ORIGINAL ARTICLE

Safety and Efficacy of Mitapivat in Pyruvate Kinase Deficiency

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ABSTRACT

BACKGROUND

Pyruvate kinase deficiency is caused by mutations in *PKLR* and leads to congenital hemolytic anemia. Mitapivat is an oral, small-molecule allosteric activator of pyruvate kinase in red cells.

METHODS

In this uncontrolled, phase 2 study, we evaluated the safety and efficacy of mitapivat in 52 adults with pyruvate kinase deficiency who were not receiving red-cell transfusions. The patients were randomly assigned to receive either 50 mg or 300 mg of mitapivat twice daily for a 24-week core period; eligible patients could continue treatment in an ongoing extension phase.

RESULTS

Common adverse events, including headache and insomnia, occurred at the time of drug initiation and were transient; 92% of the episodes of headache and 47% of the episodes of insomnia resolved within 7 days. The most common serious adverse events, hemolytic anemia and pharyngitis, each occurred in 2 patients (4%). A total of 26 patients (50%) had an increase of more than 1.0 g per deciliter in the hemoglobin level. Among these patients, the mean maximum increase was 3.4 g per deciliter (range, 1.1 to 5.8), and the median time until the first increase of more than 1.0 g per deciliter was 10 days (range, 7 to 187); 20 patients (77%) had an increase of more than 1.0 g per deciliter in the hemoglobin level at more than 50% of visits during the core study period, with improvement in markers of hemolysis. The response was sustained in all 19 patients remaining in the extension phase, with a median follow-up of 29 months (range, 22 to 35). Hemoglobin responses were observed only in patients who had at least one missense *PKLR* mutation and were associated with the red-cell pyruvate kinase protein level at baseline.

CONCLUSIONS

The administration of mitapivat was associated with a rapid increase in the hemoglobin level in 50% of adults with pyruvate kinase deficiency, with a sustained response during a median follow-up of 29 months during the extension phase. Adverse effects were mainly low-grade and transient. (Funded by Agios Pharmaceuticals; ClinicalTrials.gov number, NCT02476916.)

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Pyruvate kinase deficiency is an autosomal recessive enzymopathy in red cells that is caused by mutations in *PKLR*. These genetic alterations lead to a deficit of pyruvate kinase activity in red cells and to hemolytic anemia of variable severity.[1-8] In addition to anemia, pyruvate kinase deficiency is associated with serious complications that include gallstones, pulmonary hypertension, extramedullary hematopoiesis, and iron overload and its sequelae, which occur regardless of the degree of anemia or transfusion burden.[9,10] Patients most commonly have compound heterozygous mutations in the gene encoding the L and R isozymes of pyruvate kinase (*PKLR*), with more than 300 mutations described; most patients have at least one missense mutation.[9,11] Red-cell pyruvate kinase deficiency results in impaired glucose utilization and reduced ATP generation in red cells, which leads to compromised red-cell membrane homeostasis and hemolysis.[1,12-14]

Current management strategies, including blood transfusion and splenectomy, are supportive only and introduce both short- and long-term risks.[9,15-19] Hematopoietic stem-cell transplantation has been described in a small number of patients but has been associated with substantial risks of graft-versus-host disease and death.[20] No specific disease-modifying therapy exists.

Mitapivat (AG-348) is an oral, small-molecule allosteric activator of red-cell pyruvate kinase.[21] In vitro experiments have shown that mitapivat activates wild-type and a variety of mutant red-cell pyruvate kinase enzymes. Mitapivat increases pyruvate kinase activity ex vivo in red cells obtained from patients with pyruvate kinase deficiency.[21] In a dose-escalation study involving healthy volunteers,[22] investigators reported an acceptable safety profile and changes in glycolytic intermediates that were consistent with glycolytic pathway activation, findings that supported further investigation of mitapivat as a potential targeted treatment for pyruvate kinase deficiency. In this phase 2, multicenter study, we evaluated the safety and efficacy of mitapivat in adults with pyruvate kinase deficiency who were not receiving regular red-cell transfusions.

METHODS

STUDY DESIGN AND OVERSIGHT

We conducted the study at eight sites in North America and six sites in Europe. After a screening period of no more than 6 weeks, patients were randomly assigned in a 1:1 ratio to receive open-label mitapivat at a dose of 50 mg or 300 mg twice daily for a 24-week core period. The selection of the two doses was based on the results of a dose-escalation study involving healthy volunteers.[22] Randomization was stratified according to the *PKLR* mutation (R510Q vs. R486W vs. R479H vs. all other mutations) to maintain balance for the most frequently expected mutations. Eligible patients could opt to continue treatment in an extension phase, which is ongoing.

The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol (available with the full text of this article at NEJM.org) was approved by the institutional review board or ethics committee at each study center. (Details are provided in the Supplementary Appendix, available at NEJM.org.) All the patients provided written informed consent before screening. The study was designed and analyzed by the sponsor (Agios Pharmaceuticals) in collaboration with the authors. A data and safety monitoring board consisting of the treating investigators and representatives of the sponsor reviewed the data on an ongoing basis. The manuscript was drafted by the first and last authors; all the authors contributed to reviews and revisions. A professional medical writer paid by the sponsor assisted the authors in the preparation of the manuscript. The authors vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

PATIENTS

Adults (≥18 years of age) were eligible for participation if they had received a diagnosis of pyruvate kinase deficiency, as documented by lower activity of pyruvate kinase than that of other age-dependent enzymes in red cells and by the presence of at least two mutations in *PKLR* on genotyping. The hemoglobin level was 12.0 g per deciliter or less in men and 11.0 g per deciliter or less in women. None of the patients had received transfusions of more than 3 units of red cells in the previous 12 months or any transfusions in the previous 4 months.

