Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in adults with non-transfusion dependent α-thalassaemia or β-thalassaemia: an open-label, multicentre, phase 2 study

Kevin H M Kuo, D Mark Layton, Ashutosh Lal, Hanny Al-Samkari, Joy Bhatia, Penelope A Kosinski, Bo Tong, Megan Lynch, Katrin Uhlig, Elliott P Vichinsky

Summary

Background Patients with non-transfusion-dependent thalassaemia (NTDT), although they do not require regular blood transfusions for survival, can still accrue a heavy burden of comorbidities. No approved disease-modifying therapies exist for these patients. We aimed to investigate the safety and efficacy of mitapivat (Agios Pharmaceuticals, Cambridge, MA, USA), a pyruvate kinase activator, in adults with non-transfusion-dependent (NTD) α-thalassaemia or NTD β-thalassaemia.

Methods In this open-label, multicentre, phase 2 study, patients were recruited from four academic clinical study sites in Oakland, CA, and Boston, MA, USA; Toronto, ON, Canada; and London, UK. Patients were eligible if they were aged 18 years or older, with NTDT (including β-thalassaemia with or without α-globin gene mutations, haemoglobin E β-thalassaemia, or α-thalassaemia), and a baseline haemoglobin concentration of 10·0 g/dL or lower. During a 24-week core period, mitapivat was administered orally at 50 mg twice daily for the first 6 weeks followed by an escalation to 100 mg twice daily for 18 weeks thereafter. The primary endpoint was haemoglobin response (a ≥1·0 g/dL increase in haemoglobin concentration from baseline at one or more assessments between weeks 4 and 12). Efficacy and safety were assessed in the full analysis set (ie, all patients who received at least one dose of study drug). This study is registered with ClinicalTrials.gov, NCT03692052, and is closed to accrual.

Findings Between Dec 28, 2018, and Feb 6, 2020, 27 patients were screened, of whom 20 were enrolled (15 [75%] with β-thalassaemia and five [25%] with α-thalassaemia) and received mitapivat. The median age of patients was 44 years (IQR 35–56), 15 (75%) of 20 patients were female, five (25%) were male, and ten (50%) identified as Asian. 16 (80% [90% CI 60–93]) of 20 patients had a haemoglobin response (p<0·0001), five (100%) of five with α-thalassaemia and 11 (73%) of 15 with β-thalassaemia. 17 (85%) patients had a treatment-emergent adverse event, and 13 had a treatment-emergent event that was considered to be treatment related. One serious treatment-emergent adverse event occurred (grade 3 renal impairment), which was considered unrelated to study drug, resulting in discontinuation of treatment. The most commonly reported treatment-emergent adverse events were initial insomnia (ten [50%] patients), dizziness (six [30%]), and headache (five [25%]). No patients died during the 24-week core period.

Interpretation These efficacy and safety results support the continued investigation of mitapivat for the treatment of both α-thalassaemia and β-thalassaemia.

Funding Agios Pharmaceuticals.

Lancet 2022; 400: 493–501

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Division of Haematology, University of Toronto, Toronto, ON, Canada (K H M Kuo MD); **Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK** (Prof D M Layton MB BS); **Division of Hematology, University of California San Francisco Benioff Children's Hospital, Oakland, CA, USA** (Prof A Lal MD, E P Vichinsky MD); **Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA** (H Al-Samkari MD); **Agios Pharmaceuticals, Cambridge, MA, USA** (J Bhatia MD, P A Kosinski MS, B Tong PhD, M Lynch MSN, K Uhlig MD)

Correspondence to: Dr Kevin H M Kuo, Division of Haematology, University of Toronto, Toronto, ON, M5G 2C4, Canada
kevin.kuo@uhn.ca

Introduction

Thalassaemia is a haemoglobinopathy caused by mutations of the α-globin or β-globin gene, or both, that result in imbalanced globin chain production.[1,2] Non-transfusion-dependent thalassaemia (NTDT) is of increasing global significance because of migration of individuals from typically high incidence areas (eg, the Mediterranean and southeast Asia) to typically low incidence areas (eg, the USA and the UK), and is one of the most frequent haemoglobinopathies identified in some screening programmes for newborn babies (eg, California's Newborn Screening Program).[3] Patients with NTDT do not require regular blood transfusions for survival, but still have a substantial burden of disease-related comorbidities and complications and a reduced quality of life.[1]

Patients with NTDT typically present with symptoms of anaemia that might be precipitated by intercurrent infection when aged 2 years or older.[4] Over time, these patients can develop several comorbidities as a result of ineffective erythropoiesis, haemolysis, and associated anaemia and iron overload. Complications of NTDT include extramedullary haematopoietic pseudotumours, leg ulcers, thrombosis, pulmonary hypertension, abnormal liver function, heart failure, osteoporosis, and

For more on **the California Newborn Screening Program** see https://www.cdph.ca.gov/Programs/CFH/DGDS/Pages/nbs/default.aspx

Research in context

Evidence before this study

Patients with non-transfusion-dependent (NTD) thalassaemia develop many comorbidities and complications resulting from ineffective erythropoiesis, haemolysis, anaemia, and iron overload. We searched PubMed for publications in English, from database inception up to Dec 31, 2018, of clinical trials on drug treatments of NTD α-thalassaemia or NTD β-thalassaemia using the terms ("alpha-thalassemia/drug therapy"[Majr:NoExp] OR "beta-thalassemia/drug therapy"[Majr:NoExp] OR "thalassemia/drug therapy"[Majr:NoExp]) NOT ("Epidemiologic Study Characteristics"[MeSH] OR "chelation therapy"[MeSH] OR "iron overload"[MeSH:NoExp] OR "thalassemia major" OR "major, thalassemia" OR "beta-thalassemia major" OR "deferiprone" OR "deferasirox" OR "deferoxamine"). We identified 48 publications, of which 33 were excluded because they were not trials (eg, review article and preclinical study), did not include patients with NTD α-thalassaemia or NTD β-thalassaemia, or did not investigate drug therapies. The 15 remaining studies covered a range of pharmacological interventions in patients with α-thalassaemia or β-thalassaemia, including hydroxycarbamide and recombinant erythropoietin; however, none of these therapies have been approved for patients with NTD thalassaemia or are widely used in these patients.

