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College of Medicine

Role Of AG 348 In Treatment Of Thalasemia In Non-Transfusion Dependent Thalasemia

Submitted to the Council of the College of Medicine, Diyala University, In Partial Fulfillment of Requirements for the Bachelor Degree in medicine and general surgery.

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﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَ الَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ﴾

صدق الله العظيم

[المجادلة: 11]

Dedication

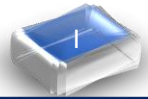
- To.. Our Beloved Country, IRAQ..

- And, We put our humble effort in the hands of those who made us see the light of knowledge ... our distinguished teachers..

- And, to my father & mother..

- And, to all colleagues of the profession studying in the field of medicine..

ELAF..



Acknowledgment

By the name of Allah, we start our project and we are thankful to Allah for helping us to complete this project and giving us the power and determination to do it faithfully and honestly.

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Abstract

Background: Thalassemia is a group of inherited blood disorders compromised red blood cell survival. The condition is characterized by ineffective erythropoiesis and peripheral hemolysis, with resultant anemia. Adenosine triphosphate supply appears to be insufficient in thalassemic RBCs to maintain RBC membrane fitness and clearance of globin precipitates. Mitapivat (AG-348) is an oral, small-molecule, allosteric activator of the RBC-specific form of pyruvate kinase (PK-R). PK-R is a key enzyme for maintaining energy homeostasis in RBCs, as they rely almost exclusively on the process of glycolysis to generate ATP. patients with PK deficiency who were not regularly transfused, oral mitapivat was well tolerated and induced rapid, durable hemoglobin (Hb) increases. Mitapivat increased ATP levels; reduced markers of ineffective erythropoiesis; and improved anemia, RBC survival, and indices of iron overload. Increased ATP synthesis mediated via PK-R activation by mitapivat may improve the survival of thalassemic RBCs in the bone marrow and/or peripheral circulation, and thus represents mechanism to treat patients with thalassemia. The review aimed to explain the role of AG 348 in treatment of thalasemia in non-transfusion dependent thalassemia.

Methods: A study was performed using the keywords “Non-transfusion-dependent thalassaemia”, “mitapivat”, “pyruvate kinase activator, pyruvate kinase deficiency”, and “thalassemia”. Only articles written in English were included in this study. We retained studies originating from randomized controlled trials, registries and included studies from year 2017 to 2020. The studies included patients with thalassaemia.

Results: study in Division of Hematology, University of Toronto, Canadain 20 patients (15 female) who received mitapivat, the mean age (SD) was 45.3 (11.8) yrs (range 29–67); mean (SD) BL Hb was 7.9 (1.4) g/dL (range 5.1–9.8). At Wk 6, all pts were escalated to 100 mg BID. Nineteen pts (95.0%) completed the core period, one discontinued due to adverse event (AE);17 pts continued to the extension period. Sixteen pts (80.0%, 90% CI 59.9, 92.9; $p < 0.0001$) achieved a Hb response, including 5/5 pts with α-thalassemia and 11/15 pts with β-thalassemia. Mean (SD) time to first Hb increase >1.0 g/dL was 4.5 (3.2) wks, and mean Hb (SD) increase from BL during Wks 12–24 was 1.3 (0.6) g/dL. A sustained response was achieved in 65% of pts, including 5/5 pts with α-thalassemia and 8/15 pts with β-thalassemia. Hb increases occurred across the spectrum of BL levels.

Conclusions: These results demonstrate that PKR activation with mitapivat was well tolerated and improved anemia, hemolysis, and ineffective erythropoiesis, and may represent a novel therapeutic approach for pts with α- or β-thalassemia. Primary endpoint with 80% of patients achieving a Hb response. Thalassemic RBCs have reduced ATP levels. Mitapivat may improve RBC survival in thalassemia by increasing ATP production. Mitapivat increased ATP and improved RBC parameters of β-thalassemia, and increased ATP levels ex vivo in human β-thalassemia RBCs.

Keywords: Non-transfusion-dependent thalassaemia, mitapivat, pyruvate kinase activator, pyruvate kinase deficiency, and thalassemia.