DOSE SELECTION

Changes in dose were permitted during the core period on the basis of safety, side-effect profile, and hemoglobin response. Increases in dose were

allowed if the hemoglobin level remained below the lower limit of the normal range (<13.0 g per deciliter in men and <11.6 g per deciliter in women) after at least 12 weeks of treatment. Dose decreases were allowed for adverse events thought to be related to mitapivat or if the hemoglobin level exceeded the midpoint of the normal range (>15.0 g per deciliter in men and >13.5 g per deciliter in women). Early in the trial, two patients had acute hemolysis after the abrupt discontinuation of the 300-mg dose of mitapivat following a rapid increase in the hemoglobin level. An additional seven patients for whom dose reduction was necessary because of a robust hemoglobin response underwent dose tapering, according to a protocol amendment, without acute hemolysis.

Patients initially were eligible for the extension phase for continued evaluation of safety if they did not have side effects that precluded the continued administration of mitapivat and were having clinical benefit, as determined by the investigator. Subsequently, after a protocol amendment, patients who did not have an increase from baseline of at least 1.0 g per deciliter in the hemoglobin level as evaluated in at least three of the last four measurements were withdrawn from the extension phase of the study. Patients who continued in the extension phase and were receiving doses of more than 25 mg of mitapivat twice daily underwent a tapering of the dose and continued to receive an individualized dose that maintained the hemoglobin level at a value that was no lower than 1.0 g per deciliter below the value that was reported before the taper began.

END POINTS

The primary objective of this study was to assess the safety and side-effect profile of mitapivat administration in patients with pyruvate kinase deficiency. We assessed safety by monitoring the incidence of adverse events, laboratory measurements (hematologic and chemical analyses, urinalysis, coagulation, and endocrine analysis), and findings on physical examination, 12-lead electrocardiography, and dual-energy x-ray absorptiometry (DXA) scans. Serum levels of sex hormones (testosterone, estradiol, and estrone) and DXA scans were monitored because of reversible, mild, off-target aromatase inhibition that had been observed in preclinical studies (authors' unpublished data) and in healthy volunteers.[22] We also evaluated adverse events, treatment-related adverse events, serious adverse events, and specific events of interest (acute hemolysis after abrupt mitapivat discontinuation, osteoporosis, elevations in liver enzymes, hypertriglyceridemia, and insomnia).

Secondary objectives were characterizations of the pharmacokinetic and pharmacodynamic profiles of mitapivat and clinical efficacy, as measured by changes in hemoglobin and markers of hemolysis. Baseline levels of pyruvate kinase protein in red cells were measured as described previously.[21]

STATISTICAL ANALYSIS

For the primary evaluation of the safety and side-effect profile of mitapivat, we determined that the enrollment of 25 patients in each of the two dose groups would provide a probability of 72% of observing a rate of adverse events of 5% in either group and a 93% probability of observing a rate of 10%. The safety analysis included all the enrolled patients who had received at least one dose of mitapivat. We summarized adverse events and other categorical safety measurements according to frequency distributions for the overall population and randomly assigned starting dose.

The efficacy analysis included all the patients who had received mitapivat for at least 3 weeks. Analyses of hemoglobin response included the number and proportion of patients who had an increase of more than 1.0 g per deciliter in the postbaseline hemoglobin level in more than 50% of assessments during the core period. In a post hoc analysis, these patients were defined as having had a hemoglobin response. For analyses of the hemoglobin response according to genotype, *PKLR* mutations were categorized into missense mutations and non-missense mutations, such as truncations or frameshift mutations. To account for dose modifications, we conducted some analyses according to the dose of mitapivat that the patients received for the longest duration in the core period (called the actual dose) rather than according to the randomly assigned dose.

RESULTS

PATIENTS

Of the 65 patients who had undergone screening, 52 were eligible for participation and were randomly assigned to receive mitapivat twice daily at a dose of 50 mg (27 patients) or 300 mg (25 patients)