Added value of this study

To our knowledge, this is the first trial to assess pyruvate kinase (PK) activation in patients with NTD α-thalassaemia or NTD β-thalassaemia as a potential disease-modifying therapy. Mitapivat (Agios Pharmaceuticals, Cambridge, MA, USA), an oral small molecule, activates PK to catalyse the final step of the glycolytic pathway. We found that 80% (16 of 20) of patients treated with mitapivat 50 mg twice daily for 6 weeks and then 100 mg twice daily for 18 weeks had a haemoglobin response, defined as an increase of 1·0 g/dL or more in haemoglobin concentration from baseline at one or more assessments between weeks 4 and 12; this response was observed in both patients with α-thalassaemia (five [100%] of five) and with β-thalassaemia (11 [73%] of 15). Directional improvements in markers of haemolysis and erythropoiesis were also observed. Mitapivat was well tolerated with a safety profile consistent with the label for its US Food and Drug Administration-approved indication in PK deficiency.

Implications of all the available evidence

The landscape of therapeutics in thalassaemia is rapidly evolving. Guidelines suggest there might be a benefit for off-label use of haemoglobin F inducers, such as hydroxycarbamide, or for erythropoietin stimulators in some patient subgroups of thalassaemia, but state that further research is needed. Luspatercept, an agent targeting ineffective erythropoiesis, is also under regulatory review for the treatment of anaemia in patients with NTD β-thalassaemia. We found that mitapivat might be a new, oral, disease-modifying treatment for haemolytic anaemia in patients with NTD α-thalassaemia or NTD β-thalassaemia, for which further investigations in phase 3 trials are ongoing.

metabolic disorders.[2,5–7] Chronic anaemia is associated with long-term negative outcomes in patients with NTDT.[6] In a study involving patients with non-transfusion-dependent (NTD) β-thalassaemia, the severity of anaemia was found to correlate with the risk of complications, with each 1 g/dL decrease in haemoglobin concentration estimated to be independently associated with an average increase of 0·75 comorbidities.[6] A higher proportion of patients with haemoglobin concentrations below 10 g/dL developed comorbidities than among those with a concentration of 10 g/dL or higher.[8,9]

Sporadic transfusions and iron chelation therapy are the mainstay of management for complications of NTDT.[10] Luspatercept is approved for transfusion-dependent thalassaemia and is being investigated for use in NTD β-thalassaemia.[11] Although hydroxycarbamide has shown promise in improving haemoglobin concentrations and associated comorbidities in patients with NTD β-thalassaemia, and is approved by the US Food and Drug Administration (FDA), the UK Medicines and Healthcare products Regulatory Agency, and the European Medicines Agency for the treatment of sickle cell disease, a substantial proportion of patients are non-responders, and γ-globin induction is ineffective in patients with NTD α-thalassaemia (ie, haemoglobin H disease).[12–15] Because of the high global burden of NTDT, new disease-modifying treatments targeting the underlying pathophysiology are needed.[11]

Despite diverse genotypes and varied phenotypic expression, clinical manifestations across all thalassaemias are largely derived from the imbalance in globin chain production in red blood cells, which results in metabolic oxidative stress. Clearance of excess globin chains creates an increased demand for adenosine triphosphate (ATP)-driven mechanisms.[2,16–19] Efficient ATP generation is essential for maintaining red blood cell function and membrane integrity;[9,20] however, ATP supply is often insufficient to meet the increased metabolic demand in thalassaemic red blood cells, resulting in premature cell death[2,20] and ultimately chronic anaemia due to ineffective erythropoiesis and haemolysis.[1,2]

Pyruvate kinase (PK) catalyses the final step of the glycolytic pathway and is crucial for generating ATP and maintaining red blood cell health. Mitapivat (AG-348; Agios Pharmaceuticals, Cambridge, MA, USA) is a first-in-class, oral, small molecule, allosteric activator of PK that has been approved by the FDA for the treatment of haemolytic anaemia in adults with PK deficiency.[21] Mitapivat activates both wild-type and mutant forms of red cell PK (PKR) enzymes in vitro, leading to increased ATP production in red blood cells.[22]

Phase 2 and 3 clinical trials in patients with PK deficiency have found that treatment with mitapivat reduced transfusion burden in patients who receive regular transfusions and improved haemoglobin concentrations in patients who did not regularly receive transfusions.[23–25] In a proof-of-concept phase 1 study in sickle cell disease, treatment with mitapivat increased haemoglobin concentrations, providing support for wild-type PKR as a potential treatment target in other haemolytic anaemias.[26]

The pharmacological action of mitapivat is hypothesised to increase ATP production in thalassaemia and mitigate harmful effects on red blood cells.[2,9,16,17,20] This hypothesis is supported by preclinical data showing that mitapivat ameliorated ineffective erythropoiesis, iron overload, and anaemia in the *Hbb*$^{th3/+}$ murine model of β-thalassaemia intermedia.[27] Furthermore, mitapivat increased PKR activity and ATP concentrations ex vivo in red blood cells from patients with β-thalassaemia,[28] providing mechanistic support for PK activation as a potential therapeutic approach.

Here, we report the efficacy and safety of mitapivat in an open-label, multicentre, phase 2 study of patients with NTD α-thalassaemia or β-thalassaemia. The primary objective of this study was to assess the efficacy of mitapivat in increasing haemoglobin concentrations during a core 24-week period.