Contents

List of figures

Introduction

Thalassemia is a group of disorders it is one of the most common monogenic diseases worldwide. Having been historically clustered in the Mediterranean, North Africa, and South Asia, thalassemias are now encountered in other regions around the globe with the more recent immigration movements towards areas of lower prevalence [1,2]. Thalassemia result from an inherited imbalance between chains of hemoglobin, instigating ineffective erythropoiesis [3].

Non-transfusion dependent thalassemia (NTDT) is a group of thalassemic disorders including patients who do not require frequent blood transfusions for survival. Those patients have Hemoglobin H disease or reduced expressivity of the b genes (homozygous β+ or compound heterozygous β+/β0 in addition to other variants where the end result remains α/β imbalance. Patients with NTDT may still require occasional or more frequent red blood cell (RBC) transfusion therapy in certain circumstances including but not limited to significant infection, pregnancy, periods of rapid growth, or surgery [2]. Over the years, many individual studies have showed a clear variation in the complications seen between transfusion dependent (TDT) and non transfusion dependent (NTDT) thalassemic patients. The difference in management (i.e., limited transfusion, limited chelation, more frequent splenectomies) is an attributable factor in such discrepancy in the multimorbidity profiles of TDT and NTDT [3].

Inherited hemoglobin disorders can be divided into two main groups. The first group includes structural hemoglobin variants, such as hemoglobin S, C, and E. The second group includes the alpha (α)- and beta (β)-thalassemias which result from the defective synthesis of the α- or β-globin chains of adult hemoglobin A. Inheritance of such disorders follows a typical Mendelian-recessive manner whereby asymptomatic heterozygous parents, or carriers, pass on one copy of a defective gene to their children. The high prevalence of hemoglobin mutations in particular parts of the world often leads to simultaneous inheritance of two different thalassemia mutations from each parent or co-inheritance of thalassemia together with structural hemoglobin variants. Thus there are a wide variety of clinically distinct thalassemia syndromes [4].

Since the hallmark of disease in these syndromes is ineffective erythropoiesis، peripheral hemolysis, and subsequent anemia, transfusion-dependence has been an essential factor in characterizing the various thalassemia phenotypes and their severity. For instance, a diagnosis of β-thalassemia major entails lifelong regular transfusion requirement for survival. The main concern with transfusion-dependence is secondary iron overload، which if left untreated leads to target-organ toxicity and death [5].

However, considerable advances have been made, in iron overload assessment and management strategies for transfusion dependent patients, especially in the last decade, and these have translated into improved patient survival. Non-transfusion- dependent thalassemias (NTDT) is a term used to label patients who do not require lifelong regular transfusions for survival, although they may require occasional or even frequent transfusions in certain clinical settings. NTDT encompasses three clinically distinct forms: β-thalassemia intermedia, hemoglobin E/β-thalassemia (mild and moderate forms), and α-thalassemia intermedia (hemoglobin H disease). Although patients with hemoglobin S/β-thalassemia and hemoglobin C/β-thalassemia may have transfusion requirements similar to NTDT patients، these forms have other specific characteristics and management peculiarities and are better considered as separate entities [6].

NTDT are primarily to be found in the low- or middle-income countries of the tropical belt stretching from sub- Saharan Africa, through the Mediterranean region and the Middle East, to South and Southeast Asia. This is primarily attributed to the high frequency of consanguineous marriages in these regions, as well as to a conferred resistance of carriers to severe forms of malaria in regions where the infection has been, or is still, prevalent.3-4 Improvements in public health standards in these regions have also helped to improve survival and the number of affected patients. Increasing incidences of these disorders in other areas of the world, such as North Europe and North America, previously relatively unaffected by these conditions, have also been reported [7].