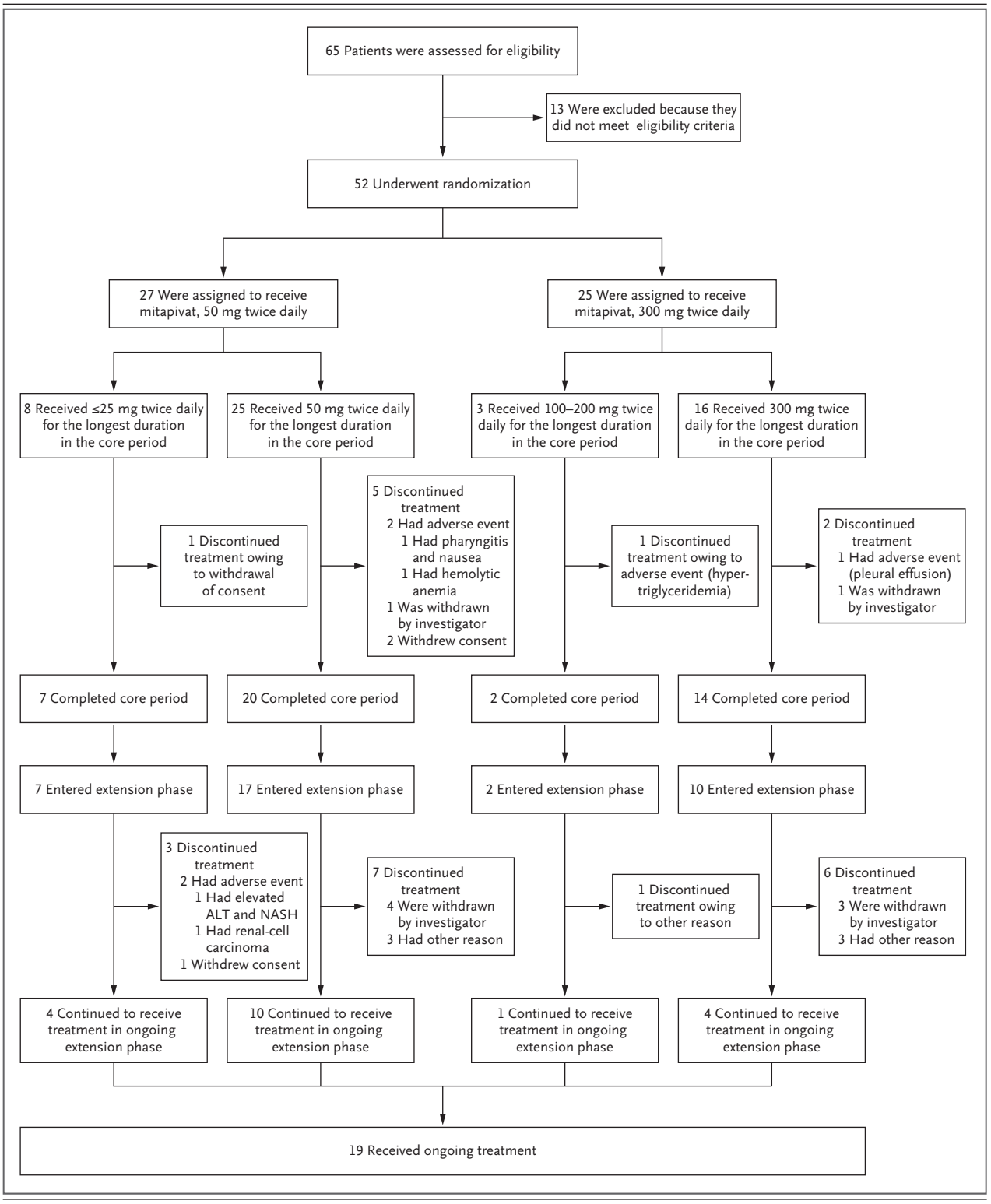

Figure 1 (facing page). Enrollment during Core Period and Extension Phase, According to Actual Dose of Mitapivat.

Shown are the numbers of patients who received the listed doses of mitapivat for the longest duration during the core period of the study, which was defined as the actual dose. According to the protocol, dose adjustments were allowed for reasons of safety, side-effect profile, and hemoglobin response. Provided in the Supplementary Appendix are a summary of dose adjustments (Table S1) and patients' assignments according to the randomized dose (Fig. S1). Of the 13 patients who were excluded during screening, 1 patient withdrew consent; 1 withdrew consent and had a concurrent medical condition; 3 had a concurrent medical condition, although 1 of these patients was successfully rescreened and enrolled; 1 was undergoing transfusion; 6 had a hemoglobin level that exceeded the entry criterion; and 1 did not complete screening assessments in the allotted time, although this patient was successfully rescreened and enrolled. ALT denotes alanine aminotransferase, and NASH nonalcoholic steatohepatitis.

(Fig. 1, and Fig. S1 in the Supplementary Appendix). Of the 52 patients, 43 (83%) completed the 24-week core period; 36 patients (69%) entered the extension phase of the study, and 19 (37%) continue to participate in the ongoing extension, with a median treatment duration of 29 months (range, 22 to 35 months) (Table 1). The doses of mitapivat that were administered and the rate of treatment adherence are summarized in Table S1 in the Supplementary Appendix.

The demographic and baseline clinical characteristics of the patients are shown in Table 1. The relative prevalence of *PKLR* mutation types was consistent with the literature, with 81% of the patients having at least one missense mutation. The median hemoglobin level at baseline was 8.9 g per deciliter (range, 6.5 to 12.3); 48% of the patients had a history of treatment with iron chelation despite the absence of regular red-cell transfusions. The majority of patients had undergone splenectomy (83%) and cholecystectomy (73%).

SAFETY

All 52 of the patients who were included in the safety analysis had at least one adverse event, the majority of which were grade 1 or 2 in severity (Table 2). The most common adverse events were headache (in 24 patients), insomnia (in 22 patients), and nausea (in 21 patients). These events resolved within 7 days after the initiation of treatment in 60 of 65 episodes (92%) of headache, in 16 of 34 episodes (47%) of insomnia, and in 25 of 32 episodes (78%) of nausea. Headache and insomnia most often occurred within 2 weeks after drug initiation. Of the 52 patients, 19 (37%) had adverse events of grade 3 or higher, as reported by the investigator. Of these patients, 9 (17%) had 11 events that were deemed by the investigator to be possibly or probably related to mitapivat, including hypertriglyceridemia (in 4 patients [6%]), hemolytic anemia (in 2 [4%]), and hemolysis, dizziness, headache, renal-cell carcinoma in the left kidney, and insomnia (in 1 each [2%]).[23] The patient with renal-cell carcinoma had a kidney lesion that had been present at the time of enrollment and was identified retrospectively.

