and rifampicin, rabbits showed extremely high levels of urinary PBG excretion (Fig. 5c). The ALA increase in rabbits was delayed with respect to PBG, and its accumulation was 10 times lower than that of PBG (Supplementary Fig. 8a). The administration of a single dose of hPBGD mRNA (0.5 mg kg^{-1}) 2 h after the third AIA dose normalized urinary PBG accumulation within 24 h (Fig. 5c) and

Fig. 4 | Therapeutic efficacy of multi-dose i.v. administration of hPBGD mRNA against three consecutive phenobarbital-induced acute attacks in AIP mice. In the multidose study, mice were treated with a single injection of 0.05 mg kg^{-1} ($n = 5$), 0.2 mg kg^{-1} ($n = 5$) or 0.5 mg kg^{-1} ($n = 5$) hPBGD mRNA or 0.5 mg kg^{-1} luciferase mRNA ($n = 5$) 1 d after the first phenobarbital injection in each cycle of the phenobarbital challenge. A group of AIP mice ($n = 5$) received hemin for three consecutive days (8 mg kg^{-1}, i.p. injection of normosang) starting 24 h after the first phenobarbital dose. A supplementary cohort of AIP mice ($n = 4$) received repeated i.v. doses of saline. Mice were housed in metabolic cages, and urine samples were collected after 24 h during each cycle of phenobarbital administration. **a**,**b**, Urinary PBG excretion over time (**a**) and quantification of the total peak area of urinary PBG excretion for each acute attack (**b**). $^{**}P = 0.001$, $^{***}P < 0.001$ versus mice treated with hPBGD mRNA (0.5 mg kg^{-1}); two-tailed Student's *t*-test. $^{+++}P < 0.001$ versus mice treated with luciferase mRNA; one-way ANOVA followed by Bonferroni post-test. **c**,**d**, Four hours after the last phenobarbital injection in each cycle, several functional assays were performed. **c**, Motor coordination was measured using the rotarod test. Challenge D0: $^{+++}P < 0.001$ versus luciferase mRNA. Challenge D13: $^{+}P = 0.017$ hPBGD mRNA (0.2 mg kg^{-1}) versus luciferase mRNA; $^{++}P = 0.001$ hPBGD mRNA (0.5 mg kg^{-1}) versus luciferase mRNA; $^{+++}P < 0.001$ hemin versus luciferase mRNA; $^{++}P = 0.001$ wild-type mice versus luciferase mRNA. Challenge D27: $^{+}P = 0.034$ hPBGD mRNA (0.05 mg kg^{-1}) versus luciferase mRNA; $^{++}P = 0.003$ hPBGD mRNA (0.2 mg kg^{-1}) versus luciferase mRNA; $^{+++}P < 0.001$ versus luciferase mRNA; one-way ANOVA followed by Bonferroni post-test. **d**, Pain measurement using the MGS scale. Challenge D0: $^{+}P = 0.028$ hPBGD mRNA (0.05 mg kg^{-1}) versus luciferase mRNA; $^{+++}P < 0.001$ versus luciferase mRNA. Challenge D13: $^{++}P = 0.002$ hemin versus luciferase mRNA; $^{+++}P < 0.001$ versus luciferase mRNA. Challenge D27: $^{+++}P < 0.001$ versus luciferase mRNA; $^{*}P = 0.013$ versus hPBGD mRNA (0.5 mg kg^{-1}); one-way ANOVA followed by Bonferroni post-test. **e**, Action potentials in the compound muscle were evoked by proximal stimulation of the sciatic nerve and were measured 8 h after the last phenobarbital challenge. Data are mean ± s.d. from one experiment. $^{+++}P < 0.001$ versus luciferase mRNA; one-way ANOVA followed by Bonferroni post-test. $^{*}P = 0.014$ hPBGD mRNA (0.2 mg kg^{-1}) versus hPBGD mRNA (0.5 mg kg^{-1}); $^{**}P = 0.009$ hPBGD mRNA (0.05 mg kg^{-1}) versus hPBGD mRNA (0.5 mg kg^{-1}); $^{*}P = 0.02$ hemin versus hPBGD mRNA (0.5 mg kg^{-1}); $^{***}P < 0.001$ versus hPBGD mRNA (0.5 mg kg^{-1}); two-tailed Student's *t*-test. **f**, Hepatic expression of *Alas1* after a 4-d period of phenobarbital challenge in wild-type and AIP mice injected with one dose of control luciferase or hPBGD mRNA (0.5 mg kg^{-1}) or three doses of hemin (8 mg kg^{-1}, i.p. injection of normosang). All groups included $n = 4$ animals, except the AIP group without phenobarbital, which included $n = 6$ animals. Subsequently, 24 h after the fourth phenobarbital dose, animals received an additional dose of phenobarbital (90 mg kg^{-1}) and were euthanized 20 min later to study the hepatic expression of *Alas1*. $^{*}P = 0.048$ versus wild-type mice without phenobarbital treatment; $^{**}P = 0.005$ wild-type mice treated with phenobarbital versus wild-type mice without phenobarbital treatment; $^{**}P = 0.009$ hemin-treated mice treated with phenobarbital versus wild-type mice without phenobarbital treatment; $^{***}P < 0.001$ versus wild-type mice without phenobarbital treatment; two-tailed Student's *t*-test.

prevented ALA accumulation (Supplementary Fig. 8a). The total peak area before and after a single administration of 0.5 mg kg^{-1} hPBGD mRNA confirmed a rapid normalization of urinary PBG (Fig. 5b) and ALA (Supplementary Fig. 8b) accumulation.