Treatment Options for Non-Transfusion-Dependent Thalassemia

The currently available treatment options for NTDT are primarily supportive; therefore, medical management of NTT takes a more symptom-focused approach by symptoms as arise. Symptomatic marrow expansion is treated with hydroxyurea, transfusion, or radiation therapy endocrine dysfunction is treated with hormone replacement therapies, vitamin D bisphosphonates, and calcium supplementation ulcers are treated with hydroxyurea plus erythropoietin (EPO) Or platelet-derived growth factor (PDGF), hygiene and transfusions and thrombotic events are treated with anticoagulation therapies. While the current standard of care for patients with TD includes regular blood transfusion and iron chelation therapy blood transfusions are not part of the routine treatment strategy for patients with NTDT, but may be required in the event of infection pregnancy or surgery [8].

Splenectomy may be useful for patients with more severe forms of NTDT, as it is associated with an increase in Hb concentrations and improvement in growth and development [9].

Removal of excess iron is essential in patients with NTT with iron overload Iron overload is frequently derived transfusion therapy but can also be caused by chronic hemolysis, ineffective erythropoiesis, and hypoxia, leading to increased intestinal iron absorption through suppression of the regulatory protein hepcidin. While iron accumulation in patients with NTT is slower than that observed in patients with TDT, it is a cumulative process that eventually leads to excess iron levels that are clinically significant and may impact overall survival [10].

Proposed Mechanism of Action of AG-348

AG-348 is an orally available potent broad-spectrum activator of the RBC-specific form of pyruvate kinase (PKR) L of 4 pyruvate kinase isoenzymes expressed in human tissues from 2 separate Both PKR and the liver-specific for of pyruvate kinase (PKL) are splice isoforms of the PKLR gene while pyruvate kinase muscle isozyme (PKM)L PKM2 both expressed from the PKM gene AG-348 is an allosteric activator of the PKR, PKL, PKM2 isoenzymes with similar activity for each Pyruvate kinase enzymatically catalyzes the metabolic conversion of phosphoenolpyruvate (PEP) and adenosine diphosphate (ADP) into pyruvate and ATP as the final glycolysis Mature RBC rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP Thus PKR is a key enzyme for maintaining energy homeostasis in erythrocytes [11].

AG-348 acts by directly binding to the PKR tetramer and allosterically enhancing the affinity for its substrate PEP. Pharmacology studies have confined the potency of AG-348 in activating wild-type (WT) and mutant PKR enzyme activity and modulating ATP and 2,3-diphosphoglycerate (2.3-DPG) levels in healthy adult subjects In preclinical studies AG-348 has also been shown to have acceptable absorption, distribution metabolism and excretion (ADME) and toxicology profiles RBC in patients with thalassemia suffer from an imbalance in the ratio between the G- and p-chains of Hb. This has the potential to impose several forms of metabolic stress On the cells specifically in the form of excess generation of reactive oxygen species (ROS) and an increased demand of ATP-dependent proteolytic mechanisms to clear excess globin chains This leads to the hypothesis that increased ATP synthesis through activation of PKR by AG-348 in thalassemic red cells may result in increased cell survival in the bone marrow or peripheral circulation [12].

Mitapivat (AG-348) is a small-molecule activator of pyruvate kinase that binds to an allosteric pocket to stabilize the active tetrameric form and enhance its affinity for its substrate، phosphoenolpyruvate. A phase 2 clinical trial of mitapivat in adult patients with pyruvate kinase deficiency who were not receiving regular transfusions demonstrated that the molecule elicited a rapid and sustained increase in Hb levels in approximately 50 % of treated patients and was generally well tolerated, with adverse events being mainly low grade and transient. Interim analysis of an ongoing phase 2 study of mitapivat in patients with non–transfusion-dependent thalassemia (NCT03692052) demonstrated that 92.3% (12 of 13 evaluable patients) achieved an Hb increase from baseline of ≥ 1 g/dL, including 4 of 4 patients with α-thalassemia and 8 of 9 patients with β-thalassemia [13].

Owing to imbalanced globin chain production, thalassemic RBCs have increased ATP demand to maintain cell fitness (Figure 1). Activation of WT PK-R in thalassemic RBCs may enhance glycolysis and increase RBC ATP levels, leading to improved cell fitness and survival.