A total of 18 serious adverse events were reported in 15 patients, all as single events with the exception of pharyngitis and hemolytic anemia (in 2 patients each [4%]). With the exception of nasopharyngitis, the cumulative safety profile (during the core phase plus the extension phase) remained similar to that observed in the core period, which indicated no change in the safety profile to date with extended treatment.

Changes from baseline in sex hormone levels, the result of off-target aromatase inhibition, were observed in male patients, with most levels of testosterone and estradiol remaining within the normal range (Fig. S2 in the Supplementary Appendix).[22] Hormone levels returned to baseline in 4 patients who discontinued mitapivat for reasons unrelated to aromatase inhibition and for whom samples were available, a finding that was consistent with the reversibility that had been observed in healthy volunteers previously.[22] In the female patients, the interpretation of data with respect to sex hormones was confounded by variability in menopausal status and the use of hormonal contraception; these data are the subject of further investigation.

In 49 patients who were evaluated, there was no worsening of bone mineral density as determined with the use of DXA scans of the total

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

Characteristic	Mitapivat, 50 mg Twice Daily (N=27)	Mitapivat, 300 mg Twice Daily (N=25)	All Patients (N=52)
Sex — no. (%)			
Female	9 (33)	11 (44)	20 (38)
Male	18 (67)	14 (56)	32 (62)
Median age (range) — yr	28 (18–58)	40 (20–61)	34 (18–61)
Race — no. (%)†			
White	22 (81)	21 (84)	43 (83)
Asian	2 (7)	1 (4)	3 (6)
Not reported	2 (7)	1 (4)	3 (6)
Other	1 (4)	2 (8)	3 (6)
PKLR mutation type — no. (%)			
Missense/missense	15 (56)	17 (68)	32 (62)
Missense/non-missense	6 (22)	4 (16)	10 (19)
Non-missense/non-missense	6 (22)	4 (16)	10 (19)
Median hemoglobin (range) — g/dl	9.6 (6.9–12.3)	8.6 (6.5–12.0)	8.9 (6.5–12.3)
Splenectomy — no. (%)‡	23 (85)	20 (80)	43 (83)
Cholecystectomy — no. (%)	19 (70)	19 (76)	38 (73)
Chelation therapy before enrollment — no. (%)	14 (52)	11 (44)	25 (48)
Median ferritin (range) — ng/ml	723 (41–3254)	775 (346–2518)	764 (41–3254)
Osteoporosis — no. (%)	5 (19)	3 (12)	8 (15)
Completion of 24-wk core period — no. (%)§	21 (78)	22 (88)	43 (83)

* *PKLR* encodes pyruvate kinase L and R isozymes. Percentages may not total 100 because of rounding.
† Race was reported by the patients. The 3 patients who selected "other" race were from North and West Africa.
‡ The type of splenectomy was not reported for 2 patients; all the others underwent a complete splenectomy.
§ The median treatment duration for the 52 patients who enrolled in the 24-wk core period was 16.4 months (range, 3.0 to 34.8). As of the data cutoff on August 31, 2018, the median treatment duration since randomization for the 19 patients who continued in the open-label extension phase was 28.9 months (range, 21.6 to 34.8).

hip, total lumbar spine, and femoral neck, which were obtained and interpreted locally during screening and during treatment over a median of 17 months (range, 4 to 30) (Table S2 in the Supplementary Appendix).

EFFICACY

Of the 52 patients, 26 (50%) had an increase from baseline of more than 1.0 g per deciliter in the hemoglobin level (Table S3 and Fig. S3 in the Supplementary Appendix). Among these patients, the mean maximum increase in the hemoglobin level was 3.4 g per deciliter (range, 1.1 to 5.8). The median time until the first observed increase of more than 1.0 g per deciliter in the hemoglobin level was 10 days (range, 7 to 187). Of the 26 patients, 20 (77%) had an increase from baseline of more than 1.0 g per deciliter at more than 50% of the assessments in the core period, which met the definition of a hemoglobin response. The hemoglobin response was maintained in the 19 patients who were continuing to be treated in the extension phase, all of whom had at least 21.6 months of treatment (Fig. S4 in the Supplementary Appendix). Details regarding the hemoglobin response and the baseline characteristics of the patients who had a response are provided in Tables S4 and S5 in the Supplementary Appendix.

A relationship between genotype and hemoglobin response was observed (Fig. 2A; and Tables S5, S6, and S7 in the Supplementary Appen-

dix). All the patients who had an increase from baseline of more than 1.0 g per deciliter had at least one missense mutation; none of the 10 patients who had two non-missense mutations and none of the 5 patients who were homozygous for R479H mutations (most common in the Amish population) had this level of hemoglobin response. Although the R479H mutation is a missense mutation, it leads to aberrant splicing owing to the location of the mutation at a splicing site.[24] A hemoglobin response occurred in 20 of 52 patients (38%; 95% confidence interval [CI], 25 to 53). After the removal of the 10 patients who had two non-missense mutations, a hemoglobin response occurred in 20 of 42 patients (48%; 95% CI, 32 to 64); with further removal of the 5 patients who were homozygous for R479H mutations, a hemoglobin response occurred in 20 of 37 patients (54%).