Methods

Study design and participants

This phase 2, open-label, multicentre study was run at four academic study sites in Oakland, CA, and Boston, MA, USA; Toronto, ON, Canada; and London, UK. The study consisted of a 24-week core period, for which results are reported here, followed by a 10-year extension period that is ongoing. Eligible patients were aged 18 years or older with a known medical history of thalassaemia, including β-thalassaemia with or without α-globin gene mutations, haemoglobin E β-thalassaemia, or α-thalassaemia (ie, deletional and non-deletional haemoglobin H disease), and a baseline haemoglobin concentration of 10·0 g/dL or lower. Additionally, they must be NTD, defined as having no more than 5 red blood cell units transfused during the 24-week period before day 1 of study treatment and no such transfusions in the 8 weeks before day 1 of study treatment, and have adequate organ function. For women of reproductive potential, patients needed to have a negative pregnancy test on day 1 of study treatment, and for women of reproductive potential and men with partners of reproductive potential, they must refrain from intercourse or agree to use two forms of contraception. Exclusion criteria included a known medical history of haemoglobin S and haemoglobin C thalassaemia. Full eligibility criteria are in the study protocol (appendix pp 15–103).

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The protocol and informed consent forms were reviewed and approved by each study site's institutional review board or institutional ethics committee before the start of the study. All study participants provided written informed consent for participation, including before screening procedures.

Procedures

All participants were given a starting dose of oral mitapivat 50 mg twice daily for the first 6 weeks of the 24-week core study period, followed by an escalation to 100 mg twice daily if the drug was well tolerated (appendix p 12). The doses of 50 mg twice daily and 100 mg twice daily were selected on the basis of the pharmacokinetic-ATP association characterised in a previous multiple-ascending-dose study in healthy volunteers,[29] because both healthy volunteers and patients with thalassaemia have wild-type PKR enzyme, unlike those with PK deficiency. Patients who discontinued the drug underwent a 2-week dose taper period before stopping of study drug. Blood samples for clinical laboratory assessments (haemoglobin, markers of haemolysis, and markers of erythropoiesis) and pharmacokinetic and pharmacodynamic assessments were collected at scheduled visits, including the day of study entry (day 1), followed by every 2 weeks up to week 8, and every 4 weeks up to week 24.

Haemoglobin was measured by each site's local laboratory using site standard operating procedures every 2 weeks for the first 8 weeks and then every 4 weeks up to week 24. Local laboratories did all primary and secondary laboratory assessments, except for those of haptoglobin, erythropoietin, soluble transferrin receptor, and sex steroids, which were done at ICON (Farmingdale, NY, USA and Leopardstown, Dublin, Ireland); the study's central laboratory. Exploratory laboratory assessments were also done at the central laboratory. Individual participant's baseline haemoglobin concentration, haemolysis markers (indirect bilirubin, lactate dehydrogenase [LDH], and haptoglobin), reticulocytes, iron biomarkers, and erythropoietic biomarkers were each defined as the average of all of the participant's available assessments during the screening period, up to the date of the first dose of study drug. In the analysis of haemoglobin, measurements within 8 weeks of transfusion were to be excluded. Patients who had a haemoglobin response during the 24-week core period were eligible to continue into the extension period.

Adverse events were collected continuously and classified using Common Terminology Criteria for Adverse Events (version 4.03). Causality of treatment-emergent adverse events was provided by the participant's investigator. Each site or investigator assessed the seriousness of treatment-emergent adverse events when recording the event.

Outcomes

See Online for appendix

The primary endpoint was the proportion of patients with haemoglobin response, defined as a 1·0 g/dL or greater increase in haemoglobin concentration from

baseline at one or more assessments between weeks 4 and 12, inclusive. Secondary endpoints were: the mean change from baseline in haemoglobin concentrations over a continuous 12-week interval from week 12 to week 24; the sustained haemoglobin response, defined as a patient who had a haemoglobin response and had a 1·0 g/dL or greater increase in haemoglobin concentration at two or more evaluable haemoglobin assessments out of the four scheduled assessments between weeks 12 and 24; the delayed haemoglobin response, defined as a patient who did not have an haemoglobin response, but did have a 1·0 g/dL or greater increase in haemoglobin concentration at one or more haemoglobin assessments between weeks 13 and 24; change from baseline in haemoglobin concentration over the duration of the extension period (to be reported in a future publication); time to first 1·0 g/dL or greater increase in haemoglobin concentration; change from baseline in markers of haemolysis (reticulocyte count, bilirubin, LDH, and haptoglobin); and change from baseline in markers of erythropoietic activity (nucleated red blood cells [count or percentage], erythropoietin, and soluble transferrin receptor). Nucleated red blood cells were tested in different local laboratories with different methods and units, thus these data could not be analysed.

Safety endpoints were the type, incidence and severity of adverse events and treatment-emergent adverse events; adverse events of special interest (ie, an increase in transaminase concentration of >2·5×baseline [defined as the mean of the screening and day 1 values] or an increase in aspartate aminotransferase or alanine aminotransferase grade ≥2 in severity, whichever was lower); adverse events leading to study drug dose reduction, interruption or discontinuation; and changes in clinical laboratory tests, physical examination, bone mineral density, vital signs, and 12-lead electrocardiogram over time (not reported here).