A second challenge was performed 1 month later (challenge on day 30, D30). For this challenge, three rabbits that previously received the control luciferase mRNA were injected with the hPBGD mRNA, and vice versa in two animals that had previously received hPBGD mRNA (Fig. 5b,c). Finally, a third challenge was performed 1 month later (challenge on day 60, D60). The three animals treated with hPBGD mRNA in the previous cycle received hPBGD mRNA again, and two naive animals were injected with the luciferase mRNA as controls. Repeated i.v. administration of hPBGD mRNA in rabbits maintained the same degree of reduction in PBG (Fig. 5b,c) and ALA (Supplementary Fig. 8a,b) after each administration. The generation of ADAs was also investigated in rabbits that received two doses of hPBGD mRNA. There was no significant change in anti-PBGD antibodies (data not shown). This data and the maintenance of the same degree of porphyrin precursor reduction after repeat hPBGD mRNA administration suggest that this therapy did not induce an immune response in these rabbits.

Safety of a single dose or multiple doses of the hPBGD mRNA in NHPs. Finally, safety studies after a single dose (SD arm) or repeated administration (MD arm) of 0.5 mg kg^{-1} hPBGD mRNA were performed in NHPs (Fig. 6a). The enzymatic PBGD activity was analyzed in liver biopsies taken before mRNA administration