A relationship was observed between the level of pyruvate kinase protein in red cells at baseline and the hemoglobin response (Fig. 2B). The mean (±SD) residual level of pyruvate kinase protein in red cells at baseline was 59±33% among the patients who had a hemoglobin response and 14±21% among those without a hemoglobin response.

No definitive relationship was apparent between the randomly assigned dose and the likelihood of a hemoglobin response. The actual dose received by 80% of the patients with a hemoglobin response was 50 mg or less twice daily, whereas 15% of the patients with a hemoglobin response received an actual dose of 300 mg twice daily, which shows that a hemoglobin response can be achieved with a low dose of mitapivat (Table S4 in the Supplementary Appendix). For some patients with a hemoglobin response, the magnitude of the change in the hemoglobin level varied according to the dose. However, during the extension phase, 14 patients who were receiving twice-daily doses of more than 25 mg of mitapivat underwent a dose taper; the hemoglobin levels in 11 of these patients were maintained at the lower doses. One of these patients returned to a dose of 300 mg to sustain the hemoglobin response after a dose taper in the extension phase (see Table S1 in the Supplementary Appendix). In 4 patients who did not have a hemoglobin response, the twice-daily dose was increased from 50 mg to 300 mg; however, none of these patients met the definition of having had a hemoglobin response at the higher dose. In 9 patients, the dose of mitapivat was reduced owing to a rapid hemoglobin response.

In the patients with a hemoglobin response, directionally appropriate changes in other indicators of clinical activity over time (e.g., absolute reticulocyte count, indirect bilirubin, lactate dehydrogenase, and haptoglobin) provide additional evidence of decreased hemolysis (Fig. 3, and Table S8 in the Supplementary Appendix), changes that are consistent with the expected mechanism of action of mitapivat. Decreased indirect bilirubin levels suggest an independent effect of mitapivat on bilirubin metabolism.

PHARMACOKINETICS AND PHARMACODYNAMICS

Mitapivat exposure in patients in this study was consistent with expectations on the basis of the phase 1 study in healthy volunteers (Table S9 in the Supplementary Appendix).[22] No difference in exposure was seen in patients on the basis of the hemoglobin response. In this study, there were no significant trends regarding whole-blood ATP or 2,3-diphosphoglycerate metabolite levels in patients with a hemoglobin response, a finding that contrasted with the results in healthy volunteers (Fig. S5 in the Supplementary Appendix). Changes in the average age of red cells associated with a reduction in the number of reticulocytes and reduced hemolysis could contribute to this observation, since reticulocytes have higher ATP levels than older blood cells.[25]

DISCUSSION

Mitapivat is a small-molecule, allosteric activator of pyruvate kinase in red cells with a side-effect profile that allowed continued administration in 46 of 52 patients (88%) with pyruvate kinase deficiency in this phase 2 study. Treatment with mitapivat was associated with a rapid, clinically significant increase in the hemoglobin level in approximately half the treated patients. The hemoglobin response was sustained for up to 35 months with ongoing mitapivat administration and was associated with improvement in laboratory markers of hemolysis. This study establishes proof of concept for a molecular therapy targeting the underlying enzymatic defect of a hereditary enzymopathy.

Table 2. Adverse Events.*

Adverse Event	Core Period			Core Period plus Extension Phase
	Mitapivat, 50 mg Twice Daily (N=27)	Mitapivat, 300 mg Twice Daily (N=25)	All Patients (N=52)	All Patients (N=52)
Summary of adverse events — no. of patients (%)				
At least one adverse event	26 (96)	25 (100)	51 (98)	52 (100)
At least one adverse event of grade ≥3	4 (15)	7 (28)	11 (21)	19 (37)
Grade 3	3 (11)	6 (24)	9 (17)	16 (31)†
Grade 4	1 (4)	1 (4)	2 (4)	3 (6)‡
At least one serious adverse event	5 (19)	3 (12)	8 (15)	15 (29)
At least one adverse event leading to treatment discontinuation	2 (7)	2 (8)	4 (8)	6 (12)§
Most common adverse events — no. of patients (%)				
Headache	9 (33)	14 (56)	23 (44)¶	24 (46)
Insomnia	5 (19)	16 (64)	21 (40)‖	22 (42)
Nausea	10 (37)	10 (40)	20 (38)	21 (40)
Nasopharyngitis	7 (26)	2 (8)	9 (17)	16 (31)
Hot flush	2 (7)	7 (28)	9 (17)**	9 (17)
Arthralgia	5 (19)	3 (12)	8 (15)	9 (17)
Fatigue	4 (15)	4 (16)	8 (15)	9 (17)
Vomiting	2 (7)	5 (20)	7 (13)	9 (17)
Diarrhea	3 (11)	3 (12)	6 (12)	9 (17)
Influenza	6 (22)	1 (4)	7 (13)	9 (17)
Cough	4 (15)	4 (16)	8 (15)	8 (15)
Dizziness	5 (19)	2 (8)	7 (13)	8 (15)
Oropharyngeal pain	3 (11)	4 (16)	7 (13)	8 (15)
Pyrexia	1 (4)	5 (20)	6 (12)	8 (15)
Serious adverse events — no. of events (%)				
Pharyngitis	1 (4)	1 (4)	2 (4)	2 (4)
Hemolytic anemia	1 (4)††	1 (4)‡‡	2 (4)	2 (4)
Cholelithiasis	1 (4)	0	1 (2)	1 (2)
Hemolysis	0	1 (4)‡‡	1 (2)	1 (2)
Hypertriglyceridemia	0	1 (4)	1 (2)	1 (2)
Influenza	1 (4)	0	1 (2)	1 (2)
Osteoporosis	1 (4)	0	1 (2)	1 (2)
Cellulitis	0	0	0	1 (2)
Gastroenteritis	0	0	0	1 (2)
Colitis	0	0	0	1 (2)
Enteritis	0	0	0	1 (2)
Mesenteric-vein thrombosis	0	0	0	1 (2)§§
Postprocedural hemorrhage	0	0	0	1 (2)¶¶
Ovarian cyst	0	0	0	1 (2)
Left renal-cell carcinoma	0	0	0	1 (2)‖‖
Inguinal hernia	0	0	0	1 (2)