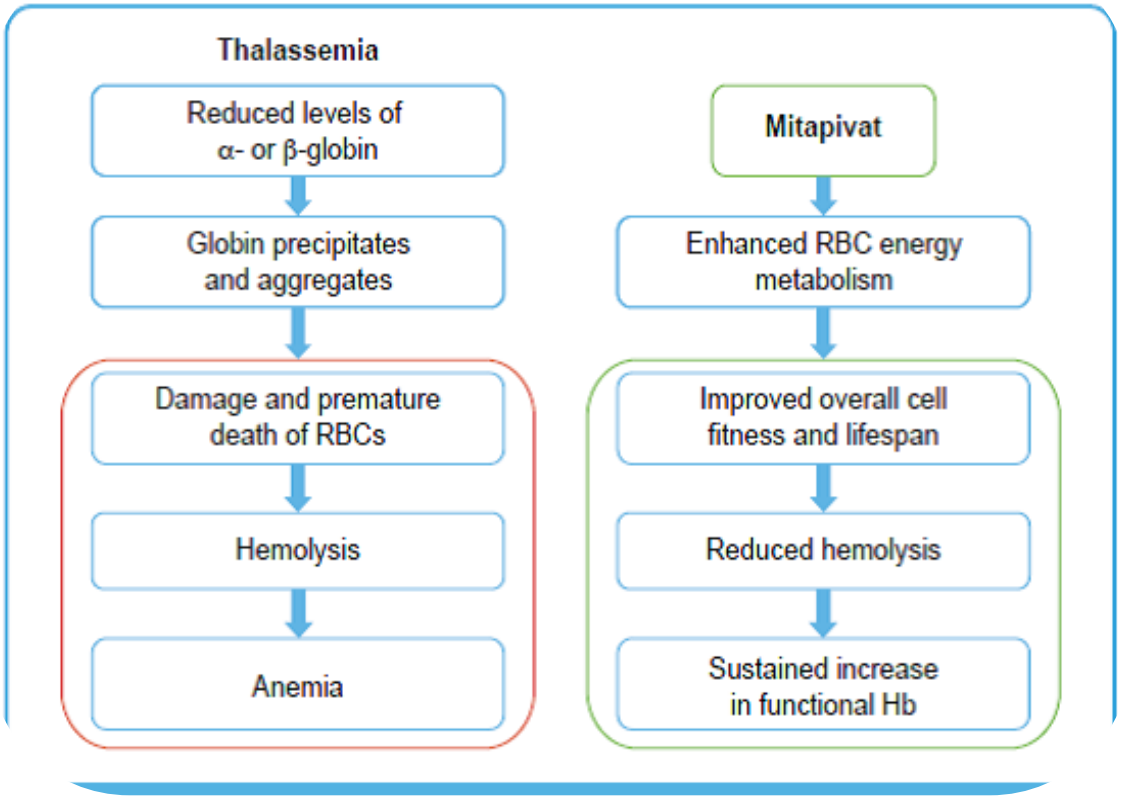

Figure 1: *Hypothesis: Mitapivat may improve thalassemic RBC survival by increasing ATP production.*

Target of review

The review aimed to explain the role of AG 348 in treatment of thalasemia in non-transfusion dependent thalassemia.

Methods

A study was performed using the key words “Non-transfusion-dependent thalassaemia”, “mitapivat”, “pyruvate kinase activator ،pyruvate kinase deficiency ،”and “thalassemia”. Only articles written in English were included in this study. We retained studies originating from randomized controlled trials, registries and included studies from year 2017 to 2020. The studies included patients with thalassaemia.

Results

In Study was conducted in Orlando, U.S [14] in 2019 they enrolled 12 of the intended 17 patients (nine with β-thalassemia and three with α-thalassemia). As of the November 14, 2019 data cutoff date, eight patients, all with β-thalassemia, were evaluable for the primary endpoint of a hemoglobin increase of ≥1.0 g/dL from baseline in at least one assessment during Weeks 4-12. All eight patients were treated with 50 mg of mitapivat twice daily for the first six weeks and escalated to 100 mg twice daily, and all patients remain on treatment (range 12.4-34.3 weeks). Seven of eight efficacy evaluable patients achieved a hemoglobin increase of ≥1.0 g/dL, and for responders the mean hemoglobin increase from baseline was 1.76 g/dL (range, 0.9–3.3 g/dL) during Weeks 4-12. The majority of adverse events were Grade 1 or 2 and consistent with previously published Phase 2 data for mitapivat in patients with pyruvate kinase (PK) deficiency. Updated results from the Phase 2 thalassemia study will be presented at a medical meeting in the first half of 2020.