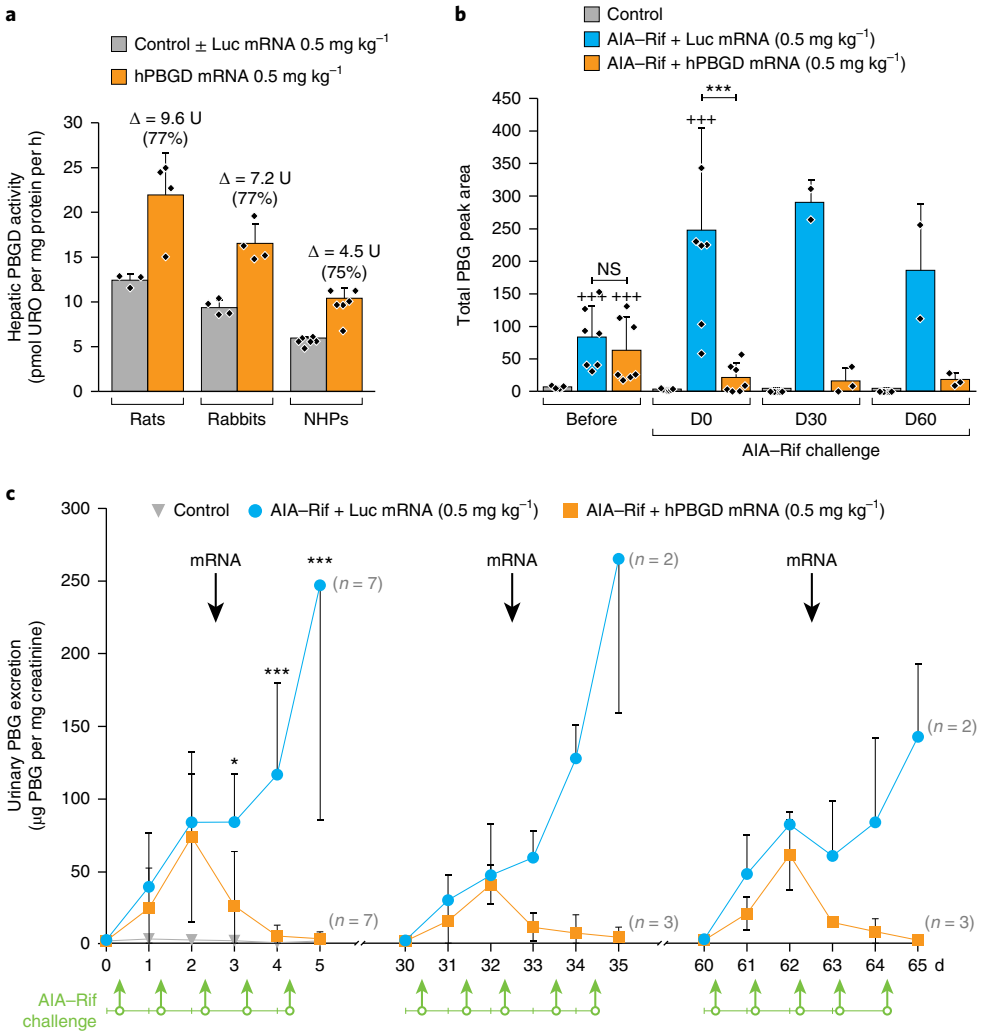


Fig. 5 | Therapeutic efficacy of a single dose or multiple doses of hPBGD mRNA against AIA- and rifampicin-induced accumulation of porphyrin precursors in rabbits. a, Increase in hepatic PBGD activity 24 h after the administration of 0.5 mg kg^{-1} of hPBGD mRNA in female rats ($n = 4$), rabbits ($n = 4$) and NHPs ($n = 6$). Data are mean ± s.d. from one assay measured in triplicate. The efficacy of hPBGD mRNA to metabolize high amounts of porphyrin precursors was assayed in seven female rabbits (about 3 kg) per group. The increase in porphyrin precursors was induced by the subcutaneous administration of AIA (350 mg kg^{-1}) in the morning and rifampicin (Rif; 200 mg kg^{-1}, i.p.) in the afternoon for five consequitive days. A dose of 0.5 mg kg^{-1} of mRNA (hPBGD or luciferase) was injected 2 h after the third dose of AIA (day 2 of challenge day 0). Urine samples were collected after 24 h. Data are mean ± s.d. from two experiments. ***$P < 0.001$ versus hPBGD mRNA (0.5 mg kg^{-1}); two-tailed Student's *t*-test. $^{+++}P < 0.001$ versus baseline values in each challenge, one-way ANOVA followed by Bonferroni post-test. A second challenge was performed a month later (challenge on D30). On this occasion, the rabbits ($n = 3$) that previously received the control luciferase mRNA were injected with the hPBGD mRNA and vice versa. Finally, a third challenge was held a month later (challenge on D60). On this occasion, the three animals treated with hPBGD mRNA in the previous cycle received the therapeutic mRNA again, and two naive animals were injected with the luciferase mRNA as controls. **b**,**c**, Repeated i.v. administration of hPBGD mRNA in rabbits maintained the same degree of reduction after each dosage as measured by the quantification of the total peak area of the urinary PBG excretion (**b**) and urinary PBG excretion over time (**c**). Data are mean ± s.d. from one experiment. *$P = 0.011$, ***$P < 0.001$ versus hPBGD mRNA (0.5 mg kg^{-1}); two-tailed Student's *t*-test.

(Fig. 6a) and in the eight different lobes of the liver taken at necropsy (24 h after the last mRNA dose for each arm). All of the NHPs that received one (SD arm) or five (MD arm) injections of hPBGD mRNA showed an increase of about 80% of the endogenous PBGD activity (Fig. 6b). At death, animal CDC056 had a clot of approximately 2 cm in diameter in the liver, probably produced during the biopsy procedure that was performed 17 d earlier. We suggest that this blood clot could have reduced the LNP distribution and limited liver transfection (Fig. 6b). No other gross pathology was observed in any of the NHPs.

To evaluate markers of liver toxicity after a single dose (SD arm, Supplementary Table 4) or after each of the five doses in the MD arm (Supplementary Table 5), serum albumin, ALP and ALT levels were measured at baseline, 2, 6 and 24 h after the administration of 0.5 mg kg^{-1} hPBGD mRNA (infusion of 17 ml in 1 h).

Liver function tests remained within normal ranges after one and five consecutive administrations of the hPBGD mRNA injection (Supplementary Tables 4 and 5, respectively). The normal range corresponds to data from a cohort of 72 age-matched female cynomolgus monkeys (*Macaca fascicularis*)[35]. Although within the normal range, an increase in the serum ALT values was observed 24 h post-injection at dose 4 and 5 (Supplementary Table 5), as recurrent ketamine administration can cause an increase in serum ALT[36]. Also, the procedure and repeated manipulation over the 24 h post-injection may favor the increase in ALT, as observed in rabbits injected with Tris-sucrose buffer (Supplementary Table 3).