Table 2. (Continued.)

* Adverse events are listed according to the number of patients who had the highest grade for a particular event. The most common adverse events occurred in at least 15% of the patients in the overall population.
† Grade 3 adverse events were colitis, diarrhea, dizziness, headache, hemolysis, hypertension, left renal-cell carcinoma, and postprocedural hemorrhage (in one patient each); hemolytic anemia and insomnia (in two patients each); and hypertriglyceridemia and nasopharyngitis (in three patients each).
‡ Grade 4 adverse events were hypertriglyceridemia, influenza, and mesenteric-vein thrombosis (in one patient each).
§ The adverse events that led to treatment discontinuation were an increase in the alanine aminotransferase level plus nonalcoholic steatohepatitis, hemolytic anemia, nausea plus pharyngitis, hypertriglyceridemia, left renal-cell carcinoma, and pleural effusion (in one patient each).
¶ Headache was transient and generally resolved within several days.
‖ Insomnia typically occurred within 14 days after the initiation of mitapivat, was self-resolving (generally in <7 days), and was not unexpected on the basis of off-target antagonistic or inverse agonist activity against the histamine H3 receptor.[23]
** Hot flush events were transient and generally reported within the first 7 days after treatment initiation and resolved without treatment within 3 days. Events did not correspond to changes in hormone levels or correlate with age or sex.
†† One patient had a progressive worsening of hemolysis and no hemoglobin response despite an increase in the dose of mitapivat to 300 mg twice daily and blood transfusions.
‡‡ After abrupt withdrawal of mitapivat, one patient had two serious adverse events, which were reported sequentially as hemolysis followed by hemolytic anemia.
§§ Mesenteric-vein thrombosis was reported in a patient who had a history of hepatic portal-vein thrombosis.
¶¶ One patient had persistent bleeding after tonsillectomy.
‖‖ One patient had an existing renal lesion before enrollment in the study. The renal-cell carcinoma did not grow at an abnormally rapid rate during the study, according to expert review. Nevertheless, it was assessed by the investigator as being associated with mitapivat.

Grade 3 or greater adverse effects that were considered by the investigator to be related to mitapivat were seen in 17% of patients who were treated daily for up to 35 months. The most commonly reported adverse events occurred soon after drug initiation and were transient. Doses of mitapivat that were significantly lower than the initial doses were associated with hemoglobin responses. Changes in sex hormone levels stayed mainly within normal ranges and did not correlate with adverse events or changes in bone mineral density.

The safety profile that was shown in this study provides equipoise for studies of mitapivat as a disease-altering therapy for patients with pyruvate kinase deficiency, who are likely to undergo therapy for years. The efficacy and safety of mitapivat continue to be studied in the extension phase as well as in two ongoing phase 3 studies (ClinicalTrials.gov numbers, NCT03559699 and NCT03548220). An exposure–response analysis of the pharmacokinetic, efficacy, and safety data from this study was used to determine the most effective strategy for individualizing doses in these trials, in which patients start at a low twice-daily dose of 5 mg, with two sequential steps for escalation to 20 mg and 50 mg on the basis of the achievement of a hemoglobin response without exceeding the upper limit of the normal range.

Preclinical data have shown that mitapivat activates pyruvate kinase activity in vitro across a broad spectrum of *PKLR* mutations, a finding that was consistent with the known binding site for mitapivat, which is distinct from the areas of the most common *PKLR* mutations.[21] In this study, mitapivat administration resulted in a robust and sustained hemoglobin response in patients with diverse *PKLR* genotypes, all of whom had at least one *PKLR* missense mutation. This result suggests that patients with at least one missense *PKLR* mutation are more likely than patients with two non-missense mutations to have a hemoglobin response to mitapivat. Most patients with pyruvate kinase deficiency have compound heterozygous *PKLR* alterations with at least one missense mutation. Therefore, mitapivat may have the potential to increase hemoglobin levels in the majority of patients with this disease.[5] This prediction requires prospective testing in patients across a broader range of genotypes and disease severity — patients who are being included in ongoing clinical trials. Patient-reported quality of life was not assessed in this phase 2 safety study, although such outcome measures are being evaluated in the ongoing phase 3 trials.