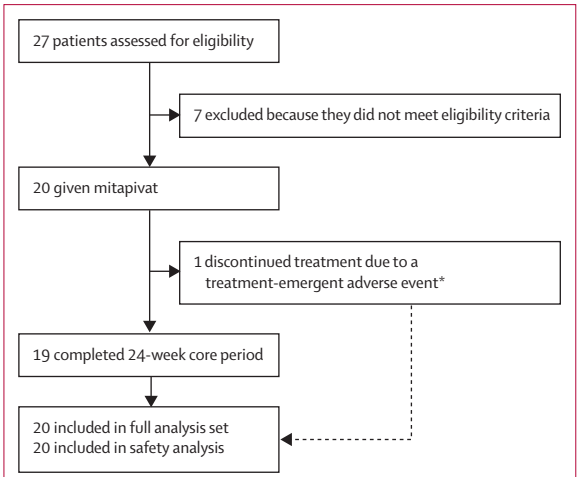

Figure 1: **Study profile**
The full analysis set and safety analysis set included all patients who received at least one dose of mitapivat. *One patient discontinued after week 4 visit due to an unrelated case of renal impairment.

Secondary pharmacokinetic endpoints were drug concentrations over time and pharmacokinetic parameters of study drug (to be reported elsewhere).

A prespecified exploratory endpoint was mean change in ATP concentration as a pharmacodynamic marker of glycolytic pathway activation. Further prespecified exploratory objectives, including pharmacodynamic markers, markers of iron metabolism, and indicators of iron overload (appendix p 20) will be reported elsewhere.

Statistical analysis

We calculated that a sample size of 17 enrolled patients would provide 80% power to reject a 30% null response rate at a one-sided 0·05 type 1 error rate when the true response rate is 60%. We did all efficacy, safety, and pharmacokinetic and pharmacodynamic analyses on the full analysis set based on the 24-week core period, which included all patients who received at least one dose of study drug. We summarised continuous variables using

	Patients with α-thalassaemia (n=5)	Patients with β-thalassaemia (n=15)*	All patients (N=20)
Sex			
Female	4 (80%)	11 (73%)	15 (75%)
Male	1 (20%)	4 (27%)	5 (25%)
Age, years	35 (35–37)	52 (35–57)	44 (35–56)
Race and ethnicity			
Asian	5 (100%)	5 (33%)	10 (50%)
White	0	4 (27%)	4 (20%)
Black or African	0	1 (7%)	1 (5%)
American	0	0	0
Native Hawaiian or other	0	1 (7%)	1 (5%)
Other	0	3 (20%)	3 (15%)
Not reported	0	1 (7%)	1 (5%)
Baseline haemoglobin, g/dL	8·37 (7·57–8·80)	8·50 (6·57–9·13)	8·43 (6·78–8·98)
<85 g/L (8·5 g/dL)	3 (60%)	7 (47%)	10 (50%)
≥85 g/L (8·5 g/dL)	2 (40%)	8 (53%)	10 (50%)
Indirect bilirubin, μmol/L	62·1 (33·6–87·2)	18·0 (14·5–23·0)	21·0 (15·5–36·1)
Previous splenectomy			
Yes	0	2 (13%)	2 (10%)
No	5 (100%)	13 (87%)	18 (90%)
Previous chelation status			
Yes	1 (20%)	2 (13%)	3 (15%)
No	4 (80%)	13 (87%)	17 (85%)
LDH, IU/L	263·0 (202·0–313·0)	245·0 (175·0–380·0)	249·0 (176·5–368·0)
Erythropoietin, IU/L	45·0 (29·0–79·0)	95·5 (30·0–141·0)	79·0 (29·0–137·0)
Reticulocyte, 10^9/L	196·0 (180·0–351·5)	86·0 (51·5–169·0)	145·8 (63·8–188·0)

Data are n (%) or median (IQR). LDH=lactate dehydrogenase. *Includes two patients with haemoglobin E β-thalassemia.

Table 1: **Patient demographics and baseline characteristics**

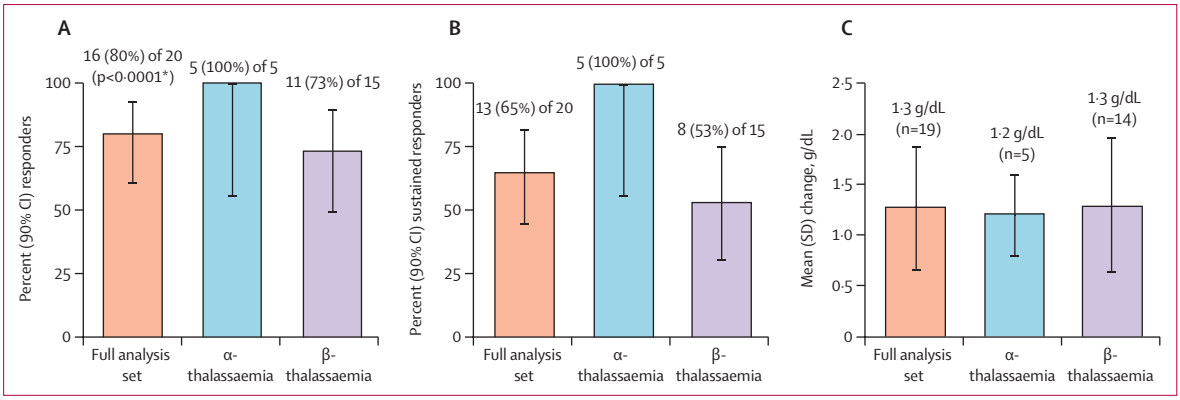

Figure 2: **Haemoglobin efficacy endpoints**
(A) Primary haemoglobin response; ≥1·0 g/dL increase in haemoglobin concentration from baseline at one or more assessment between weeks 4 and 12 (inclusive). (B) Sustained haemoglobin response; primary response during weeks 4–12 and a ≥1·0 g/dL increase in haemoglobin concentration at two or more assessments between weeks 12 and 24. (C) Mean change in haemoglobin concentration from baseline over the 12 week interval between weeks 12 and 24. Data above bars are n (%) of N or mean (n of N), with error bars showing either 90% CI or SD. *One-sided p value based on binomial exact test.

number of patients, mean (SD), and median (IQR). We summarised categorical variables using counts and percentages. In the primary analysis, we summarised the proportion of patients with a haemoglobin response using the full analysis set from the core period and calculated one-sided p values using the binomial exact test and two-sided exact 90% CIs using the Clopper-Pearson method.