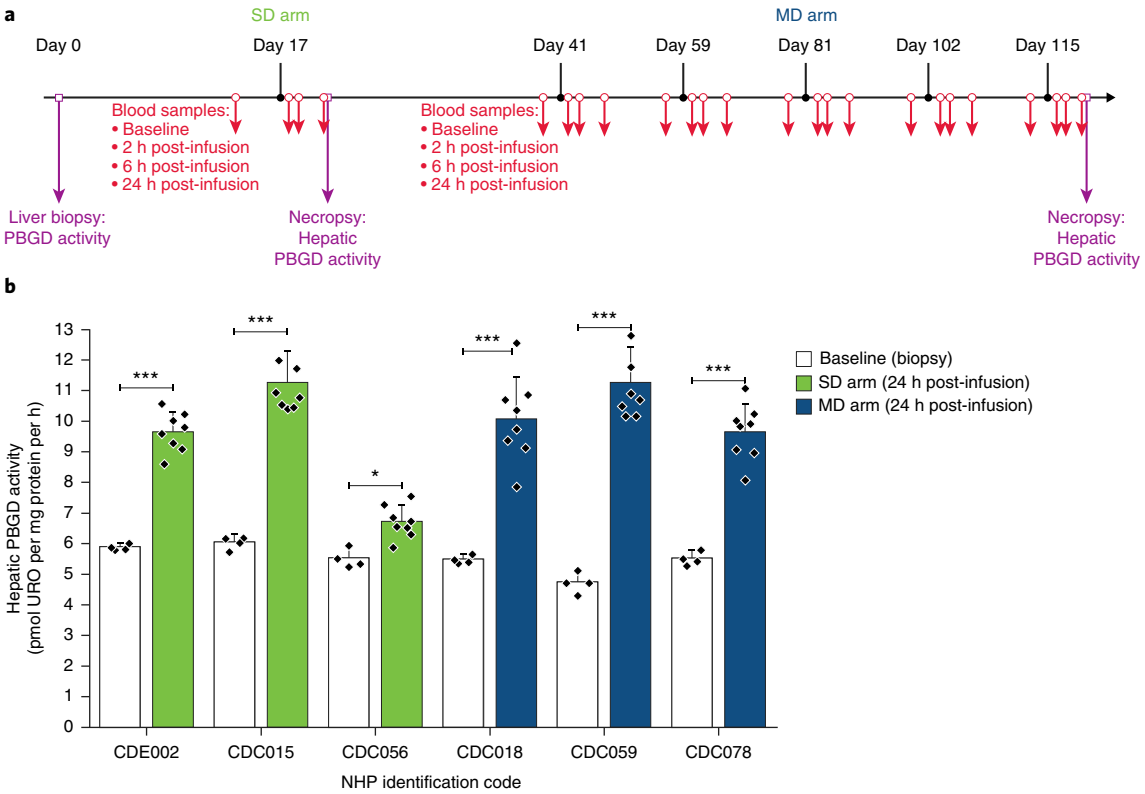


Fig. 6 | Hepatic PBGD activity 24 h after single or repeated administration of hPBGD mRNA (0.5 mg kg^{-1}) in female NHPs. a, Schematic timeline representation of the single-dose (SD, $n=3$) and multiple-dose (MD, $n=3$) arms of the study. **b**, Hepatic measurements of PBGD enzyme activity. Baseline values were obtained from four liver biopsies (two from the left side and two from the right side). NHPs were euthanized 24 h after the administration of the first dose of mRNA (SD arm) or of the fifth dose of mRNA (MD arm). Statistical differences in PBGD activity were analyzed using a two-tailed Welch's test. *$P=0.012$, ***$P<0.001$. Data are mean ± s.d.

There was no significant change in serum anti-PBGD antibody levels (Supplementary Fig. 9). This data and the fact that hepatic PBGD activity was similar in NHPs that received one or five injections suggest that recurrent administration of the hPBGD mRNA did not induce an immune response in these animals. A formal safety study of the mRNA formulation was performed as previously reported[37].

Discussion

Heme is an essential component of a variety of hemoproteins in all nucleated cells and has a crucial role in detoxification mechanisms in the liver. Hepatic heme biosynthesis is modulated by feedback control of ALAS1, the first and rate-limiting enzyme in the pathway. Because the PBGD enzyme does not participate in this control, we hypothesized that an overexpression of the PBGD protein in hepatocytes could be a safe way to metabolize high levels of porphyrin precursors that are produced during acute attacks in patients with AIP.

Clinically, AIP is divided into sporadic disease (80–90% of patients with active disease) and recurrent disease (5% of patients). Patients with sporadic disease would benefit considerably from effective acute therapies that could resolve attacks by rapidly increasing PBGD activity. However, the current therapeutic options only work to suppress exacerbation by reducing accumulation of upstream substrates.

In recurrent disease, hemin supplementation, which downregulates the heme biosynthesis pathway, is the current standard of care. However, hemin infusion has been linked to the induction of hepatic *HMOX1* gene expression, the critical enzyme of heme catabolism that paradoxically reduces regulatory heme pool levels and upregulates heme synthesis, thus increasing the risk of recurrent attacks[16]. This mechanism may underlie the reported decrease in the therapeutic benefit of chronic hemin in some patients.

Here, we report that hPBGD mRNA formulated in LNPs may represent a promising alternative that can address the need for an acute intervention in sporadic disease and to chronically normalize heme pathway activity in recurrent disease. In models of acute AIP, hPBGD mRNA administration led to expression of hPBGD protein in the liver in as little as 2 h, with maintained activity throughout the entire duration of the attack (mean duration of an acute attack in humans is 7 d)[27]. hPBGD mRNA administration rapidly normalized ALA and PBG accumulation in the urine of AIP mice. The duration of effect from a single administration was improved over hemin therapy, which requires recurrent doses to reduce porphyrin precursor accumulation to similar levels (Fig. 4a,b and Supplementary Fig. 6a,b) and to reverse pain symptoms (Fig. 3d) and hypertension (Fig. 3b). In models of acute porphyria (AIP mice and induction of porphyrin precursors using AIA and rifampicin in rabbits) repeated administration of hPBGD mRNA showed sustained therapeutic benefits and was well-tolerated. The translatability of this approach was confirmed in a repeated-dose administration study in NHPs, which demonstrated maintenance of supraphysiologic PBGD activity that was well-tolerated, as shown by no significant alterations in serum liver function tests or antibodies against hPBGD. Therefore, we report sustained efficacy and tolerability of hPBGD mRNA after single and repeated administration in all three species tested (mouse, rabbit, NHP).