“These data demonstrate proof of concept that activation of wild-type PKR has the potential to convey clinical benefit in thalassemia and provides compelling evidence to broaden mitapivat clinical development in this disease,” said Chris Bowden, M.D., chief medical officer at Agios. “The safety and tolerability profile observed in this trial and in adults with pyruvate kinase deficiency supports the continued investigation of mitapivat treatment across severe, lifelong hemolytic anemias such as pyruvate kinase deficiency, thalassemia and sickle cell disease”.

Mitapivat Phase 2 Trial in Thalassemia

The ongoing Phase 2 study is evaluating the efficacy, safety, pharmacokinetics and pharmacodynamics of treatment with mitapivat in adults with non-transfusion-dependent β- and α-thalassemia (NTDT). This study includes a 24-week core period followed by a 2-year extension period for eligible participants. The primary endpoint is hemoglobin response. Approximately 17 participants with NTDT who have a baseline hemoglobin concentration of ≤10 g/dL will be enrolled. The initial dose of mitapivat is 50 mg twice daily with one potential dose-level increase to 100 mg twice daily, at the week six visit based on the participant's safety and hemoglobin (Hb) concentrations. With a total of 17 patients enrolled, the study would have 80% power to reject a ≤30% response rate at a one-sided 0.05 type 1 error rate.

Activate: A placebo-controlled trial with a 1:1 randomization, expected to enroll approximately 80 patients who do not receive regular transfusions. The primary endpoint of the trial is the proportion of patients who achieve a sustained hemoglobin increase of ≥1.5 g/dL.

Activate-T: A single arm trial of up to 40 regularly transfused patients with a primary endpoint of reduction in transfusion burden over six months compared to individual historical transfusion burden over prior 12 months.

In addition, another study in Division of Hematology, University of Toronto, Canada[15] in 20 patients (15 female) who received mitapivat, the mean age (SD) was 45.3 (11.8) yrs (range 29–67); mean (SD) BL Hb was 7.9 (1.4) g/dL (range 5.1–9.8). At Wk 6, all pts were escalated to 100 mg BID. Nineteen pts (95.0%) completed the core period, one discontinued due to adverse event (AE);17 pts continued to the extension period. Sixteen pts (80.0%, 90% CI 59.9, 92.9; $p < 0.0001$) achieved a Hb response, including 5/5 pts with α-thalassemia and 11/15 pts with β-thalassemia. Mean (SD) time to first Hb increase >1.0 g/dL was 4.5 (3.2) wks, and mean Hb (SD) increase from BL during Wks 12–24 was 1.3 (0.6) g/dL. A sustained response was achieved in 65% of pts, including 5/5 pts with α-thalassemia and 8/15 pts with β-thalassemia. Hb increases occurred across the spectrum of BL levels. Directional improvements in markers of erythropoiesis and hemolysis were also observed. The safety profile was consistent with previously published mitapivat studies. There was one serious grade 3 unrelated AE of renal impairment, leading to treatment discontinuation. There were three non-serious grade 3 AEs leading to dose reduction:

(1) Resulted in a permanent dose reduction to 50 mg BID.

(2) Successfully rechallenged at 100 mg BID. The most common non-serious AEs occurring in ≥ 25% of pts were initial insomnia (10/20), dizziness (6/20), and headache (5/20). Dose escalation to 100 mg BID was well tolerated and not associated with any increase in AEs. Twelve pts had DXA scans at BL and 6 months; no notable changes in bone mineral density were observed.