We used SAS (version 9.4) for all analyses. The trial is registered at ClinicalTrials.gov, NCT03692052.

Role of the funding source

The study was designed and analysed by the study sponsor (Agios Pharmaceuticals) in collaboration with the authors; the study sponsor also funded medical writing assistance and contributed to data collection, data analysis, data interpretation, writing of the manuscript, and decision to submit.

Results

Between Dec 28, 2018, and Feb 6, 2020, 27 patients were screened, of whom 20 were enrolled (15 [75%] with β-thalassaemia and five [25%] with α-thalassaemia), received mitapivat, and included in the full analysis set (figure 1). Of those who enrolled, 19 (95%) patients completed the core treatment period, with one patient who had β-thalassaemia discontinuing after week 4 due to a treatment-emergent adverse event unrelated to the study drug (renal impairment). All patients except the one patient who discontinued early received an escalated dose of mitapivat at week 6, from 50 mg twice daily to 100 mg twice daily. One patient who completed the core period but did not have a haemoglobin response did not enter the extension period; all remaining 18 patients entered the extension period.

The median age of patients at baseline was 44 years (IQR 35–56), and most patients were female (15 [75%] of 20; table 1). All patients with α-thalassaemia were of Asian race and a third of patients with β-thalassaemia identified as Asian. Median baseline haemoglobin concentration was 8·43 g/dL (IQR 6·78–8·98). Baseline values for LDH, bilirubin, and erythropoietin varied between individuals, but on average were increased above normal laboratory references. Genotype data were available for 19 patients: 14 (93%) of 15 with β-thalassaemia and five (100%) of five with α-thalassaemia. A wide range of genotypes was observed (appendix pp 7–9).

The primary endpoint of haemoglobin response was observed in 16 of 20 patients (80% [90% CI 60–93]; p<0·0001), including five of five (100% [55–100]) with α-thalassaemia and 11 of 15 (73% [49–90]) with β-thalassaemia (figure 2A). A sustained haemoglobin response was observed in 13 (65%) patients (figure 2B), including all patients with α-thalassaemia (five [100%]) and eight (53%) with β-thalassaemia. Of the 16 patients with primary haemoglobin response, 13 (81%) had a response while receiving 50 mg twice daily in the first 6 weeks, and three (19%) had a response while receiving 100 mg twice daily in the following 6 weeks. Additionally, a delayed haemoglobin response (ie, after week 12 and after dose increase to 100 mg twice a day) was observed in two (10%) patients, both of whom had β-thalassaemia (two [13%] of 15).

Haemoglobin assessments were available for 19 (95%) of 20 patients at weeks 12–24. The mean change from baseline in haemoglobin concentration over the 12-week interval between weeks 12 and 24 was 1·3 g/dL (SD 0·6). Findings were similar in those with α-thalassaemia (1·2 g/dL [0·4]) and β-thalassaemia (1·3 g/dL [0·7]; figure 2C). The increases in haemoglobin concentration occurred across the spectrum of genotypes and baseline haemoglobin readings (figure 3; appendix p 13).

The mean time to first haemoglobin increase of 1 g/dL or more among responders was 4·5 weeks (SD 3·2), and improvements were maintained over the duration of the 24-week core period (figure 4).

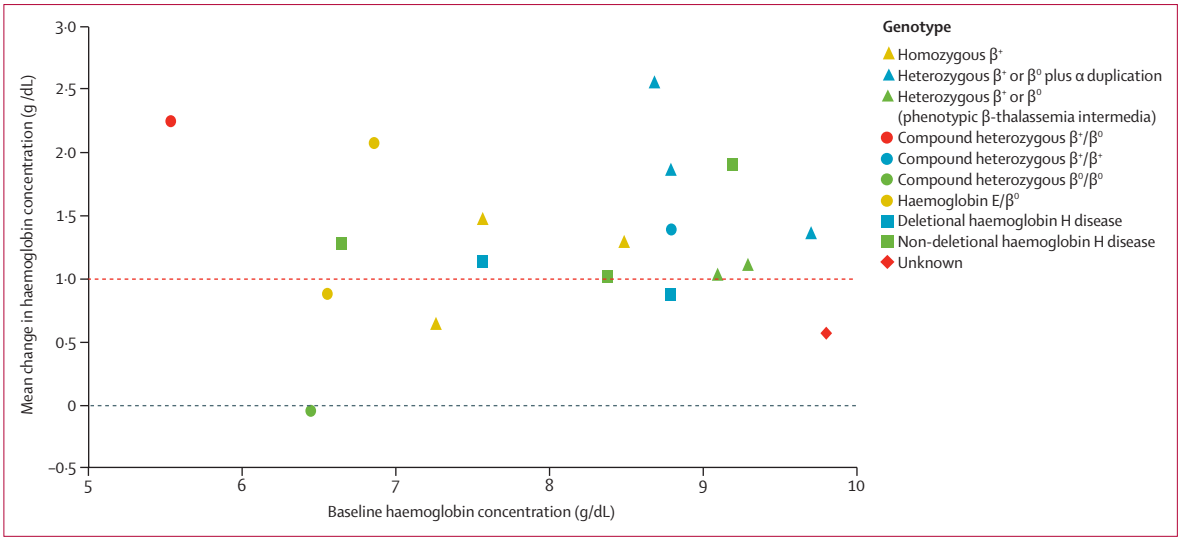

Figure 3: **Mean baseline haemoglobin concentration and change in concentration from baseline averaged over weeks 12–24, by patient and their genotype**
Data are shown for 19 patients with genotype information; one patient discontinued before week 12 (homozygous β+). Baseline is at zero; red dashed line indicates 1·0 g/dL above baseline. β+=reduced β globin; β0=absence of β globin. *All patients with non-deletional haemoglobin H disease had the Constant Spring mutation.