The dominant clinical feature of acute and recurrent AIP is the neurovisceral attack, characterized by severe abdominal pain, often complicated by peripheral neuropathy and elevated systolic

blood pressure. In the mouse model of AIP, hPBGD mRNA not only addressed the biochemical abnormalities but also showed the ability to rapidly protect against pain, motor discoordination and disturbances in nerve conduction velocity. hPBGD mRNA also normalized blood pressure abnormalities that are characteristic of an acute attack.

The rapid and sustained correction of symptoms by hPBGD mRNA could help to reduce the duration of inpatient hospitalization during acute attacks, which occur in both sporadic and recurrent forms of AIP disease (5 d, range 3–32, in 26 patients infused with hemin or 4 d, range 2–22, in 15 patients without hemin treatment)[27]. This treatment might also help to reduce pain. Finally, the ability to chronically administer hPBGD mRNA might prevent the need for more invasive interventions to reduce the number of attacks in this subset of patients, including liver transplantation[38] or gene therapy.

In summary, we demonstrate the efficacy and safety of hPBGD mRNA in small and large animals. The approach holds promise as a singular therapeutic intervention that can address both the sporadic and recurrent clinical presentations of AIP.

Received: 21 March 2018; Accepted: 10 August 2018;
Published online: 08 October 2018

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Acknowledgements

P.B., M.A.A. and A.Fo. thank J. Prieto for his enthusiastic and continuous support of our research on porphyria. We thank S. Arcelus and I. Alkain for technical assistance; J. L. Lanciego, A. Rico, L. Guembe and A. Benito for their helpful technical and scientific support with NHPs; P. Harper and E. Sardh for supplying liver explants from patients with porphyria. T1 and T2 mouse strains were provided by U. A. Meyer (Biozentrum of University of Basel, Basel, Switzerland). This study was supported in part by grants from the Spanish Fundación Mutua Madrileña, Spanish Fundación Eugenio Rodríguez Pascual and Spanish Institute of Health Carlos III (FIS) cofinanced by European FEDER funds (grant numbers PI09/02639, PI12/02785, PI15/01951 and PI16/00668 funds). P.B. is supported by a Miguel Servet II (CPII15/00004) contract from Instituto de Salud Carlos III.

Author contributions

L.J., P.B., L.T.G., C.M.L., M.A.A., P.G.V.M. and A.Fo. designed in vitro and animal experiments. L.J., P.B., D.J., A.S., A.Fr., J.-S.P., X.Z. and A.Fo. performed the experiments and processed animal samples and tissues. D.J., A.S., and A.Fo. performed behavior assays in AIP mice and rabbits. E.S. and A.S. performed mitochondrial function studies. K.B. performed IHC analysis. M.A. and A.Fo. performed electrophysiological studies

in AIP mice and rabbits. A.P., A.S., D.J. and A.Fo. performed blood pressure studies. L.J., L.T.G., K.E.B., M.Ka., W.B., S.S., E.S.K., T.S., M.Ke., C.M.L. and P.G.V.M. designed and produced mRNA formulations. L.J., L.T.G., C.M.L., A.Fo. and P.G.V.M. supervised mRNA production and supported administrative, technical and logistic tasks for sending and receiving samples. L.J., P.B., L.T.G. and A.Fo. performed all statistical analyses. L.J., P.B., L.T.G., A.S., D.J., E.S., M.A., A.P., P.G.V.M. and A.Fo. analyzed the data. L.J., P.B., L.T.G., M.A.A., C.M.L., P.G.V.M. and A.Fo. wrote the manuscript, assisted by A.S. and D.J for figures and tables. All authors performed a critical revision of the manuscript for important intellectual content and final approval of the manuscript.

Competing interests

L.J., L.T.G., A.Fr., K.E.B., K.B., M.Ka., W.B., J.-S.P., X.Z., S.S., E.S.K., T.S., M.Ke., C.M.L. and P.G.V.M. are employees of Moderna Therapeutics, Inc. focusing on the development of therapeutic approaches for rare diseases. The other authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41591-018-0199-z.

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Correspondence and requests for materials should be addressed to P.G.V.M. or A.F.

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Methods

Production of LNPs containing modified mRNAs encoding luciferase or the hPBGD protein. The mRNAs encoding luciferase were synthesized in vitro by T7 RNA polymerase-mediated transcription from a linearized DNA template, which incorporates the 5′ and 3′ untranslated regions and a poly-A tail, as previously described[25]. hPBGD mRNA utilized Cap1 and full replacement of uridine with 5-methoxyuridine to increase mRNA potency (Supplementary Fig. 10a,b). After purification, the mRNA was diluted in acetate buffer to the desired concentration and formulated for i.v. delivery[23,24]. For detailed information on mRNA formulation in LNPs, see Supplementary Methods.