Since mitapivat directly binds and activates residual mutant red-cell pyruvate kinase enzyme, it is hypothesized that a minimal level of full-length red-cell pyruvate kinase protein may be required for pyruvate kinase activation by mitapivat. In this study, a hemoglobin response was associated with the residual level of pyruvate

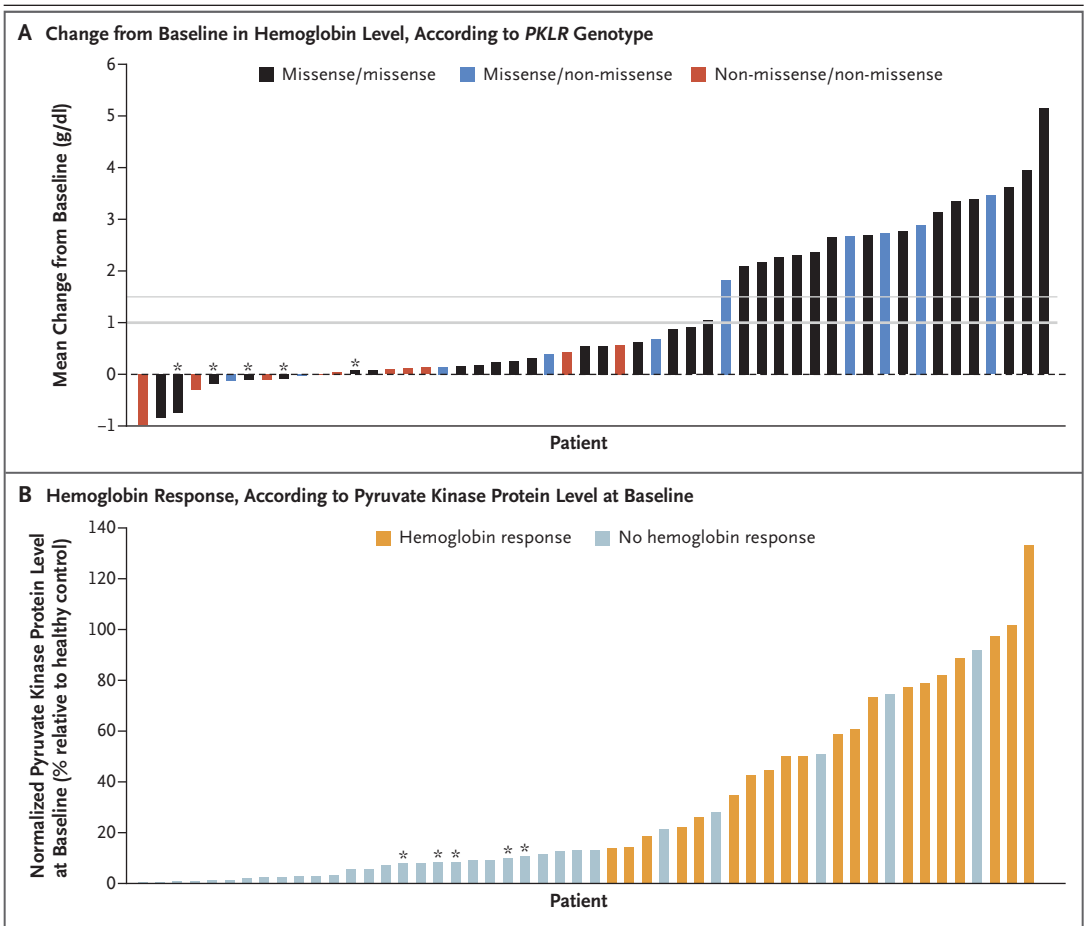

Figure 2. Change in Hemoglobin According to *PKLR* Genotype and Hemoglobin Response According to Pyruvate Kinase Protein Level at Baseline.

Panel A shows the mean change from baseline in the hemoglobin level during the 24 weeks of the core period, according to the patient's *PKLR* genotype category. Of the 52 patients who received mitapivat, 20 (38%) — all of whom had at least one missense mutation — had a mean increase of more than 1.0 g per deciliter in the hemoglobin level (indicated by a thick gray line); 19 patients had a mean increase of at least 1.5 g per deciliter (thin gray line) (Table S6 in the Supplementary Appendix). A mean increase of more than 1.0 g per deciliter in the hemoglobin level did not occur in any of the 5 patients who were homozygous for the R479H mutation (as indicated by an asterisk) or in any of the 10 patients who were homozygous for non-missense mutations (as indicated by a red bar). Panel B shows the patients who had a hemoglobin response (defined as an increase from baseline of >1.0 g per deciliter in the hemoglobin level at >50% of the assessments during the core period) and those who did not have a response, according to the baseline level of pyruvate kinase protein in red cells, a value that has been normalized to the hemoglobin level (an approximation of the total red-cell protein level) and to the pyruvate kinase protein level measured in a sample obtained from a healthy control (to allow for interassay comparisons). Patients with an increased baseline level of pyruvate kinase protein were more likely to have a hemoglobin response to mitapivat. However, since there were exceptions to this observation, the baseline level of pyruvate kinase protein is not a consistent predictor of hemoglobin response. Patients who were homozygous for the R479H mutation are indicated by asterisks. In 1 patient, the baseline level of red-cell pyruvate kinase protein was not available, so the day 15 sample was used. The data bars in Panels A and B are not aligned for each patient, so a 1:1 comparison of the individual data bars in the two panels is not possible.

kinase protein in red cells at baseline. This finding is consistent with previous ex vivo observations and provides further evidence that mitapivat is working by means of its proposed mechanism of action. Nevertheless, differing degrees of hemoglobin response in patients with equivalent

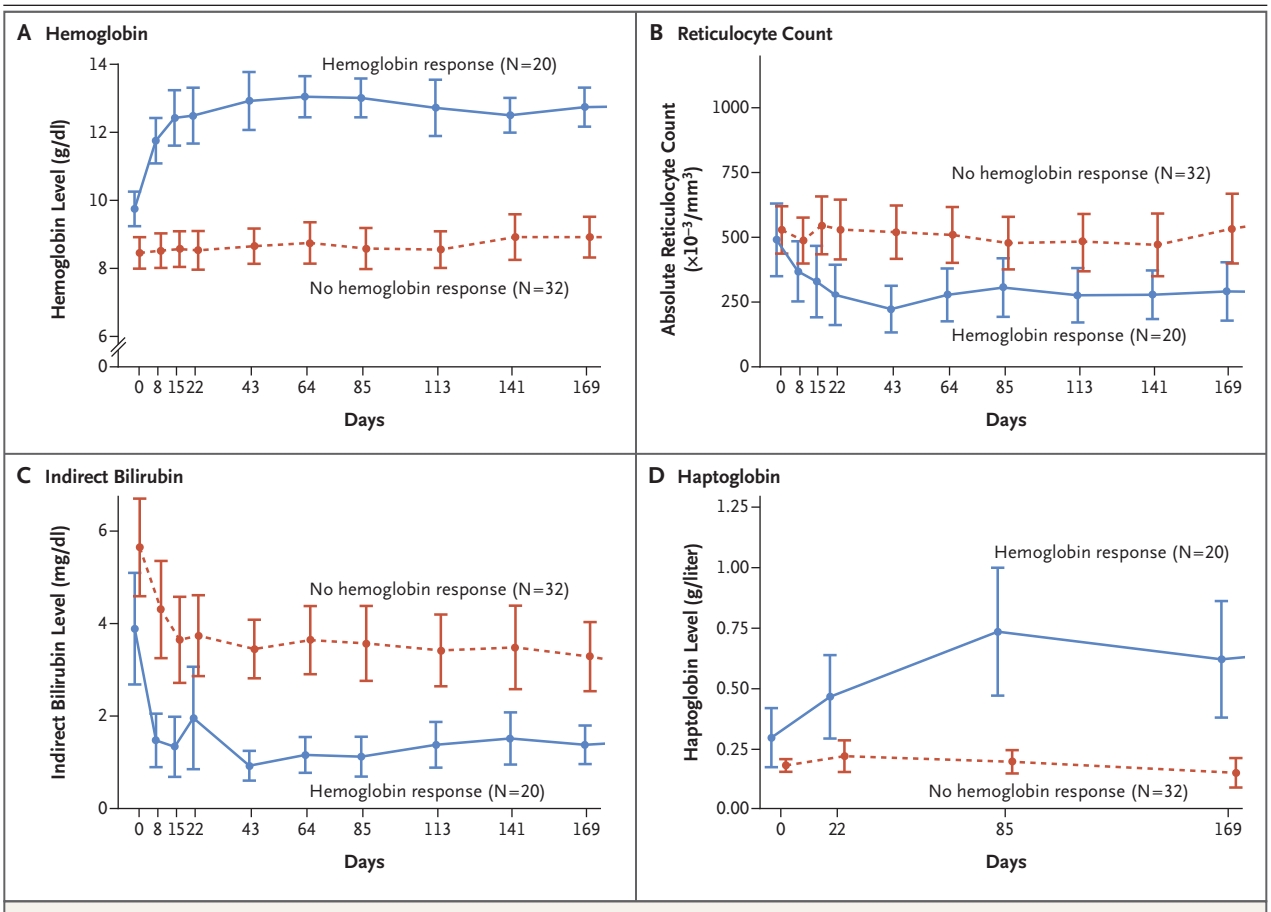

Figure 3. Hematologic Measures in Patients with and Those without a Hemoglobin Response.

Shown are the mean levels of hemoglobin (Panel A), the absolute reticulocyte count (Panel B), indirect bilirubin (Panel C), and haptoglobin (Panel D) during the 24-week core period, according to whether the patients had a hemoglobin response or no hemoglobin response. The hemoglobin response was sustained during nearly 3 years of follow-up in the extension phase (Fig. S4 in the Supplementary Appendix). The indirect bilirubin levels decreased in all the patients. However, the lactate dehydrogenase levels (Table S8 in the Supplementary Appendix) and reticulocyte counts changed only in the patients with a hemoglobin response. Thus, there may be an independent effect of mitapivat on bilirubin metabolism. The I bars indicate 95% confidence intervals. In Panel B, the values for the reticulocyte count per cubic millimeter are presented at 0.001 of the actual value. To convert the values for bilirubin to micromoles per liter, multiply by 17.1.

levels of red-cell pyruvate kinase protein suggest that this protein level is not a reliable predictor of hemoglobin response.

The data from this study indicate that the adverse-event profile for mitapivat at the adjusted dose aimed at increasing the hemoglobin level appears to allow for long-term administration of the drug. In addition to finding that 88% of the patients could continue to receive mitapivat without unacceptable adverse events, we showed that the subsequent pyruvate kinase activation resulted in clinically significant hemoglobin increases and improvement in hemolytic markers in approximately half the patients with pyruvate kinase deficiency, particularly those with a *PKLR* genotype that included at least one missense mutation.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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