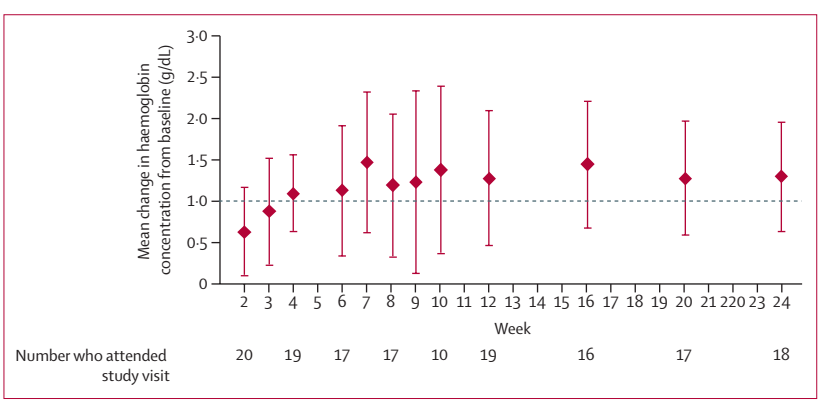

Figure 4: **Mean change in haemoglobin concentration from baseline over time**
Datapoints are means, with error bars showing SD, with number of patients who attended each study visit shown along the x-axis. All patients were escalated to 100 mg twice daily by week 6; one patient had a permanent dose reduction because of a non-serious grade 3 adverse event.

A trend in decreasing concentrations of markers of haemolysis (total bilirubin and LDH concentrations) was observed in both patients with α-thalassaemia and with β-thalassaemia, with decreases observed as early as week 2 (figure 5). Decreases in erythropoietin concentrations were also observed in both patients with α-thalassaemia and with β-thalassaemia (figure 5). The greatest change in markers of haemolysis and erythropoiesis was observed in the first 8 weeks of treatment and improvements were maintained over the duration of the core period.

Overall, 17 (85%) of 20 patients had a treatment-emergent adverse event during the core period, of whom 13 (76%) had a treatment-emergent adverse event that was considered to be treatment related (table 2; appendix p 11). Most treatment-emergent adverse events were deemed non-serious by the investigators and reported as either grade 1 or grade 2 in severity. The most commonly reported treatment-emergent adverse events (occurring in ≥10% of patients) were initial insomnia (difficulty in falling asleep), dizziness, and headache (table 3), with median durations of 34·0 days (IQR 5–146) for initial insomnia, 2·0 days (1·5–3·0) for dizziness, and 14·5 days (2·0–853·0) for headache. Grade 3 or worse treatment-emergent adverse events were reported in five (25%) patients and included initial insomnia, arthralgia, renal impairment, anaemia, and positional vertigo (appendix p 11). Initial insomnia was the only grade 3 or worse treatment-emergent adverse event considered to be treatment related. All other treatment-related treatment-emergent adverse events were grade 2 or lower and non-serious.

There was one serious treatment-emergent adverse event of renal impairment (grade 3), leading to dose reduction and then discontinuation of study treatment after week 4 (appendix p 11). This treatment-emergent adverse event was not considered to be related to study drug, but rather due to fluctuation in the patient's estimated glomerular filtration rate, with a transient decrease during treatment and further decreases were seen intermittently after study drug discontinuation (data not shown). Two other patients had treatment-related treatment-emergent adverse events that led to a dose reduction: one patient with two treatment-emergent adverse events (abdominal distension and dyspepsia) required prolonged dose reduction, and one patient required temporary dose reduction due to initial insomnia. One patient had a temporary dose interruption due to benign positional vertigo that was not considered related to study drug by their investigator. No deaths occurred during the 24-week core period.

Increases in whole blood ATP concentrations were observed in all patients (appendix p 10). By week 6, at the

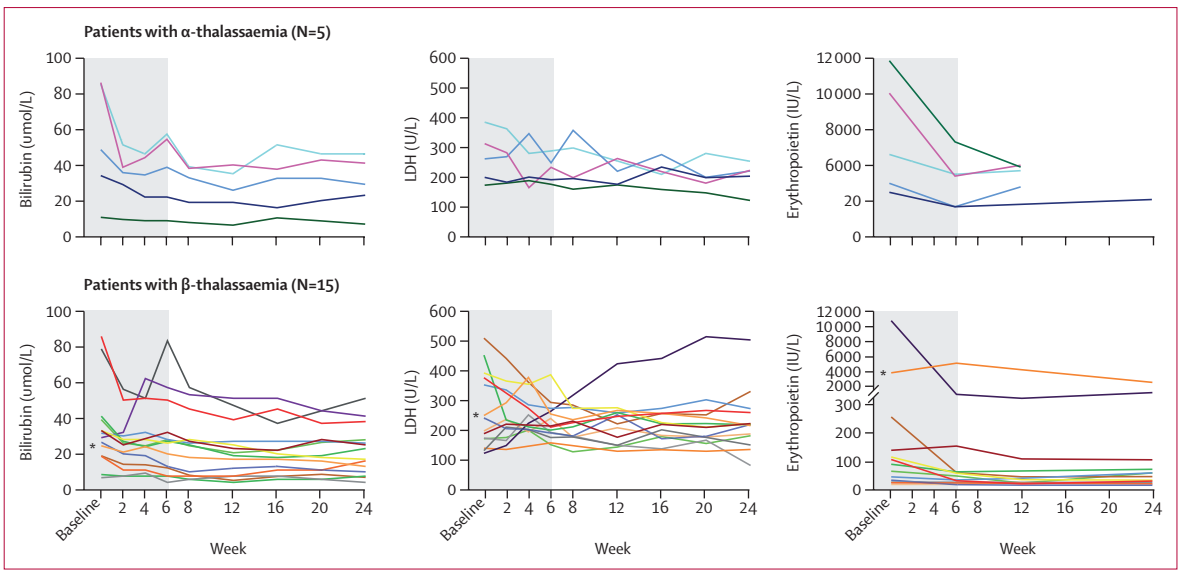

Figure 5: **Change in total bilirubin (A) and LDH (B; markers of haemolysis) and erythropoietin (C) over time, by thalassaemia subtype**
Each line shows the longitudinal data for an individual patient. Shaded area indicates the mitapivat 50 mg twice daily dosing period, with vertical dashed lines indicating study visits. For the α-thalassaemia erythropoietin analysis, week 24 data were missing for four of five patients because of COVID-19-related factors. EPO=erythropoietin. LDH=lactate dehydrogenase. *Non-responder.

end of the 50 mg twice daily dosing period, ATP concentrations had a mean increase of 78% from baseline (coefficient of variation 83%; n=11) and remained increased thereafter.

Discussion

In this open-label, multicentre, phase 2 study, we established proof of concept for mitapivat, an oral, first-in-class activator of pyruvate kinase, as a potential treatment for patients with NTDT. 80% of all patients (100% of those with α-thalassaemia and 73% of those with β-thalassaemia) had a haemoglobin response in this first interventional study of a potentially disease-modifying treatment for both α-thalassaemia and β-thalassaemia.

Haemoglobin responses were observed within the first few weeks of mitapivat treatment, along with increases in ATP concentrations and reductions in markers of haemolysis and erythropoiesis. Such changes support the hypothesised mechanism of action that improvements in haemoglobin concentration occurred as the result of improved red blood cell maturation or increased cell survival (ie, an amelioration of the underlying pathophysiological processes of this disease), or both, rather than stimulation of erythropoiesis via an increase of erythropoietin.[30]

Responses were observed across a diverse range of disease genotypes, suggesting that mitapivat's mechanism of action of improving the red blood cell energetics might provide a unifying approach in the treatment of thalassaemias, because all share features of ineffective erythropoiesis and haemolysis to some degree. The rapid and substantial haemoglobin responses observed across the spectrum of baseline haemoglobin concentrations suggest an absence of correlation between the two factors.

Haemoglobin responses were sustained over the 24-week core period, and the magnitude of response remained stable. Increased haemoglobin concentrations are associated with reduced comorbidities. A recently published 10-year retrospective cohort study of 51 patients with NTD β-thalassaemia showed significantly reduced morbidity-free survival in patients with haemoglobin concentrations of less than 10 g/dL, with an adjusted four-times increased risk for the development of comorbidities compared with patients with a haemoglobin concentration of 10 g/dL or higher.[9] Another retrospective cohort study of 415 patients with NTD β-thalassaemia found that a haemoglobin concentration of 10 g/dL or lower was independently associated with a 7·6-times increase in mortality risk compared with those with a concentration higher than 10 g/dL.[31] In a retrospective analysis of 2033 patients with NTD β-thalassaemia from 13 international thalassaemia centres of excellence from eight countries (Italy, Iran, Pakistan, the USA, Oman, Egypt, Greece, and Saudi Arabia), the median age at death was 46·3 years (IQR 28·3–61·9).[31] A subset of patients (254 [12·5%] of 2033 patients) was eventually placed on regular transfusion programmes, while the remaining patients (1779 [87·5%]) received only sporadic transfusions or no transfusions at all. All-cause mortality was significantly higher in patients who did not receive regular transfusions than in those who received transfusions regularly.[31] Taken together, these data suggest that untreated anaemia in NTDT predicts worse survival and risk for comorbidities, and that improving anaemia might reduce the burden of disease associated

	Grade 1	Grade 2	Grade 3	Any grade
Patients who had a treatment-emergent adverse event	4 (20%)	8 (40%)	5 (25%)	17 (85%)
Adverse event				
Initial insomnia	6 (30%)	3 (15%)	1 (5%)	10 (50%)
Dizziness	6 (30%)	0	0	6 (30%)
Headache	1 (5%)	4 (20%)	0	5 (25%)
Cough	4 (20%)	0	0	4 (20%)
Dyspepsia	4 (20%)	1 (5%)	0	4 (20%)
Fatigue	4 (20%)	0	0	4 (20%)
Nasal congestion	3 (15%)	1 (5%)	0	4 (20%)
Upper respiratory tract infection	1 (5%)	3 (15%)	0	4 (20%)
Abdominal pain	3 (15%)	0	0	3 (15%)
Diarrhoea	3 (15%)	0	0	3 (15%)
Ocular icterus	3 (15%)	0	0	3 (15%)
Pain	2 (10%)	1 (5%)	0	3 (15%)
Pain in extremity	2 (10%)	1 (5%)	0	3 (15%)
Abdominal distension	2 (10%)	1 (5%)	0	3 (15%)
Nausea	2 (10%)	1 (5%)	0	3 (15%)
Oropharyngeal pain	3 (15%)	0	0	3 (15%)
Upper abdominal pain	2 (10%)	1 (5%)	0	2 (10%)
Back pain	1 (5%)	1 (5%)	0	2 (10%)
Chest discomfort	2 (10%)	0	0	2 (10%)
Rash erythematous	2 (10%)	0	0	2 (10%)
Pyrexia	2 (10%)	0	0	2 (10%)
Rash	2 (10%)	0	0	2 (10%)
Vomiting	1 (5%)	1 (5%)	0	2 (10%)

No grade 4 or 5 treatment-emergent adverse events occurred.

Table 2: **Most common treatment-emergent adverse events (occurring in ≥10% of patients) in the safety population (N=20)**

with NTDT, underscoring the importance of the findings reported here.

Mitapivat was generally well tolerated throughout the 24-week core study period, at both the initial 50 mg twice daily dose and the increased 100 mg twice daily dose. The tolerability profile of mitapivat was consistent with that reported in other haemolytic anaemias, specifically PK deficiency and sickle cell disease.[24–26] Common adverse events, including insomnia, occurred early after drug initiation and were transient. One serious treatment-emergent adverse event of renal impairment (grade 3) was reported but was considered to not be related to treatment by the patient's investigator, but instead more likely to be due to renal haemodynamic changes. The major elimination pathway of mitapivat is metabolism in the liver, making it unlikely to affect or be affected by kidney function. In the two patients who withdrew from treatment (n=1) and had interrupted treatment (n=1), no treatment-emergent adverse event of acute haemolysis occurred. The protocol included a 2-week dose taper as a pre-emptive measure to reduce the risk for acute haemolysis after abrupt drug discontinuation, because this adverse event was seen in another study[23] in two patients with PK deficiency who were treated with a dose of 300 mg twice daily and quickly had haemoglobin responses followed by acute haemolysis after abrupt drug withdrawal. We expected the risk of acute haemolysis to be lower in our patient population than in this previous study, because we used a lower dose of mitapivat and our patient population have a wild-type PK enzyme, not a deficient one, providing them with baseline enzymatic activity upon drug discontinuation.

The absence of a placebo group in our study precludes full understanding of insomnia associated with mitapivat in thalassaemia; this adverse event will be further assessed in ongoing placebo-controlled trials (NCT04770753 and NCT04770779). The long-term safety and benefits of mitapivat in this population will continue to be assessed as part of the planned 10-year extension period.

Limitations of this study include the single-arm, open-label trial design without a control group, which restricts the inferences that can be made. However, this design is unlikely to have biased the results of haemoglobin concentrations and markers of haemolysis and erythropoietic activity, which underpin the key efficacy endpoints. Due to the study design and the small sample size, we did no hypothesis testing on changes in markers of haemolysis and erythropoietin; these markers will be assessed in larger randomised, placebo-controlled, phase 3 studies. The sequential dose escalation prevents definitive inferences regarding dose-dependent effects. Most patients had an increase in haemoglobin of 1·0 g/dL at a dose of 50 mg twice daily; however, some patients had this increase only after their dose was increased to 100 mg twice daily, and we cannot determine whether these increases in haemoglobin concentration were due to the dose increase or longer duration of drug exposure. The relatively small number of patients in each genotype subgroup restricts the generalisability of our results for each subgroup and warrants confirmation in a larger sample. Safety information of the study drug in thalassaemia remains limited to this sample but is complemented by the congruent safety profile in studies of mitapivat in patients with PK deficiency, a condition that shares pathophysiological features with thalassaemia.

In summary, this study establishes proof of concept that activation of PK by the oral agent mitapivat improves anaemia in patients with NTD α-thalassaemia and β-thalassaemia. Mitapivat showed a tolerable safety profile consistent with that of previous studies in other haemolytic anaemias. Together, these efficacy and safety results provide rationale for the continued investigation of mitapivat for the treatment of both α-thalassaemia and β-thalassaemia.

Contributors

KHMK, DML, EPV, AL, HA-S, KU, ML, PAK, and JB developed or modified the protocol. KHMK, DML, AL, EPV, and HA-S collected the data. BT, KU, ML, PAK, and JB developed the statistical analysis plan and interpreted the analyses, with input from all the authors. BT, PAK, and KHMK had access to and verified the underlying study data. All authors contributed to the drafting, review, and revision of this manuscript, and vouch for the adherence of the study to the protocol and the accuracy of the results.

Declaration of interests

KHMK reports consultancy fees from Agios Pharmaceuticals, Alexion, Apellis, bluebird bio, Celgene, Forma, Pfizer, and Novartis; honoraria from Alexion and Novartis; membership on an advisory committee for Agios Pharmaceuticals and Bioverativ/Sanofi/Sangamo; and research funding from Pfizer. DML reports consultancy fees from Agios Pharmaceuticals and membership on the Board of Directors or advisory committee for Agios Pharmaceuticals and Cerus. AL reports research funding from bluebird bio, Celgene, Insight Magnetics, La Jolla Pharmaceutical Company, Novartis, Protagonist Therapeutics, Terumo Corporations, and Forma; consultancy fees from Agios Pharmaceuticals and Chiesi USA; and membership on the Board of Directors or advisory committee for Celgene and Protagonist Therapeutics. HA-S reports consultancy fees from Agios Pharmaceuticals, argenx, Dova/Sobi, Moderna, Novartis, Rigel, and Forma and research funding from Agios Pharmaceuticals, Amgen, and Dova. JB, PAK, BT, ML, and KU are employees and shareholders of Agios Pharmaceuticals. EPV reports consultancy fees and research funding from Agios Pharmaceuticals, bluebird bio, Global Blood Therapeutics, Novartis, and Pfizer.

Data sharing

Qualified researchers can request access to related clinical study documents. Please send your data sharing requests to datasharing@agios.com. The following considerations will be taken into account as part of the review: (1) ability for external researcher to reidentify trial participants such as small rare disease or single-centre trials; (2) language used in data and requested documents (eg, English or other); (3) informed consent language with respect to allowance for data sharing; (4) plan to re-evaluate safety or efficacy data summarised in the approved product labelling; and (5) potential conflict of interest or competitive risk. We follow the International Committee of Medical Journal Editors (ICMJE) guidelines for data sharing, and more details can be found online.

Acknowledgments

This study was funded by Agios Pharmaceuticals. We thank the patients, their families, and all investigators involved in this study. Medical editorial support, including figure preparation, formatting, proofreading, and submission, was provided by Michelle Mancher (Onyx Medica, London, UK), supported by Agios Pharmaceuticals.

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