Up to 57 different hPBGD mRNA constructs were synthesized and screened in HeLa cells in vitro (1.5 µg RNA transfected for 24 h in HeLa cells plated at $3–4 \times 10^5$ cells per well on six-well plates using Lipofectamine MessengerMAX). PBGD expression was measured by western blot analysis (anti-PBGD antibody ab129092, Abcam) using β-actin as a control protein, and hPBGD mRNA number 8 was selected for use in further in vivo experiments (Supplementary Fig. 1).

Genetic mouse model of AIP. The mouse model of AIP was generated by crossbreeding T1 (C57BL/6 *Pbgd*$^{tm1(neo)Uam}$), and T2 (C57BL/6 *Pbgd*$^{tm2(neo)Uam}$) mouse strains as previously described[26]. T1 and T2 mouse strains were provided by U. A. Meyer (Biozentrum of University of Basel, Basel, Switzerland). Compound heterozygote T1/T2 mice (AIP) exhibit 33% of the normal PBGD activity but low porphyrin precursor accumulation. To overload the deficient enzymatic step and biochemically imitate a human porphyric attack, mice were intraperitoneally (i.p.) injected with four increasing doses of phenobarbital at 24-h intervals (75, 80, 85 and 90 mg kg^{-1} body weight)[19]. Experimental protocols were approved by the Ethics Committee of the University of Navarra (CEEA050–16) and the Institute of Public Health of Navarra (2016/292147) according to European Council Guidelines.

Mice were injected in the tail vein with the indicated doses of hPBGD mRNA in a final volume of 300 µl. Porphyrin precursors (ALA and PBG) were quantified in urine samples 24 h after treatment using a quantitative ion exchange column method[19] and normalized to urine creatinine values. Pain measurement (MGS[28,29]), motor coordination (rotarod test, Ugo Basile 7650) and footprint analysis[20] were performed after the end of each phenobarbital challenge (starting 4 h after the last phenobarbital dose). Electrophysiological studies were performed after recurrent challenges as described elsewhere[19]. Blood pressure was measured using a noninvasive tail-cuff system using an MRBP single-animal tail-cuff blood-pressure multichannel system (IITC Life Science).

All the animals were euthanized the day after the last dose of phenobarbital. Organ samples were stored in cold 0.9% NaCl solution for immediate enzyme determination[19], liquid nitrogen for LC–MS/MS and qPCR analysis and formaldehyde for paraffin preparation. Intrahepatic PBGD distribution was measured by immunohistochemistry (IHC) using a human PBGD antibody (Novus, NBP2-33600, Novus Biologicals). For more detailed information on antibodies, see the Reporting Summary. The antibody was titrated to minimize the staining of endogenous PBGD. Images were captured at 20× magnification with a Pannoramic 250 Flash II (3DHISTECH) digital slide scanner. Image analysis was performed on PBGD IHC images using the 'area quantification' v.1.0 analysis algorithm using the Halo image analysis platform v.2.1.1637.11 (Indica Labs). The percentage of the positive area above a threshold of DAB intensity was calculated. Final readout was the percentage of the positive PBGD area over the total nuclear pixel area plus PBGD pixel area (µm^2).

Mitochondrial function was measured as the OCR of isolated mouse liver mitochondria in the Seahorse XF24 Analyzer. In order to analyze the maximal respiratory capacity driven by complex II–IV in a manner independent of the tricarboxylic acid (TCA) cycle, we added oligomycin, an inhibitor of ATP synthase (complex V)[39], and rotenone and succinate to the reaction to prevent any complex I activity.

Absolute quantification of hPBGD protein was determined by LC–MS/MS. Mouse livers were homogenized in a buffer containing 100 mM ammonium bicarbonate and 8 M urea. Each sample was spiked with an isotopically labeled signature peptide specific for hPBGD (ASYPGLQFEIIAMSTTGDK, natural C and N atoms on lysine are fully replaced by ^{13}C and ^{15}N isotopes, respectively; Thermo Scientific Pierce) as an internal standard. Measurements were performed in a Thermo Easy 1000 nano-UPLC, Orbitrap Fusion Mass Spectrometer as previously described[25].

PBGD activity in tissue homogenates was determined by measuring the conversion of PBG to uroporphyrin[40]. In brief, 1 g tissue was homogenized at 4 °C in four volumes of a 1.15% KCl solution. The homogenate was centrifuged at 12,000 r.p.m. at 4 °C for 20 min, and the clear supernatant without any cellular debris was used the same day for protein determination (Bradford assay, using an albumin standard) and PBGD activity. The supernatant samples were diluted 1:3 with phosphate buffer (pH 7.6), DTT, Cl_2Mg and Triton X-100; and 100 µl of this mixture was preincubated with 1.8 ml of 0.1 M Tris-HCl (pH 8.1) for 3 min at 37 °C. Next, the mixture was incubated in the dark with 0.5 ml PBG substrate 1 mM, for 60 min at 37 °C. The reaction was stopped with 350 µl cold 40% trichloroacetic acid, and the uroporphyrinogen formed was oxidized to uroporphyrin by light exposure. Uroporphyrins were measured quantitatively in a PerkinElmer LS50B spectrofluorometer with an excitation peak (λ_{ext}) at 405 nm and window emission peak (λ_{em}) values between 550 and 660 nm. The PBGD activity was expressed in terms of pmol uroporphyrin per mg protein per h using appropriate standards.