In another study was conducted in university medical center Utrecht Netherlands [16], about AG-348 (Mitapivat), an allosteric activator of red blood cell pyruvate kinase, increases enzymatic activity, protein stability, and ATP levels over a broad range of PKLR genotypes, Fifteen adult, transfusion independent, PK-deficient patients were enrolled for study (median age: 44.0 years; range: 20.0-51.5). PK deficiency was confirmed by demonstrating compound heterozygosity or homozygosity for mutations in PKLR (Sanger sequencing). Whole blood from 15 healthy volunteers was used as control samples.

Baseline patients' characteristics displayed varying degrees of anemia. In addition, most patients had strongly elevated reticulocyte counts, a prominent feature of PKD patients, in particular after splenectomy. Most patients showed reduced red cell PK activity and reduced PK thermal stability. Since the activity of many red cell enzymes is red cell age-dependent, 36 we also compared PK activity to hexokinase (HK) activity. This PK/HK ratio was clearly decreased in all patients, indicating a relatively strong decrease in activity of PK ($r=-0.677$, $P<0.01$). When causative mutations where classified as missense (M) or non-missense (NM) a genotype to phenotype correlation was identified. All four patients without splenectomy were of the M/M genotype, which is in line with the lower likelihood of splenectomy in this group. Similarly, these patients had generally higher Hb levels and lower reticulocyte counts.

In 15 patients ex vivo treatment with AG-348 resulted in increased enzymatic activity in all patient cells after 24 hours (mean increase 1.8-fold, range 1.2-3.4). ATP levels increased (mean increase 1.5-fold, range 1.0-2.2) similar to control cells (mean increase 1.6-fold, range, 1.4-1.8). Generally, PK thermos ability was strongly reduced in PK-deficient RBCs. Ex vivo treatment with AG-348 increased residual activity 1.4 to >10-fold than residual activity of vehicle-treated samples. Protein analyses suggests that a sufficient level of PK protein is required for cells to respond to AG-348 treatment ex-vivo, as treatment effects were minimal in patient cells with very low or undetectable levels of PK-R. In half of the patients, ex vivo treatment with AG-348 was associated with an increase in RBC deformability.

In additions, Study conducted in Division of Hematology، Massachusetts General Hospital [17], support that Mitapivat has been shown to significantly upregulate both wild-type and numerous mutant forms of erythrocyte pyruvate kinase (PKR), increasing adenosine triphosphate

(ATP) production and reducing levels of 2,3-diphosphoglycerate. Given this mechanism, mitapivat has been evaluated in clinical trials in a wide range of hereditary hemolytic anemias, including pyruvate kinase deficiency (PKD), sickle cell disease, and the thalassemias. The clinical development of mitapivat in adults with PKD is nearly complete‹ with the completion of two successful phase III clinical trials demonstrating its safety and efficacy. Given these findings, mitapivat has the potential to be the first approved therapeutic for PKD. Mitapivat has additionally been evaluated in a phase II trial of patients with alpha and beta-thalassemia and a phase I trial of patients with sickle cell disease, with findings suggesting safety and efficacy in these more common hereditary anemias. Following these successful early-phase trials, two phase III trials of mitapivat in thalassemia and a phase II/III trial of mitapivat in sickle cell disease are beginning worldwide. Promising preclinical studies have additionally been done evaluating mitapivat in hereditary spherocytosis, suggesting potential efficacy in erythrocyte membranopathies as well. With convenient oral dosing and a safety profile comparable with placebo in adults with PKD, mitapivat is a promising new therapeutic for several hereditary hemolytic anemias.

Discussion

The results of Orlando [14] study demonstrate that PKR activation with mitapivat was well tolerated and improved anemia, hemolysis, and ineffective erythropoiesis, and may represent a novel therapeutic approach for patients with α- or β-thalassemia, the study met its primary endpoint with 80% of patients achieving a Hb response.

These data support the hypothesis that increased ATP synthesis mediated via PK-R activation by mitapivat may improve the survival of thalassemic RBCs in the bone marrow and/or peripheral circulation, and thus represents a novel mechanism to treat patients with thalassemia.