Efficacy studies of hPBGD mRNA administration in large animals. Efficacy studies were also performed in female wild-type rabbits (2.7 kg). A single dose of mRNA (control luciferase or hPBGD at 0.5 mg kg^{-1}, i.v.) in a total volume of 16 ml were injected in awake rabbits through the ear vein at a rate of 2 ml min^{-1}. Rabbits that were infused with the same volume of saline buffer constituted the control group. Bleeding was performed predose, at 2 h and 6 h and at death (24 h post-injection) for clinical chemistry studies in serum samples. Rabbits were fasted overnight and injected with a sedative cocktail of ketamine HCl. Then, the rabbits received i.p. injections of 10 ml of 10% chloral hydrate followed by exsanguination by whole-body perfusion (left ventricle puncture) with 0.5 l Ringer solution at a constant speed of 2 l h^{-1}. Rabbits were euthanized at 24 h after mRNA administration, and samples from four different liver lobes were collected for PBGD activity measurements.

Efficacy was assessed in rabbits challenged with 2-allyl-2-isopropylacetamide (AIA, compound ID: 299–78–5, Wuxi Apptec) (400 mg kg^{-1}, subcutaneous injection) in the morning and the antibiotic rifampicin (sodium rifampicin, Sanofi-Aventis SA) (200 mg kg^{-1}, i.p.) in the afternoon for five consecutive days. Previous studies[41–43] have described AIA as an inducer of acute porphyria attacks in rats[43], and also in rabbits[41] and monkeys[44]. AIA disrupts heme and increase ALAS1 activity. However, the administration of one or two doses of 300–400 mg kg^{-1} of AIA in female New Zealand rabbits (body weight range from 3 to 3.5 kg) (Granja San Bernardo) only showed moderate ALA and PBG accumulation that was rapidly normalized within a few hours after the drug administration. To further increase ALA and PBG accumulation and duration, AIA was administered in synergy with rifampicin for five consecutive days. The porphyrinogenic activity of the rifampicin causes an increased heme demand for hemoprotein induction and a partial block in heme biosynthesis due to reduced protoporphyrinogen oxidase activity (EC 1.3.3.4)[45].

The efficacy of the hPBGD mRNA (0.5 mg kg^{-1}, i.v.) administration after a single ($n = 3$) or multiple doses ($n = 3$) was assessed in female wild-type NHPs (*M. fascicularis*) weighing 3–4 kg. The total volume of the mRNA formulation (5 ml kg^{-1} of body weight) was administered under anesthesia through the leg vein at a rate of 17 ml h^{-1}. As described in the rabbit study, serum ADAs and biochemical parameters were measured at baseline and 2, 6 and 24 h after mRNA administration. Before each blood extraction, a sedative cocktail of ketamine HCl was administered by intramuscular injection. Animals were euthanized 24 h after the start of infusion as described for rabbits. Samples from eight liver lobes were also taken to measure PBGD activity. Baseline hepatic PBGD activity was determined in liver samples from individual animals taken with needle biopsy (16*G* needles from BioPince, Argon Medical Devices) under ultrasound guidance before mRNA administration (Fig. 6a).

Only female rabbits and NHPs were used because acute attacks are more prevalent in women than in men[46]. Experimental protocols in large animals were approved by the Ethics Committee of the University of Navarra (CEEA142-16) and the Institute of Public Health of Navarra, Spain (Doc: 2017/50445) according to European Council Guidelines.

Determination of PBGD activity in human liver biopsies. Human liver biopsies from patients with AIP, obtained after consent from the patients and Institutional Review Board approval, were provided by P. Harper (Karolinska University Hospital, Porphyria Center, Sweden). Healthy liver tissue samples were collected from percutaneous liver biopsies performed because of mild alterations of liver function after obtaining consent from the patients and approval by the Institutional Review Board (University of Navarra, Pamplona, Spain). PBGD activity was measured as described above for animal liver samples.

Statistical analysis and additional resources. Data are shown as mean ± s.d. Statistical analyses were performed using GraphPad Prism v.7.01 (GraphPad Software). Before statistical analysis, data were transformed using the formula $\log(1 + x)$ to normalize the variances. Comparisons between two groups were analyzed by Student's *t*-tests. In the case of comparisons between more than two groups, data were analyzed using one or two-way ANOVA, and pairwise comparisons were made using Bonferroni's multiple comparison tests. $P < 0.05$ was used to indicate statistically significant differences.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Corresponding author(s): Paolo G.V. Martini (E-mail: Paolo.Martini@modernatx.com) , Antonio Fontanellas (E-mail: afontanellas@unav.es)
NMED-A91084

Reporting Summary

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	GraphPad Prism® 7.01 (GraphPad Software, Inc., La Jolla, CA), Microsoft Excel 2010
Data analysis	GraphPad Prism® 7.01 (GraphPad Software, Inc., La Jolla, CA), Halo™ image analysis platform V2.1.1637.11 Area Quantification V1.0 analysis algorithm

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
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Publicly available datasets or restrictions on data availability: Not applied. None of the figures have associated raw data.