The finding in study of Utrecht [16] indicate that ex vivo treatment with AG-348 effectively increases PK-R activity over a broad range of PKLR genotypes as assessed by enzymatic activity as well as cellular ATP levels. Findings are consistent with those previously reported by Kung et al.,[18] and further extend those findings through the evaluation of a substantially larger cohort of PK-deficient blood samples, including a comprehensive baseline metabolomics analysis, and assessment for the first time of the effects of PK deficiency on parameters such as red cell deformability and ex vivo expansion of erythroid progenitors.

The data illustrate that AG-348 has the potential to stabilize a diverse range of different mutant PK-R molecules in vitro, but also demonstrate the complex variability that is introduced by the compound heterozygous state of most patients.

In addition, data report the effects of AG- 348 on ex vivo produced PK-R deficient RBC. In presence of AG-348 we observed a trend towards a slightly improved proliferation of erythroid progenitor cells in both healthy controls and PK-deficient patients of various PKLR genotypes. Further studies are warranted to establish this more firmly, but it could indicate that, apart from acting on mature RBC in the circulation, AG-348 could have a potential beneficial effect on both normal and PK-deficient early stage erythroid development. In addition, show that ex vivo produced (mutant) PK-R can be activated to a similar degree as (mutant) PK-R from RBC from patients and healthy controls. This was particularly evident for patients 7 and 15, who also showed the most pronounced effect of AG-348 on PK-R protein levels.

More importantly, genotype-phenotype correlations could explain these conflicting results as different genotypes result in different complications, indicating that different mutations could result in a large variability in the occurrence of ineffective hematopoiesis [19].

The improvement in RBC deformability as measured by osmotic gradient ektacytometry after ex vivo treatment with AG-348 in half of the patients. However, this technique likely underestimates the effect of ATP increase by AG-348. Although ektacytometry is an important and established method in the diagnosis of RBC membrane and hydration disorders, it does have some limitations. Ektacytometry reflects the passive deformation of RBC in contrast to filter- based assays or microfluidics which forces RBC to actively deform; the latter is thought to be more dependent on ATP. This could explain the finding of only a small correlation with ATP levels and deformability measured in RBC concentrates intended for transfusion [20].

The results show only a slight difference in RBC deformability between PK-deficient patients and healthy controls, with the lowest deformability observed in M/NM patients. This is in line with recent findings using the same technique, but contrasts with earlier findings based on a filtration-based assay [21].

These results are in line with previous studies that show that reticulocytes of PK-deficient patients are preferentially removed by the spleen,[22] probably due to their compromised deformability as a result of insufficient ATP levels [24]. It has been suggested that such a decreased deformability could be due to the decreased hydration as a result of K+ efflux through PIEZO1-mediated Ca2+ influx and activation of Gardos channels. However, the osmoscan data show no signs of dehydration of RBC (i.e., decreased Ohyper), which is clearly measurable on RBC of patients with hereditary xerocytosis, where dehydration is thought to occur through the same mechanism.

Collectively, the data presented in this study support the hypothesis that drug intervention with AG-348 effectively up-regulates PK-R enzymatic activity and increases stability in PK-deficient RBC over a broad range of PKLR genotypes.

Conclusions

- These results demonstrate that PKR activation with mitapivat was well tolerated and improved anemia, hemolysis, and ineffective erythropoiesis, and may represent a novel therapeutic approach for pts with α- or β-thalassemia. Primary endpoint with 80% of patients achieving a Hb response.
- Thalassemic RBCs have reduced ATP levels.
- Mitapivat may improve RBC survival in thalassemia by increasing ATP production.
- Mitapivat increased ATP and improved RBC parameters of β-thalassemia, and increased ATP levels ex vivo in human β-thalassemia RBCs.
- An ongoing, phase 2, open-label, multicenter study examines the effect of mitapivat on Hb in non–transfusion-dependent patients with thalassemia.
- The concomitant increase in ATP levels suggests that glycolytic pathway activity may be restored. AG-348 treatment may represent an attractive way to correct the underlying pathologies of PK deficiency.

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