Field-specific reporting

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Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	To calculate the sample size we used the computer application: http://www.biomath.info/power/ttest.htm
Data exclusions	None
Replication	All studies have been repeated at least twice except those assays performed in rabbits and non-human primates.
Randomization	The animals were randomly assigned to the groups.
Blinding	This manuscript focused on preclinical. Assays were not blind because the administration and sample collection and processing carried out by the same researchers.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☐	☒ Human research participants

Unique materials

Obtaining unique materials	The mRNAs encoding luciferase were synthesized in vitro by T7 RNA polymerase-mediated transcription from a linearized DNA template, which incorporates the 5′ and 3′ untranslated regions (UTRs) and a poly-A tail. hPBGD mRNA utilized Cap1 and full replacement of uridine with 5-methoxyuridine to increase mRNA potency. After purification the mRNA was diluted in acetate buffer to the desired concentration and formulated for i.v. delivery. Lipid nanoparticle formulations were prepared using a modified procedure of a method previously described for siRNA. Briefly, heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate was dissolved in ethanol at a molar ratio of 50:10:38.5:1.5 (ionizable lipid:DSPC:cholesterol:PEGDMG). The lipid mixture was combined with a 6.25 mM sodium acetate buffer (pH 5) containing mRNA at a ratio of 3:1 (aqueous:ethanol) (N:P = 5.67) using a microfluidic mixer (Precision Nanosystems, Vancouver, BC, Canada). Formulations were dialyzed against phosphate-buffered saline (pH 7.4) in dialysis cassettes for at least 18 h. Formulations were concentrated using Amicon ultra centrifugal filters (EMD Millipore, Billerica, MA, USA), passed through a 0.22-µm filter, and stored at 4 °C until use. All formulations were tested for particle size, RNA encapsulation, and endotoxin and were found to be between 80 nm – 100 nm in size, with greater than 90% encapsulation and <1 EU/mL endotoxin. Alternatively 2-(dinonylamino)-1-(4-(N-(2-(dinonylamino)ethyl)-N-nonylglycyl)piperazin-1-yl)ethan-1-one was employed as the ionizable lipid at molar ratios between 30 and 40%.

Antibodies

Antibodies used	human PBGD antibody (Lot. R60556, Novus, NBP2-33600, Novus Biologicals, Littleton, USA). Online database: https://www.novusbio.com/products/hmbs-antibody_nbp2-33600). Suitable for IHC. Human HMBS antibody (Lot: GR97596-9 (0.165 mg/ml, Abcam ab129092). Online database: http://www.abcam.com/hmbsantibody-epr8105-ab129092.html Suitable for western blot.
Validation	Novus, NBP2-33600: This antibody was developed against a recombinant protein corresponding to amino acids: EFSAIILATAGLQRMGWHNRVGQILHPEECMYAVGQGALGVEVRAKDQDILDLVGVLHDPETLLRCIAERAFLRHLEGGCSVPVAVHTAMKDGQLY LTGGVWSLDGSDSIQETMQATIHVPAQHEDGPEDDPQLV. Specificity of human HMBS antibody verified on a Protein Array containing target protein plus 383 other non-specific proteins. Human (100%), Mouse (93%), Rat (94%). Isotype: IgG. Polyclonal. Immunogen affinity purified. Host: rabbit. Human HMBS antibody (Abcam ab129092): This product is a recombinant rabbit monoclonal antibody. Immunogen: Synthetic

peptide corresponding to residues in Human HMBS (P08397). Rabbit monoclonal to HMBS. Species reactivity: Reacts with: Mouse, Rat, Human. Clone number: EPR8105.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HeLa cell lines from ATCC
Authentication	None of the cell lines used here were authenticated.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	HeLa cells were used in this study for an initial in vitro screening.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials	Male and female mice [C57BL/6, wild type, T1 (C57BL/6-pbgdtm1(neo)Uam), and T2 (C57BL/6-pbgdtm2(neo)Uam)] at 6-7 weeks old, female wild-type rabbits with mean weights of 2.7 kg (New Zealand), female wild-type non-human primates weighing 3 to 4 kg (Macaca fascicularis) were used. Experimental protocols were approved by the Ethics Committee of the University of Navarra (CEEA050-16 for mice and CEEA142-16 for Rabbits and non human primates) and the Institute of Public Health of Navarra (Doc: 2016/292147 and Doc: 2017/50445) according to European Council Guidelines.

Human research participants

Policy information about studies involving human research participants

Population characteristics	The characteristics of the two patients with recurrent active chronic AIP are described in Wahlin S et al. Transpl Int. 2010 Jun;23(6):e18-21. Briefly, the first AIP patient was a 24-year-old woman and the second AIP patient was a 55-year-old woman. Both of them had pronounced neuropathy and renal impairment and they underwent a liver-renal transplantation. Healthy liver tissue samples were collected from percutaneous liver biopsies performed because of mild alterations of liver function. Recruitment: Liver explant from AIP patients were obtained after liver-renal transplantations. Healthy liver explants were collected due to mild alterations of liver function. All samples were obtained after patients' consent and institutional review board approval.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging