CLINICAL RESEARCH PROJECT

Protocol # 19H0097
IND # 142982
IND Holder: NHLBI OCD

Date: April 1, 2021

Title: A Pilot Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of escalating multiple oral doses of AG-348 in subjects with stable sickle cell disease

Abbreviated title: A Dose-Finding study of AG-348 in sickle cell disease

Other identifying words: HbS polymerization, pyruvate kinase, 2,3- diphosphoglycerate and ATP in red blood cells, acute sickle pain.

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Subjects:
Number : N=15 (to enroll 20-25, over approximately 1 year, for 15 patients who have completed the full study of multiple doses)

Sex: Male & Female Age range: 18-70 years

Project involves ionizing radiation?	No (medically and research indicated)
Off-site project?	Yes (for Pharmacokinetics and Pharmacodynamics assays of IND only)
Multi-institutional project?	No
DSMB Involved?	Yes
Tech Transfer?	CRADA

Table of Contents

Contents

PRECIS

Sickle cell disease (SCD) is a multisystem disorder associated with episodes of acute clinical events and progressive organ damage. Episodic pain, triggered by micro-vasoocclusion induced by 'sickled' red blood cells, is the most common acute complication and the leading cause of hospitalization. Management strategies for SCD have evolved very slowly, and treatment of acute pain is still limited to supportive therapy with opioid medication. Until recently in 2017, the only approved therapy for SCD was hydroxyurea (HU), indicated to reduce frequency of acute painful crises but not universally effective. In addition to HU, transfusions with normal red blood cells are widely used to treat severe sickle crises, but this strategy has limitations (not uniformly accessible, accompanied by risks of alloimmunization, hemolytic transfusion reactions and transfusional iron overload). The only curative treatment is bone marrow transplantation, but this option carries significant risks and is limited by the availability of histocompatible donors.

As the root cause of SCD is polymerization of deoxy-HbS, there is a strong rationale for exploring agents that could inhibit/reduce the polymerization process itself. HbS polymerizes only when deoxygenated, its oxygenation is influenced by a few factors, one key factor being the 2,3- diphosphoglycerate (2,3-DPG) concentration in the red blood cell. Increased intracellular 2,3-DPG decreases oxygen binding and stabilizes the deoxygenated form (T form) of hemoglobin. In addition, increased 2,3-DPG concentration decreases intracellular red cell pH further promoting HbS polymerization. 2,3-DPG is an intermediate substrate in the glycolytic pathway, the only source of ATP production in red blood cells. Pyruvate kinase (PK) is a key enzyme in the final step of glycolysis; PK converts phosphoenolpyruvate to pyruvate, creating 50% of the total red cell ATP that is essential for maintaining integrity of the red cell membrane. Reduced PK activity leads to accumulation of the upstream enzyme substrates, including 2,3-DPG, that favours polymerization of deoxy-HbS. In humans with SCD, and even in sickle carriers who are generally asymptomatic, reduced oxygen affinity will favour deoxygenation of HbS and its polymerisation, and thus sickling. Indeed, the combination of PK deficiency and sickle cell trait causing an acute sickling syndrome has been previously reported in two cases.

Current approaches to reduce HbS polymerization include fetal haemoglobin induction via multiple strategies and drugs that targets HbS polymerization through increasing affinity of hemoglobin for oxygen (eg. Oxbryta™ / Voxelotor / GBT440). Increasing red cell PK (PK-R)

activity, leading to a decrease in intracellular 2,3-DPG concentration, presents a potentially attractive therapeutic target for thwarting HbS polymerization and acute sickle pain. AG-348 is a novel, orally bioavailable, small molecule allosteric activator of PK-R, that is currently in Phase II/III clinical trials in humans with PK deficiency (NCT02476916, NCT03548220 / AG348-C-006; NCT03559699 / AG348-C-007). Overview of the preclinical AG-348 data and other data support dose-dependent changes in blood glycolytic intermediates consistent with glycolytic pathway activation at all multiple ascending doses tested, supporting the potential role of AG-348 in the treatment of sickle cell disease. The overall objective of the present study is to determine the clinical safety and tolerability of AG-348 in subjects with SCD.

1.0 OBJECTIVES

Primary Objective:

1.1 To assess the clinical safety and tolerability of multiple escalating doses of AG-348, an allosteric activator of the enzyme pyruvate kinase, in subjects with stable sickle cell disease (SCD).

Secondary Objectives:

1.2 To assess the pharmacokinetics of AG-348 in stable SCD subjects after multiple escalating oral doses.

1.3 To understand the mechanisms of action of AG-348 on the glycolytic pathway in sickle cell disease through laboratory studies. Specific pharmacodynamics objectives include: the levels of 2,3-DPG, PK-R and ATP, and oxygen dissociation sickling in red blood cells from stable SCD subjects treated ex-vivo as well as in-vivo.

1.4 To evaluate the relationship between AG-348 pharmacokinetics and safety parameters.

2.0 BACKGROUND

2.1. Sickle cell disease

Sickle cell disease (SCD) is an inherited hemoglobin disorder caused by the presence of hemoglobin S (HbS) which results from the substitution of valine for glutamic acid in position 6 of the β chain of hemoglobin (βGlu6Val), a consequence of a specific single base substitution in the β-globin gene (HBB c.20A>T; rs334). SCD is an autosomal recessive disorder, individuals with only one copy of the β^S allele are carriers of the condition (HbAS). This sickle cell 'trait' is a benign condition and generally does not cause any clinical disability. The genetic causes of SCD include homozygosity for the rs334 mutation (HbSS) (generally known as sickle cell anemia; SCA) and compound heterozygosity between rs334 and mutations that lead to other structural variants of β-globin (such as HbC), or to reduced levels of β-globin production (β-thalassemia). In patients of African ancestry, HbSS is the majority cause of SCD (65-70%), followed by HbSC (about 30%), with HbS/β-thalassemia being responsible for the majority of the rest.

2.2 Epidemiology

The HbS mutation is a prototype of a balanced polymorphism. Carriers for the β^S gene (HbAS) are protected from falciparum malaria and have a survival advantage over normal (HbAA) individuals [1]. This is, however, offset by the increased fatalities in HbSS individuals. Although the sickling disorders occur predominantly in individuals of African descent, the disease is also prevalent throughout the Mediterranean, Middle East and parts of India, the Caribbean, and South and

Central America, where falciparum malaria was endemic. Within these regions the HbS gene frequency ranges from 10–30%. Due to the recent global population migration, however, SCD has become an important part of clinical practice in many regions, that are well beyond its early geographic regions, including the Caribbean islands, Brazil, the United States, the United Kingdom, France and many parts of other European countries [2].

Around the world, it is estimated that more than 300,000 children are born with sickle cell anemia each year [3]. In the US, SCD affects an estimated 100,000 Americans (vast majority African-Americans and the rest of Hispanic descent), and about one in 365 African-American newborns [4].

2.3 Clinical Manifestations

Individuals with SCD have chronic hemolytic anemia interrupted by acute and recurrent clinical events, that vary greatly in frequency and severity, the most common being acute pain, frequently referred to as acute vaso-occlusive crises (VOC).

Symptoms do not develop in babies until after 4–6 months of age when adult type hemoglobin becomes the predominant hemoglobin. Until then, neonates are protected from the effects of SCD due to the presence of residual fetal hemoglobin (HbF). Hand-foot syndrome, or dactylitis, is often one of the first indications of SCD, presenting as painful swelling of the bones of the hand or foot [5]. At this stage, the infant is usually anemic with mild jaundice and has a palpably enlarged spleen. Splenomegaly usually resolves due to repeated infarctions of the spleen and it is unusual to be able to feel the spleen after the first decade of life, although functional hyposplenism may occur in as many as 90% of children with SCA between the ages of 6 months and 3 years. Typically, these children have a hemoglobin level that varies between 6 and 8 gm/dL and a reticulocyte count of 10 to 20%.

The clinical course of SCD is notoriously variable. Progressive damage to a range of organs is a constant feature of the disease. The chronic hemolysis of SCD is punctuated by acute exacerbations of the illness, the most common manifestations are the vaso-occlusive crises characterized by acute painful episodes due to blockage of small vessels triggered by the sickled erythrocytes and tissue infarction. Microvascular occlusion occurs in different organs including: spleen, as acute splenic sequestration – commonly in the first two years of life; lungs as “acute chest syndrome” (ACS). ACS presents with a clinical picture (including cough, shortness of breath and signs of consolidation and hypoxemia) that is difficult to distinguish from acute pneumonia. Vaso-occlusion affecting the CNS manifests either as fits, transient neurological symptoms resembling ischemic attacks, or a fully developed stroke. Vaso-occlusion of the outflow vessels from the corpora cavernosa by sickled erythrocytes causes priapism, an unwanted and painful erection of the penis that lasts for >2 hours.

Repeated vaso-occlusive events and the chronic uncompensated hemolytic anemia ultimately result in end organ damage and almost any organ can be affected. Virtually every patient with SCD has some form of renal impairment, with progressive inability to concentrate urine, polyuria, nocturia and enuresis, which is common in children. Eventually the glomerular damage causes

chronic renal failure, particularly in patients over 40 years of age. Other common clinical conditions include gallstones, recurrent chronic leg ulceration (more common in those patients with severe anemia) and proliferative retinopathy leading to progressive visual loss, which is more common in patients with HbSC disease. The vertebral bodies, femoral and humeral heads are particularly prone to infarction and avascular necrosis of the femoral head may lead to total disability, frequently requiring a total hip prosthesis.

Acute anemia, usually defined as a decline in hemoglobin of >2g/dL from steady state levels [6], is a common feature of painful crises but can also result from sequestration of erythrocytes in deep vascular beds, most commonly within the spleen (acute splenic sequestration) or liver (acute hepatic sequestration), temporary marrow aplasia secondary to Parvovirus B19 infection [7], or from increased hemolysis secondary to serious acute infections or delayed transfusion reactions. Infection is the major cause of death in children, with hyposplenism increasing the susceptibility to encapsulated bacteria, in particular *Steptococcus pneumonia*. Causes of death in adults are more variable and include infection, acute chest syndrome, liver failure, stroke and heart failure.

2.4 Economic Impact of SCD

Less than 50 years ago in 1960, SCD was regarded as a "disease of childhood" [8] and few children survived beyond their teens, while 25 years later, the Cooperative Study of Sickle Cell Disease (CSSCD) reported a median age at death of 42 years for males and 48 years for females with HbSS and that 85% HbSS patients will survive to adulthood [9]. More recent studies confirmed that the majority of newborns (94 to 99%) in well-resourced countries will now survive to adulthood [10-12] but early mortality remains. Survival of patients with SCD in well-resourced and medium-resourced settings, has improved greatly in the last 60 years, and continues to improve [13-15]. Nonetheless, the life expectancy of patients with SCD is still shortened by 20 to 30 years compared to the general population [4,16,17]. Patients with SCD also have a poor quality of life beset with varying degrees of multi-system organ damage and chronic pain, further impacted by intermittent unpredictable episodes of acute pain. In a two-year study of 1,189 SCD patients in Illinois, 8,403 hospital admissions were reported. From these hospitalizations, over 97% of admitting diagnoses were for acute pain crises, of which 86% required emergency services as the primary source of admission [18]. A study of 4,294 patients who were enrolled in Florida Medicaid program demonstrated that a lifetime cost of care in average was $460,151 per patient with SCD [19]. The majority (80.5%) of SCD-related costs were associated with inpatient hospital care. Few options are available for treating acute pain crises, which are largely managed symptomatically with fluids and analgesics.

2.5 Pathophysiology of Sickle Cell Disease

The root cause of SCD is polymerization of deoxy-HbS. Low oxygen conditions predispose deoxy-HbS to polymerize which causes the red blood cells to become distorted and bizarrely shaped,

some adopting a “sickled” shape. Sickled red blood cells are rigid and adhesive, and together with white blood cells and platelets, cause blockages in the fine blood vessels (microvascular occlusion) leading to ischemia and depletion of oxygen to tissues. The body experiences this as an intense pain episode (known as acute painful crisis), acute organ damage, stroke or even acute multi-organ failure, depending upon the site and severity of the vascular blockage. Sickled RBCs have a shortened lifespan of 16 days compared to the average of 120 for normal RBCs, leaving patients with SCD in a constant state of uncompensated hemolytic anemia. The chronic anemia and recurrent vaso-occlusive events result in multiple organ co-morbidities and reduced life expectancy.

2.6 Available treatments for SCD

Management strategies for SCD have evolved very slowly, and treatment of SCD remains a serious unmet medical need. Until July 2017, when Endari (L-glutamine oral powder) was licensed by the US Food and Drug Administration (FDA), hydroxyurea (HU) was the only disease-modifying drug ever to be licensed for the treatment of SCA by the FDA. HU is the first of two FDA-approved anti-sickling agents. HU is indicated to reduce the frequency of painful crises but its use is limited by its side-effect profile, including neutropenia and thrombocytopenia [20,21]. The clinical effectiveness of HU is largely due to induction of HbF, but it is not universally effective. HbF is not evenly distributed unlike the rare natural genetic variants (Hereditary Persistence of Fetal Hemoglobin, HPGH) in which the HbF increase is evenly distributed among the red blood cells . Voxelotor is the second anti-sickling agent that was approved by the FDA in November 2019 for the treatment of SCD in adults and pediatric patients 12 years of age and older. Voxelotor inhibits sickling by preferentially binding to the high-oxygen affinity, non-polymerizing R conformation of hemoglobin, thereby reducing the concentration of the polymerizing T conformation [23]. Other treatment options include transfusions with normal blood to alleviate symptomatic anemia, treat severe sickle crises (eg, acute chest syndrome), and treat cerebrovascular stroke [24]. Blood transfusions, however, have substantial limitations: the treatments are expensive, not uniformly accessible, and are accompanied by risks including alloimmunization, hemolysis, and transfusional iron overload. Another option is to reduce/prevent frequency of VOC in those patients with recurrent acute pain, an indication for Adakveo® (crizanlizumab / anti-P selectin) that was approved by FDA in November 2019 [25]. The only curative treatment is bone marrow transplantation from a histocompatible donor [26,27], but bone marrow transplantation carries significant risks and is limited by the availability of histocompatible donors [28].

As curative options are limited to a small subgroup, and current treatment options are not universally effective, there is a need for novel therapies that target the other mechanisms of sickling [29]. HU has multiple mechanisms of action that affect various points on the pathophysiological pathway of SCD but the main mechanism of action of HU is the therapeutic induction of fetal hemoglobin (HbF) which has an inhibitory effect on HbS polymerization. However, the inter-individual response to HbF induction by HU is highly variable. HU-induced HbF increase is

unevenly distributed among the RBCs (F-cells), resulting in limited protection from sickling unlike the pancellular HbF increase seen in patients with sickle hereditary persistence of fetal hemoglobin (S/HPFH) [30]. Few other therapies developed for the treatment of SCD seek to target the underlying mechanism of the disease, i.e., preventing the polymerization of HbS and sickling of red blood cells (RBCs) that cause the clinical complications of SCD (Bunn 1997).

2.7 Current Experimental Nongenetic Drug Therapies

The last 2 decades have witnessed a surge in the development of drugs that target HbS polymerization [23,29], and those that prevent or reverse vaso-occlusion [25,31]. The best established strategy for mitigating HbS polymerization is the induction of fetal hemoglobin (HbF) synthesis, and both gene therapy and pharmacological approaches are actively being investigated [32,33]. Induction of HbF is a major component of the efficacy of HU therapy but the variability in HbF increase and its uneven distribution among the RBCs results in limited protection from sickling [22]. In recent years, advances in unravelling the molecular mechanisms controlling globin gene expression has led to alternative approaches to HbF induction that fall into 2 groups - those that affect chromatin regulators (such decitabine on DNA methylation and histone deacetylase (HDAC) inhibitors) and, the others that affect DNA-binding transcription factors [34-36]. Another HbF-inducing agent under investigation is Metformin, a prescription drug currently in use for type II diabetes mellitus (NCT02981329).

A second ant-sickling agent that was approved by the FDA in November 2019 for the treatment of SCD is voxelotor. Voxelotor is an allosteric hemoglobin modifier that binds to the N-terminal valine residue of the α-globin chain of HbS and increases its oxygen affinity. To date, there is no evidence that it reduces frequency of pain crises, organ damage and survival, although the increase in hemoglobin is dose-dependent and accompanied by decreased markers of hemolysis, suggestive of reduced sickling [23]. There is also some concern that Hb molecules with the drug bound are in a conformation that delivers very little oxygen, especially detrimental in a disease characterized by decreased oxygen delivery [37]. Hopefully, these concerns are addressed in current multicenter phase 3 clinical studies in both adults (http://www.clinicaltrials.gov/, NCT03036813) and children (http://www.clinicaltrials.gov/, NCT02850406).

Most of the nongenetic approaches currently being assessed in clinical trials are aimed at using drugs to ameliorate the downstream sequelae of sickle cell hemoglobin (HbS) polymerization, such as adhesion of red cells to vascular endothelium, leukocytes, and platelets, as well as inflammation, coagulation, and nitric oxide scavenging [31]. From the perspective of preventing acute painful crises, crizanlizumab, a humanized antibody to P selectin holds much promise. In a double-blind, randomized, placebo-controlled phase II trial, adult participants (on or off HU), Crizanlizumab showed a significant reduction in sickle related pain crises and low incidence of adverse events [38]. Crizanlizumab was approved by the FDA in November 2019for reducing VOCs in SCD. A phase 2 trial has shown efficacy of GMI-1070 (rivipansel), a pan-selectin inhibitor, that seems to shorten the duration of acute sickle pain crises accompanied by a marked reduction in

opioid use [39]. Rivipansel is currently undergoing phase 3 investigation (http://www.clinicaltrials.gov/, #NCT01119833).

2.8 Investigational Agent AG348 and pyruvate kinase deficiency

Investigational drug AG-348 (formerly known as AGI-1480 and AGX-0841) is a potent, broad-spectrum activator of alleles of the red blood cell (RBC)-specific form of pyruvate kinase (PKR). PKR is 1 of 4 pyruvate kinase isoenzymes expressed in human tissues from 2 distinct genes. PKR and liver-type pyruvate kinase (PKL) are generated from the *PKLR* gene by 2 separate tissue-specific promoters (https://www.ncbi.nlm.nih.gov/gene/5315), while PKM1 and PKM2 are from the *PKM* gene via differential splicing of the ribonucleic acid (https://www.ncbi.nlm.nih.gov/gene/5315). *PKLR* is located on chromosome 1q21. The difference between the red cell (PKR) and liver (PKL) isoforms is the 32 amino acids at the N- terminus generating a different promoter; red cell and liver PK are identical except for exon 1 [40]. AG-348 is an allosteric activator of PKR, PKL, and PKM2, with similar potency against each.

Pyruvate Kinase deficiency

Hereditary RBC enzymopathies are genetic disorders arising from mutations in genes encoding red cell enzymes [41,42]. These red cell enzymopathies cause a specific type of anemia termed hereditary/congenital nonspherocytic hemolytic anemia (HNSHA) [42]. Glycolysis is the only source of ATP production in red blood cells [43] (Fig. 1). Enzyme deficiencies in the glycolytic pathway (including pyruvate kinase) leads to the accumulation of upstream enzyme substrates, including 2,3-DPG (Fig. 1). PK converts phosphoenolpyruvate (PEP) to pyruvate, creating 50% of the total red cell ATP. Red cell longevity is dependent on ATP produced during glycolysis. PK deficiency leads to less intracellular ATP, and thereby shortening the red cell lifespan [44].

Mutations in *PKLR* gene cause PK deficiency in RBCs and hemolysis. PK deficiency (PKD) is rare (estimated at 1:20,000 in the general white population [45] with various prevalence estimates of 0.24%, 1.1%, <0.1%, 1.9%, and 3.2 % reported in populations of Spain, Turkey, Asia, Hong Kong (Chinese), southern Iran, and Saudi Arabia, respectively [46-50]. Over 250 *PKLR* mutations have been described, causing a range of quantitative deficiencies and dysfunctional PK enzymes [51]. The RBCs from patients with PK deficiency are characterized by changes in metabolism associated with defective glycolysis, including a deficiency in adenosine triphosphate (ATP) levels. Levels of 2,3-diphosphoglycerate (2,3-DPG), phosphoenolpyruvate (PEP), and other glycolytic intermediates upstream of the PKR reaction are reported to be elevated in patients with PK deficiency [42], reflecting the constriction of glycolysis at the PKR step. The range of *PKLR* mutations lead to a range of functional PK deficiencies, and diverse clinical manifestations of PKD, ranging from mild or fully compensated forms to life-threatening neonatal anemia, all with the usual hallmark of chronic hemolysis [44,52,53].

Drug intervention with AG-348 has been previously shown to restore glycolytic pathway activity and normalize RBC metabolism in PK deficient patients with minimal accompanying side effects. Two completed clinical Phase 1 studies (randomized, placebo-controlled, double-blind) assessed

the safety, tolerability and pharmacokinetics/pharmacodynamics of AG-348 in single ascending dose (SAD; http//:www.Clinical Trials.gov: NCT02108106), and multiple ascending dose (MAD; http//:www.Clinical Trials.gov: NCT02149966) and the results were recently published [54]. Dose-dependent changes in blood glycolytic intermediates consistent with glycolytic pathway activation were observed at all multiple ascending doses, supporting the potential role of AG348 in the treatment of sickle cell disease. The most common treatment-related AEs in AG-348-treated subjects were headache and nausea.
AG-348 is currently in Phase 2/3 clinical trials in subjects with PK deficiency.

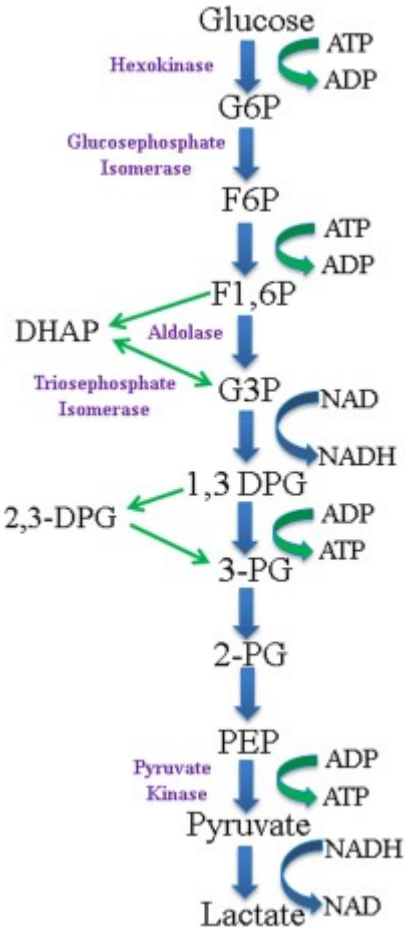


Figure 1. The Emden-Meyerhof pathway (Glycolysis). G6P, glucose-6-phosphate; F6P, fructose-6-phophate; F1,6P, fructose 1,6-phosphate; DHAP, dihydroxyacetone phosphate; G3P, glucose-3-phosphate; 1,3-DPG, 1,3-diphosphoglycerate; 2,3-DPG, 2,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate; 2-PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate (picture courtesy:[52]).

2.9 Clinical and Scientific Justification

Attempts to target downstream sequelae of the sickling process have not proved to be effective in

the reducing vaso-occlusive crisis frequency [55-63]. More effort should be directed toward preventing or mitigating the HbS polymerization process itself, the root cause of sickle cell pathology.

Reduce concentration of 2,3-diphosphoglycerate

Polymerization of HbS and its oxygenation are influenced by a few factors, one key factor is 2,3-diphosphoglycerate (2,3-DPG) concentration in RBCs [29,64]. 2,3-DPG is an allosteric effector on hemoglobin oxygen binding. It has a greater binding affinity to deoxygenated hemoglobin (the T state) than to oxygenated hemoglobin (the R state). 2,3-DPG thus stabilizes the quaternary T form of deoxy-hemoglobin and decreases oxygen binding. Thus, lowering 2,3-DPG intracellular concentration would increase the fraction of HbS in the non-polymerizing R quaternary structure [65]. In addition, decreasing 2,3-DPG concentration increases HbS solubility because of an accompanying increase in intracellular pH. Studies have shown that 2,3-DPG levels are significantly elevated in sickle cell patients and that 2,3-DPG promotes HbS polymerization [66,67]. Further, adding 2,3-DPG lowers oxygen affinity of HbS [66]. Increased 2,3-DPG concentration and decreased intracellular red cell pH have both been shown to boost deoxy-HbS polymerization [68,69]. While increased 2,3-DPG concentration and reduction of hemoglobin oxygen affinity is beneficial in anemia caused by PK deficiency [70,71], increased 2,3-DPG levels and decreased intracellular red cell pH are detrimental in the presence of HbS, as they boost deoxy-HbS polymerisation. In SCD, and even in sickle trait (where intracellular HbS concentration is 35-40%), the reduced oxygen affinity will favor deoxy-HbS polymerisation - and thus sickling [72].

2.9.1 Further Scientific Rationale

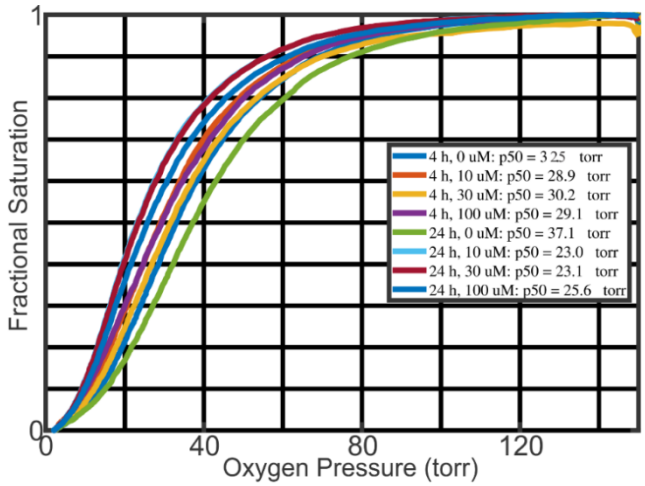
Further scientific rationale for proposing the study stems from the PI's two clinical observations:

1. Two case reports (including one from the PI's group) exist of sickle carriers, typically a totally benign condition, but with typical sickle cell phenotype [73,74]. Each of the proband had co-inherited mutations in the PKLR gene in addition to being a carrier for sickle cell. One report supported the role of 2,3-DPG in in-vitro functional assays. Unfortunately, in the second case reported by the PI, we could not obtain fresh blood for oxygen dissociation and other studies as patient had left the country.
2. Genetic association studies led by the PI in a sickle cohort in King's College London (where PI was located prior to relocation to the NHLBI) showed significant association between 4 genetic variants in the PKLR gene and hospitalization rate, a marker of sickle cell vaso-occlusive pain crises (Kate Gardner, PhD thesis King's College London; manuscript in preparation). The 4 PKLR variants are fairly common among individuals of African descent. We confirmed an allele frequency of 14% of these PKLR intronic variants in the NHLBI sickle cell cohort, and we have preliminary evidence from work here at the NHLBI that they are associated with reduced PK-R messenger activity. We are currently investigating the impact of these PKLR variants on PK, 2,3-DPG and ATP levels.

Because 2,3-DPG plays such a critical role in potentiating HbS polymerization, there is a

compelling rationale for the development of drugs that target the enzymatic pathway responsible for its remarkably high (5 mM) concentration in red blood cells. Apart from reducing 2,3-DPG levels, activation of PKR will also concomitantly generate more ATP, a key molecule for maintaining RBC membrane integrity.

Prompted by these clinical observations, we have performed studies on ex-vivo incubation of sickle RBCs with AG-348, and the preliminary data show clear left shifts in the oxygen dissociation curve for red cells from two sickle trait patients (so there is no interference resulting from fiber formation) and inhibition of sickling for cells from a patient with homozygous SS disease at concentrations of 10 micromolar (see figure 2). While encouraging, we would caution how we interpret the result from ex-vivo experimentation with respect to implications for dosing in humans, especially how it relates to the effect on downstream PD markers.



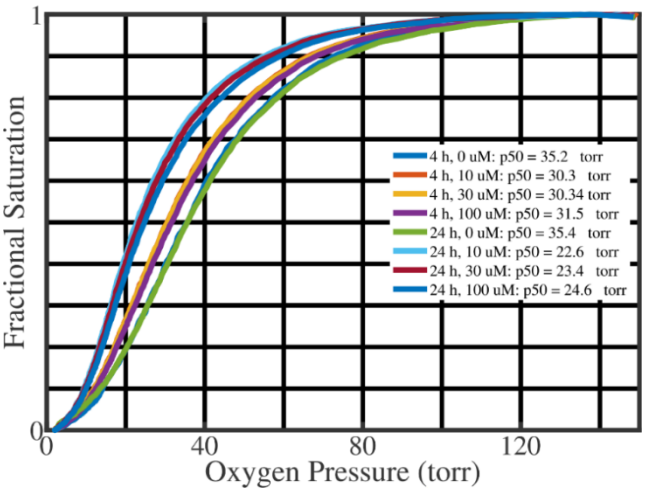


Fig. 2a. Oxygen dissociation curves in 20 mM phosphate-buffered saline, pH 7.4, 37 C for two sickle trait patients. Cells are incubated for 4 or 24 hrs in 0, 10, 30, and 100 µM AG-348.

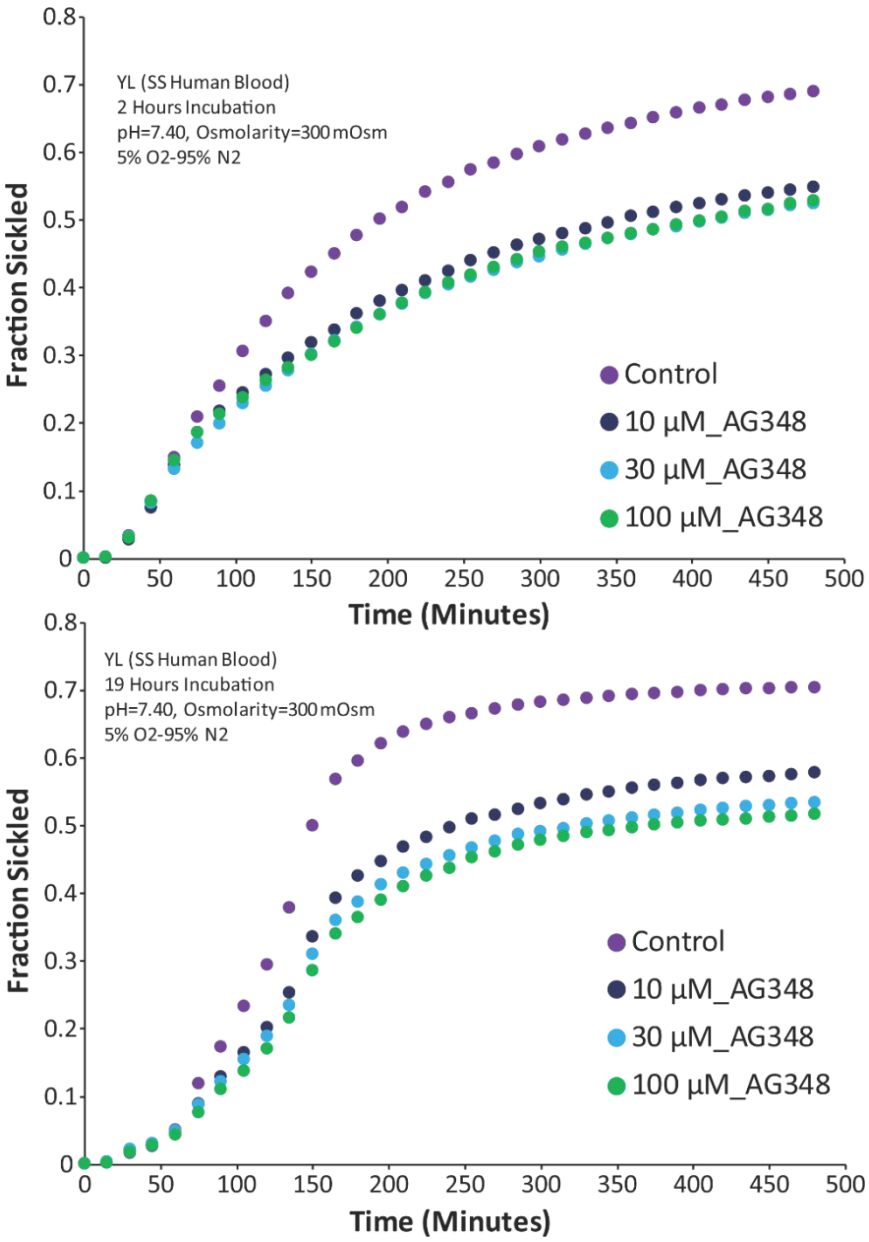


Fig. 2b. Fraction sickled vs time following deoxygenation with 95% nitrogen, 5% oxygen for homozygous SS patient after 2 hr or 19 hr incubation with 0 (control), 10, 30, and 100 µM AG-348 in 20 mM phosphate-buffered saline, pH 7.4, 37 C. Images of cells in wells of 384 well plate collected every 15 minutes in Biotek Lionheart microscope system.

We have also started recruiting for a prospective protocol in October 2018 on "**Genotype - Phenotype Correlation of *PKLR* Variants with Pyruvate Kinase, 2,3-diphosphoglycerate and Adenosine Triphosphate Activities in Red Blood Cells of Patients with Sickle Cell**

Disease", and as of December 2019, more than 300 subjects with AA, AS and SS have been enrolled and sampled. This study will inform any potential variability observed in the response of SCD patients to AG-348.

Justification for AG348

AG-348 is a novel, first-in-class, orally bioavailable, small molecule allosteric activator of the PKR, PKL, and PKM2 isoenzymes, with similar activity for each. AG-348 acts by directly binding to the PKR tetramer and allosterically enhancing its affinity for PEP. Deficiency in PK activity leads to defective glycolysis, including a buildup of PEP and the intermediate 2,3-DPG, and lowered levels of ATP, changes that impair metabolism of RBCs and their progenitors. The effect of AG-348 on PKR activity and a number of downstream pathway markers was evaluated in both human and murine RBCs and whole blood. In blood samples from PKR deficient patients, ex vivo treatment with AG-348 increased PKR activity (by 1·3- to 3·4-fold) and induced metabolic changes, including increased levels of ATP (by 1·3- to 2·4-fold), consistent with increased glycolytic pathway activity [75]. Mice with wild-type PK-R treated with AG-348 had increased PKR activity levels, as well as increased ATP and decreased 2,3-DPG levels consistent with in vivo activation of PK-R [75]. Moreover, in the Clinical Phase 1 study of healthy adult subjects, the concentration of 2,3-DPG decreased after a single dose of AG-348 in a dose-dependent manner to a minimum at 24 hours post dose and then returned to baseline values by 72 to 120 hours post dose. AG-348 was well tolerated at doses ranging from 15 to 360 mg administered q12h over 14 days, and pharmacodynamic changes in blood consistent with increased activity of the glycolytic pathway were observed at these levels. Collectively, these observations lead to the hypothesis that activation of PKR through AG-348 may lead to decreased 2,3-DPG that could have anti-HbS polymerizing effect. The concomitantly increased ATP generation may also help improve red cell survival. Of particular relevance to SCD, we noted that AG-348 showed potent activation (defined as >2-fold activation) not only of mutant isoforms of PKR, but also wild type PKR. We therefore propose this dose-escalating study of AG348 in patients with SCD in a clinical trial designed to evaluate its safety (maximum tolerated dose) and tolerability.

In the SAD and MAD studies, the concentration of 2,3-DPG decreased in a dose-dependent manner and returned to levels close to baseline by 72 hours following the final dose of AG-348. (see figure 3 below)

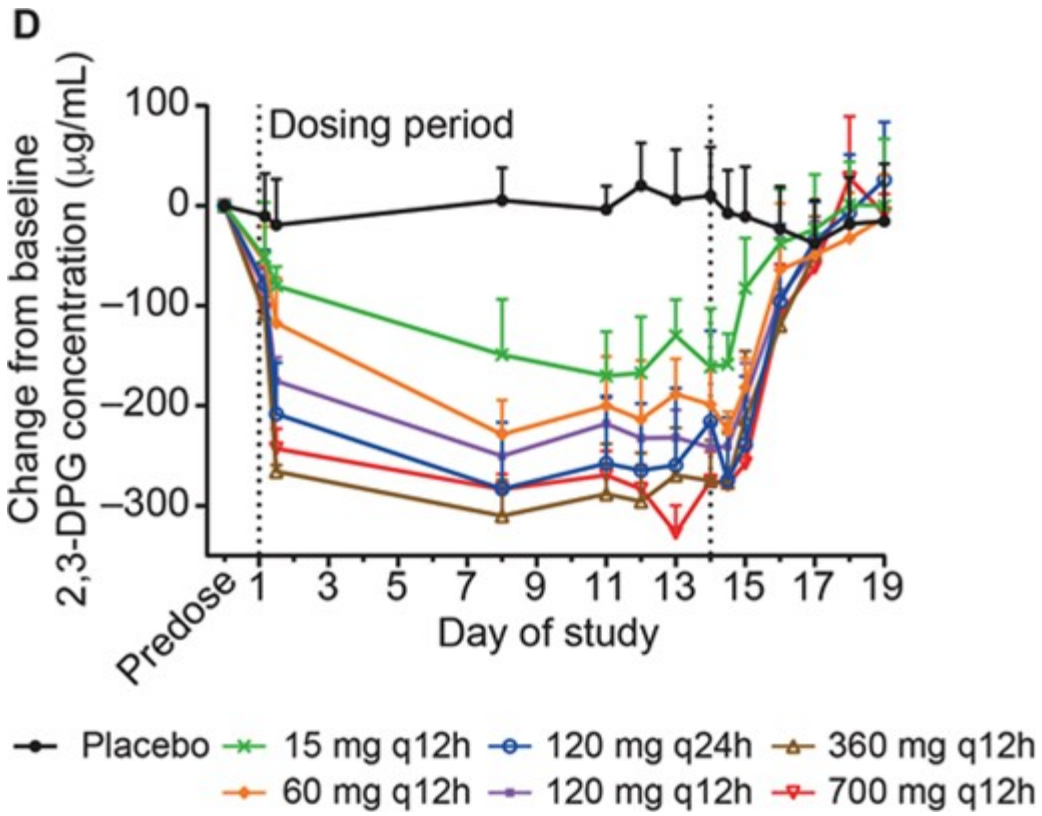


Figure 3, panel D in: Yang H et al. Clinical Pharmacology in Drug Development 2018, 00(0) 1–14.

Patients with SCD have a WT PKR enzyme as found in healthy individuals. Therefore, pharmacokinetic/PD results from the MAD study of AG-348 conducted in healthy adult subjects (NCT02149966, referenced in the protocol on page 13) have been used to select doses for the study planned to be conducted in patients with SCD. Briefly, a simple Emax (maximum effect) model was used to describe the pharmacokinetic/PD correlation between plasma AG-348 AUC0-12 and blood AUC_Net_B0-12 for 2,3-DPG using results from the MAD study. The estimated plasma AG-348 AUC0-τ that resulted in 50% maximum inhibitory effect (IAUC50) on 2,3-DPG over the dosing interval at steady-state was estimated to be 750 ng•h/mL. This corresponded to the AG-348 plasma total exposure (AUC0-τ) achieved following multiple doses of AG-348 below or near 15 mg q12h in the MAD study. Based on the mean observed values of 2,3-DPG blood AUC_Net_B0-12 and the predicted value of the maximum inhibitory effect of AG-348 on 2,3-DPG blood AUC_Net_B0-12 of 3,381 μg•h/mL, approximately 74% of the maximum inhibitory effect was achieved at a dose of 60 mg q12h in the MAD study and over 99% of the maximum inhibitory effect was achieved at a dose of 360 mg q12h in the MAD study.

In a phase 2 study (DRIVE PK), subjects with PKD were randomized to receive either 50 or 300 mg of AG-348 twice daily. Preliminary results of the DRIVE-PK study showed that several

subjects had to have their randomized dose level reduced because of excess increases in Hb or the occurrence of adverse events (AEs) such as insomnia, headache, or nausea) [54]. Dose reductions led to the resolutions of most of these AEs and the maintenance of satisfactory efficacy where applicable. Therefore, in the ongoing phase 3 studies being conducted in subjects with PKD, individual dose optimization is incorporated to allow each subject to gradually increase his/her dose of AG-348 in order to identify a dose that confers maximum benefit with minimum risk to that subject. In the phase 3 PKD studies, all subjects will start at a low dose level (5 mg BID) with 2 sequential steps for dose level increases (ie, from 5 to 20 mg BID and from 20 to 50 mg BID), depending on safety and tolerability. In a phase 2 Agios sponsored study being conducted in patients with thalassemia, doses of 50 mg and 100 mg BID are being evaluated. Given there is no prior experience of administering AG-348 in patients with SCD, the starting dose in this study was selected to be 5 mg BID (similar to the starting dose in phase 3 studies being conducted in patients with PKD), with the potential for intra-patient dose escalations to 20, 50, and 100 mg BID. In the AG-348-C-002 study, at 120 mg BID, there's ~89% inhibitory effect on 2,3-DPG compared to 700 mg BID; ~85% inhibitory effect on 2.3-DPG compared to 360 mg BID. Maximal Inhibition of 2,3-DPG was observed at about 360 mg BID. Studies of AG-348 at doses of 300mg (DRIVE-PK for PKD) suggest that 100 mg BID would have a comparable safety and tolerability profile to doses in the range of 5-50mg BID. The higher doses of 50 and 100 mg BID of AG-348 in the proposed study in adult subjects with SCD are expected to lower 2,3 DPG sufficiently to be able to study its effects on sickling, while concomitantly generating more ATP, a key molecule for maintaining RBC membrane integrity.

3.0 STUDY DESIGN

This is a non-randomized, dose-escalating clinical study designed to assess the safety and tolerability of escalating doses of AG348 in subjects with sickle cell disease (SCD).

Fifteen subjects with SCD (HbSS) will complete multiple dose levels of AG-348. Up to ten subjects will complete 3 dose levels, starting with 5 mg of AG-348 BID by mouth, escalating to 20 mg BID, and then 50 mg BID, with a dosing period of 2 weeks at each dose level. Starting with amendment #5, at least 5 subjects will complete 4 dose levels, escalating from 50mg BID to 100 mg BID for 2 additional weeks to allow for assessment of the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of the 100 mg BID dose in subjects with SCD. If fewer than 10 subjects have successfully completed the 3 dose levels at the time of approval of amendment #5, then all remaining subjects will be enrolled onto the 4-dose level escalation scheme detailed in the amended protocol. For any subjects undergoing dose escalation at the time of approval of amendment #5 (i.e. prior to initiating the drug taper), the study team will discuss the higher 100 mg BID dose level with the subject, and if interested, the subject will be reconsented to complete the 4 dose levels. If 10 subjects who are on track to complete the 3 dose levels are enrolled prior to the approval of amendment #5, then enrollment will be held until the amendment is approved to allow for at least 5 subjects to complete all 4 dose levels.

For subjects escalating to a maximum dose of 50 mg BID, subjects will be assessed for safety and tolerability every 2 weeks; in the absence of serious adverse effects, subjects will receive the escalating dose from 5 mg BID to 20 mg BID to 50 mg BID. Dose taper will start on Day 42 from their last dosing regimen of 50 mg twice daily using a rapid taper schedule over 12 days (Table 7A). For subjects escalating to a maximum dose of 100 mg BID, subjects will also be assessed for safety and tolerability every 2 weeks; in the absence of serious adverse effects, subjects will receive the escalating dose from 5 mg BID to 20 mg BID to 50 mg BID to 100 mg BID. Dose taper will start on Day 56 with subjects reducing their last dosing regimen of 100 mg twice daily to stopping all together using a rapid taper schedule over 15 days (Table 7B).

Subjects will be instructed to closely monitor for any signs or symptoms differing from baseline and to contact the study team immediately if they experience any change in their symptoms or overall health. If there is no change in their health, subjects will not need to come to the NIH for a provider visit or lab work. However, if a subject reports any symptoms concerning acute pain, the subject may be transitioned to a gradual dose taper per Investigator's discretion and potentially be asked to come to the NIH for a clinical evaluation with a provider and lab work prior to the end of the taper. The rapid and gradual dose taper are described in section 12.1.4.

At any time, the investigator can suspend or alter dosing for reasons related to safety or tolerability.

All patients may be admitted overnight for up to 2 days for observation and for completion of all blood collection time points when they commence the study, for each of the escalating dose of 20 mg BID, 50 mg BID, and 100 mg BID, and at the beginning of dose taper.

Subjects should be advised not to discontinue dosing without first speaking with the treating Investigator except in case of medical emergency; abrupt discontinuation of AG-348 may result in withdrawal hemolysis. If a subject needs to discontinue study drug at any time during the study, guidance is provided in Section 10.0 Off Study Criteria.

All study visits will be conducted at the NIH Clinical Center. Study visits conducted electronically will not be routinely used and will only be conducted under extreme circumstances for existing subjects. In these scenarios, only approved electronic technology and telehealth platforms will be used to communicate with the subject and will allow study providers to obtain a medical history, perform a limited physical exam, and assess for adverse events. The initial consent and screening procedures for new subjects should take place at the NIH. All telehealth visits will be documented in the source documents similar to a routine in-person study visit.

Even in the situation in which telehealth visits are used, subjects should have their laboratory studies drawn at the NIH if at all possible. If subjects are unable to present themselves to the NIH, the study team will directly order or will work with the subject's local provider to order necessary clinical laboratory tests and cover the cost of all study-related laboratory testing. If laboratory tests are obtained outside of the NIH, then only clinical laboratory tests for safety will be ordered, and research labs will be omitted.

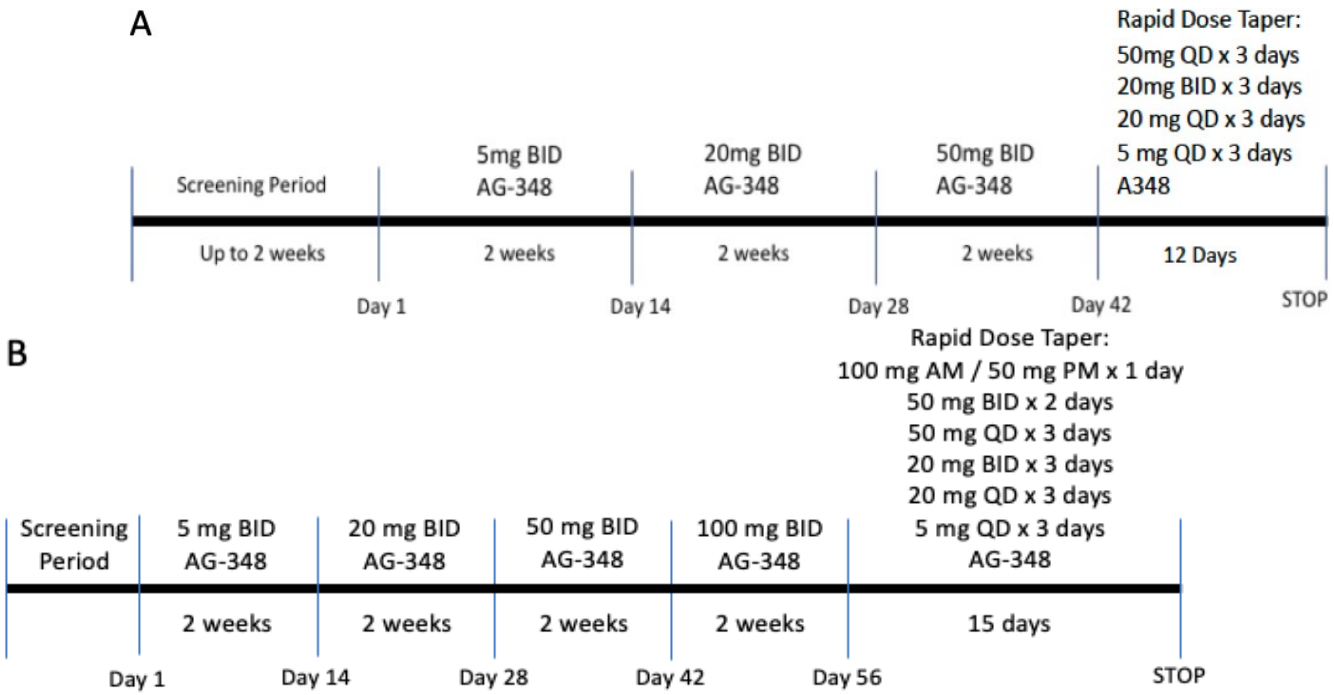


Figure 4. Dosing schemes.

Abbreviations: BID = twice daily; n/a = not applicable; QD = once daily; AM = daily in the morning; PM = daily at night.

A) Dosing scheme for subjects escalating to maximum study drug dose of 50 mg BID. Blood samples drawn for measurement of PK-R, 2,3-DPG and ATP levels, and Oxygen Sickling Assay at Screening, Day 1, Day 14 (± 3 days), Day 28 (± 3 days), Day 42 (± 3 days), Stop date (Day 54 ± 3 days) and 4 weeks (± 3 days) post-last dose, in addition to full blood counts, reticulocyte count, lactate dehydrogenase, renal and liver profile.

B) Dosing scheme for subjects escalating to maximum study drug dose of 100 mg BID. Blood samples drawn for measurement of PK-R, 2,3-DPG and ATP levels, and Oxygen Sickling Assay at Screening, Day 1, Day 14 (± 3 days), Day 28 (± 3 days), Day 42 (± 3 days), Day 56 (± 3 days), Stop date (Day 71 ± 3 days) and 4 weeks (± 3 days) post-last dose, in addition to full blood counts, reticulocyte count, lactate dehydrogenase, renal and liver profile.

4.0 SUBJECT RECRUITMENT AND REGISTRATION

Subjects with steady state sickle cell disease will be recruited from the sickle cell clinic at the NIH CC. We will also recruit subjects through NIH using traditional recruitment methods such as referrals from other protocols, outside physician referral and self-referral.

The study may also use the following strategies of recruitment including:

- ClinicalTrials.gov website
- Clinical Center Research Studies ("Search the Studies") website
- National Heart, Lung and Blood Institute (NHLBI) patient recruitment website
- Disease advocacy organizations such as the Sickle Cell Disease Association of America and Sickle Cell Anemia Research Fund website
- Twitter messages and chats with study investigators
- Facebook Posts
- Google AdWords
- Use of Clinical Center Office of Patient Recruitment services including creation and distribution of study flyers and information through pre-existing recruitment avenues such as the NIH recruitment listserv.

The DIR Patient Recruitment Office (PRO) will work with study investigators to ensure accrual goals are being met. All recruitment materials and tools will use IRB-approved language and information to include standard recruitment contacts.

5.0 ELIGIBILITY ASSESSMENT

All patients with sickle cell anemia (HbSS) will be considered for enrollment.

5.1 Inclusion Criteria

For enrollment, subjects must meet all of the following criteria during the screening period:

5.1.1 Have provided signed written informed consent prior to performing any study procedure, including screening procedures.

5.1.2 Age between 18-70 years

5.1.3 Unequivocal diagnosis of HbSS confirmed by hemoglobin electrophoresis performed on patients at least 90 days after a blood transfusion if previously transfused, or DNA genotyping

5.1.4 No transfusion in the 90 days prior to the first dose of study drug, or absence of HbA on hemoglobin analysis (by high-performance liquid chromatography; HPLC)

5.1.5 Have adequate organ function, as defined by:

a. Serum aspartate aminotransferase (AST) ≤2.5 × Upper Limit of Normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition) and alanine aminotransferase (ALT) ≤2.5 × ULN (unless the increased ALT is assessed by the Investigator as due to hepatic iron deposition).

b. Serum creatinine ≤1.25 × ULN. If serum creatinine is >1.25 × ULN, then glomerular filtration rate (based on creatinine) must be ≥60 mL/min.
c. Absolute neutrophil count ≥1.0 × 10^9/L.
d. Hemoglobin ≥ 7 g/dL
e. Platelet count ≥100 × 10^9/L.
f. Activated partial thromboplastin time and international normalized ratio ≤1.5 × ULN, unless the subject is receiving therapeutic anticoagulants.

5.1.6 For women of reproductive potential, have a negative serum or urine pregnancy test during the screening period. Women of reproductive potential are defined as sexually mature women who have not undergone a hysterectomy, bilateral oophorectomy, or tubal occlusion; or who have not been naturally postmenopausal (i.e., who have not menstruated at all for at least the preceding 12 months prior to signing informed consent and have an elevated follicle-stimulating hormone level indicative of menopause during the screening period).

5.1.7 For women of reproductive potential as well as men and their partners who are women of reproductive potential, be abstinent as part of their usual lifestyle, or agree to use 2 effective forms of contraception from the time of giving informed consent, during the study, and for 28 days for women and 90 days for men following the last dose of study treatment. An effective form of contraception is defined as hormonal oral contraceptives, injectables, patches, and barrier methods.

5.1.8 Be willing to comply with all study procedures for the duration of the study.

5.2 Exclusion Criteria

Subjects who meet any of the following criteria during screening will not receive AG348 and will not be counted toward the final enrollment count for statistical purposes:

5.2.1 Documented pyruvate kinase deficiency

5.2.2 Have a significant medical condition that confers an unacceptable risk to participating in the study, and/or that could confound the interpretation of the study data. Such significant medical conditions include, but are not limited to the following:

a. Poorly controlled hypertension (defined as systolic blood pressure [BP] >150 mmHg or diastolic BP >90 mmHg) refractory to medical management.

b. History of recent (within 6 months prior to signing informed consent) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.

c. Cardiac dysrhythmias judged as clinically significant by the Investigator.

d. Heart-rate corrected QT interval-Fredericia's method (QTcF) >480 msec with the exception of subjects with right or left bundle branch block.

e. Clinically symptomatic cholelithiasis or cholecystitis. Prior cholecystectomy is not exclusionary. Subjects with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.

f. History of drug-induced cholestatic hepatitis.

g. Iron overload sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac (e.g., clinically significant impaired left ventricular ejection fraction), hepatic (e.g., fibrosis, cirrhosis), or pancreatic (e.g., diabetes) dysfunction.

h. Have a diagnosis of any other congenital or acquired blood disorder, or any other hemolytic process as defined by a positive direct antiglobulin test (DAT), except mild allo-immunization as a consequence of transfusion therapy.

i. Positive test for hepatitis B surface antigen or hepatitis C virus (HCV) antibody (Ab) with signs of active hepatitis B or C virus infection. If the subject is positive for HCV Ab, a reverse transcriptase-polymerase chain reaction test will be conducted. Subjects with hepatitis C may be rescreened after receiving appropriate hepatitis C treatment.

j. Positive test for human immunodeficiency virus 1 or 2 Ab.

k. Active infection requiring any use of systemic antimicrobial agents (parenteral or oral) or Grade ≥3 in severity (per National Cancer Institute Common Terminology Criteria for Adverse Events) within 2 months prior to signing informed consent.

l. Diabetes mellitus judged to be under poor control by the Investigator or requiring >3 antidiabetic agents, including insulin (all insulins are considered 1 agent); use of insulin per se is not exclusionary.

m. History of any primary malignancy, with the exception of: curatively treated nonmelanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent, no known active disease present, and no treatment administered during the last 3 years.

n. Unstable extramedullary hematopoiesis that could pose a risk of imminent neurologic compromise.

o. Current or recent history of psychiatric disorder that, in the opinion of the Investigator or Medical Monitor, could compromise the ability of the subject to cooperate with study visits and procedures.

p. Are currently enrolled in another therapeutic clinical trial involving ongoing therapy with any investigational or marketed product or placebo. Sickle cell anemia subjects on hydroxyurea or L-glutamine will also be considered, provided that they have been on an unchanged dose of hydroxyurea or L-Glutamine for three months prior to enrollment.

q. Have exposure to any investigational drug, device, or invasive procedure within 3 months prior to the first dose of study treatment. All non-investigational invasive procedures within 3 months of starting study treatment may be considered as a potential exclusion criteria per the PI's discretion.

r. Have had any prior treatment with a pyruvate kinase activator.

s. Have received crizanlizumab or voxelotor in the 12 weeks prior to signing consent.

t. Have a prior bone marrow or stem cell transplant.

u. Are currently pregnant or breastfeeding.

v. Are currently receiving medications that are strong inhibitors of cytochrome P450 (CYP)3A4 or strong inducers of CYP3A4 that have not been stopped for a duration of at least 5 days or a timeframe equivalent to 5 half-lives (whichever is longer) prior to the first dose of AG-348.

w. Are currently receiving hematopoietic stimulating agents (e.g., erythropoietins, granulocyte colony stimulating factors, thrombopoietins) that have not been stopped for a duration of at least 28 days prior to the first dose of study treatment.

x. Have a history of allergy to sulfonamides if characterized by acute hemolytic anemia, drug-induced liver injury, anaphylaxis, rash of erythema multiforme type or Stevens-Johnson syndrome, cholestatic hepatitis, or other serious clinical manifestations.

y. Have a history of allergy to AG-348 or its excipients (microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, and mannitol).

6.0 SAMPLE COLLECTION, STORAGE, AND TRACKING PLAN

Collection Practices:

6.1 Clinical Evaluation of the Subject

Pre-Study Evaluation- Screening:
Testing for screening evaluation will be conducted under another NHLBI screening protocol. Alternatively, if a test result is available from any other NHLBI protocol within 90 days of enrollment, that test result can also be used for screening purposes

a. Informed Consent
b. Inclusion/Exclusion Criteria evaluation
c. Prior Concomitant medications
d. History and physical exam including prior transfusion history and review of sleep history.
e. Vital sign measurements including weight
f. Oximetry
g. Electrocardiogram (EKG)
h. CBC with differential
i. Cooximeter (venous) if hemoglobin value is not able to be obtained with a CBC
j. Reticulocyte count
k. Acute care panel (Sodium (NA), Potassium (K), Chloride (CL) Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen, , Anion Gap), Mineral panel (Albumin, Calcium, Magnesium (Mg), Phosphorus), Hepatic panel (Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin)
l. Iron Panel and ferritin
m. Coagulation screen (PT/INR, PTT, fibrinogen)
n. DAT (Direct Antiglobulin Screen)
o. Pregnancy test (serum or urine) for female subjects
p. Total protein, creatinine kinase, uric acid, lactate dehydrogenase
q. eGFR
r. Urinalysis
s. Hemoglobin electrophoresis
t. Serum HBsAg, anti-HBc, anti-HBs, anti-HCV
u. anti-HIV
v. Calculated creatinine clearance

Baseline values:

The most recent results of laboratory/procedure testing performed within 90 days of administration of the first dose of study drug will be considered subject's baseline laboratory values.

Table 1A: **Schedule of Events for Subjects Escalating to Maximum AG-348 Dose of 50 mg BID**

Procedure	**VISIT 1**	**VISIT 2**	**VISIT 3**	**VISIT 4**	**VISIT 5**	**VISIT 6**	**VISIT 7**
		Day 1± 3 days (inpatient)	**Day 14± 3 days (inpatient)**	**Day 28± 3 days (inpatient)**	**Day 42± 3 days (inpatient)**	**Day 54± 3 days**	**Follow-up± 3 days**
	Consent	**5mg BID**	**20mg BID**	**50mg BID**	**DoseTaper**	**Stop**	**4 week follow-up**
Informed consent	X						
Inclusion/exclusion criteria	X						
History and Physical with Transfusion history and sleep history	X	X	X	X	X	X	X
Vital sign measurements including weight		X	X	X	X	X	X
Oximetry		X	X	X	X	X	X
Electrocardiogram (EKG)							X
CBC with diff		X	X	X	X	X	X
*Cooximeter panel, venous		*X	*X	*X	*X	*X	*X
Reticulocyte count		X	X	X	X	X	X
Acute Care, Mineral, Hepatic panels,		X	X	X	X	X	X
Fasting lipid panel	X						X
Coagulation screen (PT/INR, PTT, fibrinogen)							X
Urine or serum pregnancy test (if applicable)		X	X	X	X	X	X
Total Protein, Creatinine Kinase, Uric Acid, Lactate		X	X	X	X	X	X
eGFR		X	X	X	X	X	X
Urinalysis							X
Hemoglobin electrophoresis		X	X	X	X	X	X
Testosterone, Total and free	X						X
Estradiol, Serum	X						X
Estrone, serum	X						X
Research labs & pharmacokinetics of AG-348							

Pharmacokinetic blood samples	X	X	X	X	X	X	X
Determination of PK-R protein	X	X	X	X	X	X	X
ATP/2,3-DPG concentration samples	X	X	X	X	X	X	X
Shift in p50 of hemoglobin from venous blood specimen	X	X	X	X	X	X	X
Percentage of sickled RBCs	X	X	X	X	X	X	X
Drug administration		X	X	X	X		
Drug accountability and pill count			X	X	X	X	
Study diary review			X	X	X	X	X
Adverse event monitoring	X	X	X	X	X	X	X

***If clinical hemoglobin value cannot be obtained with a CBC, a hemoglobin value by venous cooximeter panel will be used instead for clinical monitoring.**

Table 1B: **Schedule of Events for Subjects Escalating to Maximum AG-348 Dose of 100 mg BID**

Procedure	**VISIT 1**	**VISIT 2**	**VISIT 3**	**VISIT 4**	**VISIT 5**	**VISIT 6**	**VISIT 7**	**VISIT 8**
		Day 1 ± 3 days (inpatient)	**Day 14 ± 3 days (inpatient)**	**Day 28 ± 3 days (inpatient)**	**Day 42 ± 3 days (inpatient)**	**Day 56 ± 3 days (inpatient)**	**Day 71 ± 3 days**	**Follow-up ± 3 days**
	Consent	**5mg BID**	**20mg BID**	**50mg BID**	**100mg BID**	**DoseTaper**	**Stop**	**4 week follow-up**
Informed consent	X							
Inclusion/exclusion criteria	X							
History and Physical with Transfusion history and sleep history	X	X	X	X	X	X	X	X
Vital sign measurements including weight		X	X	X	X	X	X	X
Oximetry		X	X	X	X	X	X	X
Electrocardiogram (EKG)								X
CBC with diff		X	X	X	X	X	X	X
*Cooximeter panel, venous		*X	*X	*X	*X	*X	*X	*X
Reticulocyte count		X	X	X	X	X	X	X
Acute Care, Mineral, Hepatic panels,		X	X	X	X	X	X	X
Fasting lipid panel	X							X
Coagulation screen (PT/INR, PTT, fibrinogen)								X
Urine or serum pregnancy test (if applicable)		X	X	X	X	X	X	X
Total Protein, Creatinine Kinase, Uric Acid, Lactate		X	X	X	X	X	X	X
eGFR		X	X	X	X	X	X	X
Urinalysis								X
Hemoglobin electrophoresis		X	X	X	X	X	X	X
Testosterone, Total and free	X							X

Estradiol, Serum	X							X
Estrone, serum	X							X
Research labs & pharmacokinetics of AG-348								
Pharmacokinetic blood samples	X	X	X	X	X	X	X	X
Determination of PK-R protein	X	X	X	X	X	X	X	X
ATP/2,3-DPG concentration samples	X	X	X	X	X	X	X	X
Shift in p50 of hemoglobin from venous blood specimen	X	X	X	X	X	X	X	X
Percentage of sickled RBCs	X	X	X	X	X	X	X	X
Drug administration		X	X	X	X	X		
Drug accountability and pill count			X	X	X	X	X	
Study diary review			X	X	X	X	X	X
Adverse event monitoring	X	X	X	X	X	X	X	X

***If clinical hemoglobin value cannot be obtained with a CBC, a hemoglobin value by cooximetry panel will be used instead for clinical monitoring.**

6.2 Monitoring During AG-348 Administration for Safety and Tolerability

Time Points: See Schedule of Events Tables 1A and 1B.

a. History and physical exam, including sleep history.
b. CBC with differential
c. Cooximeter panel (venous) labs if hemoglobin value is not able to be obtained with a CBC
d. Acute care panel (Sodium (NA), Potassium (K), Chloride (CL) Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen, eGFR, Anion Gap), Mineral panel (Albumin, Calcium, Magnesium (Mg), Phosphorus), Hepatic panel (Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin), total protein, creatinine kinase, uric acid, lactate dehydrogenase
e. Calculated creatinine clearance
f. Fasting lipid panel, baseline and last visit only
g. Serum hormone levels (estradiol, estrone, and total and free testosterone), last visit only
h. Hemoglobin electrophoresis
i. Vital signs including weight
j. Reticulocyte Count on each patient visit
k. Urine or serum pregnancy (if applicable)
l. As clinically indicated anytime during the study

6.3 Research Sample Collection

6.3.1 Pharmacokinetics:

- For subjects escalating to maximum AG-348 dose of 50 mg BID, blood samples will be collected at the following time points to measure plasma concentrations of AG-348:
    - Visit 1
    - Day 1, pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 14, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 28, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 42, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 54 , ± 3 days: After last dose of drug
    - 4 weeks, ± 3 days post-last dose of AG-348

- For subjects escalating to maximum AG-348 dose of 100 mg BID, blood samples will be collected at the following time points to measure plasma concentrations of AG-348:
    - Visit 1
    - Day 1, pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 14, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 28, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 42, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 56, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 71, ± 3 days: After last dose of drug
    - 4 weeks, ± 3 days post-last dose of AG-348

6.3.2 Pharmacodynamics:

- For subjects escalating to maximum AG-348 dose of 50 mg BID, blood samples will be collected for pharmacodynamics assessments at the following time points:
    - Visit 1
    - Day 1: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 14, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 28, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 42, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 54, ± 3 days: After last dose of drug
    - 4 weeks ± 3 days post-last dose of AG-348

- For subjects escalating to maximum AG-348 dose of 100 mg BID, blood samples will be collected for pharmacodynamics assessments at the following time points:
    - Visit 1
    - Day 1: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 14, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 28, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 42, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 56, ± 3 days: pre-dose, and 1, 2, 4, and 8 hours post-dose
    - Day 71, ± 3 days: After last dose of drug
        - 4 weeks ± 3 days post-last dose of AG-348

- The following research assessments will be performed on collected samples: after signing consent, visit 1, 2, 3, 4, 5, 6, and 7 (and visit 8 only for subjects escalating to 100 mg BID AG-348 dose)

- Determination of PK-R protein levels and concentrations of 2,3-DPG, and ATP in whole blood
- Shift in p50 of hemoglobin from venous blood specimen (assessment of the 12-hour time point is optional for p50)
- Percentage of sickled RBCs
  - Under normal ex vivo conditions
  - Under hypoxic ex vivo conditions
- Investigation of the effects of AG348 on oxidation and proteomic changes in both RBC and RBC membrane
- Glycated hemoglobin S fraction

6.4 Sample Storage: Research samples will be stored with subject identifiers in the secure laboratory of Dr. Swee Lay Thein using the NHLBI's Biospecimen Inventory (BSI) tracking system in accordance with NHLBI DIR Biospecimen policy. The PI will be responsible for overseeing entry of data into an in-house password protected electronic system. Only those authorized can retrieve samples.

6.5 Intended Use: These specimens will not be read by a pathologist or used for diagnostic purposes. These studies will not be used in assessing the primary endpoint but are undertaken for descriptive or exploratory ancillary research.

6.6.1. Collaborative Efforts of Research

At the FDA laboratories, the team led by Dr. Abdu Alayash will focus on proteomic and metabolic analyses of blood taken from SCD subjects after treatment wth AG- 348. Immunological and other biological assays will determine the effects of AG-348 treatment on oxygen reactive species (ROS), Adenosine Triphosphate (ATP) and 2,3-DPG.

6.6 How Specimens/Data will be Tracked: Samples collected at the NIH will be ordered and tracked through CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. All research samples collected at the NIH under this protocol will be coded with a specific patient number. Research Samples will be logged in on the NHLBI BSI tracking system with the sample code, date drawn, and location of storage.

6.7 End of Study Procedures: The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

6.8 Loss or Destruction of Samples: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

7.0 DRUG ADMINISTRATION – AG-348

On Day 1, two additional conditions must be met before a subject, considered eligible after completing screening, can be dosed:

1. The subject must not have received a transfusion since completing Screening (see Section 5.1, Inclusion Criterion).
2. The subject, if a female of reproductive potential (see Section 5.1.6, Inclusion Criterion) must have a negative urine or serum pregnancy test.

The first dose of study drug on Day 1 should be taken at the study site following all Day 1 assessments.

Over the course of the study, subjects will attend visits on Day 1, Visit 2, Visit 3, Visit 4, Visit 5, Visit 6, and Visit 7 (and Visit 8 only for subjects escalating to 100 mg BID AG-348 dose). See Schedule and Events Tables 1A and 1B for laboratory assessments. Blood samples will be analyzed by Agios-approved laboratories for determination of PK-R protein levels and concentrations of ATP and 2,3-DPG in whole blood.

All subjects will receive an initial dose of 5 mg BID of AG-348 for 2 weeks followed by 2 dose increases, from 5 to 20 mg BID and from 20 to 50 mg BID, depending on safety and tolerability. At least 5 subjects will receive an additional dose increase from 50 mg BID to 100 mg BID; these will consist of subjects who are either undergoing dose escalation at the time of approval of amendment #5 and reconsented to escalate to the 100 mg BID dose or newly enrolled after the approval of amendment #5. Specifically, the treating clinician will assess the safety and tolerability of the current dose level before a decision is taken to escalate the dose to the next level.

7.1 Drug Accountability and Compliance:

Subjects will receive education on how to take the study medication. If they are hospitalized, for example for a pain crisis, they should take the bottle of study drug with them. Subjects will be given a diary, along with their take-home bottle of AG-348, to record when they take their study drug. The diary can also be used to record missed doses and any symptoms the subject experiences till the end of the study. Subjects will be instructed to bring back their study drug and diary at each visit.

When the study staff receives returned study medication, they will record the number of returned pills into the study drug accountability log. The remaining drug will be sent back to the NIH pharmacy.

Each subject will receive a list of NIH study contacts and phone numbers as well as a wallet card to refer to for questions or emergencies while in the study.

8.0 DATA AND BIOSPECIMEN MANAGEMENT PLAN

8.1 Data Collection:

Primary source data will be captured in the electronic medical record and case report forms (CRFs). The PI will be responsible for overseeing entry of this data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator and associate investigators, research nurses and/or a contracted data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes, as well as from progress notes forwarded from referring home physicians. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database. Laboratory values from referring home physicians will be entered into the system.

All human subjects personally identifiable information (PII), eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All protocol data will be stored in the NHLBI secured network drive (P drive) with the following security measures: restricted access, password protection, and daily off-site back-up.

8.2 End of Study Procedures:

All medical information collected from study participants at the NIH will be kept in a locked file at the Clinical Center at the NIH. Unique patient identifiers will be used to label all data. Strict standards of confidentiality will be upheld at all times.

8.3 Data Sharing:

De-identified human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion

of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter.

Research data may be shared with qualified non-collaborator recipients following publication of the primary research results after removal of PII and IRB approval. Future research use of data not defined in the research protocol may occur only after IRB review and approval or an exemption from the NIH OHSRP. Refusal of a research subject participant to permit future use of data--other than required in the protocol or by the FDA--will be honored. Limitations in data sharing and future use of data due to contractual obligations (e.g., CRADAs) or intellectual property proceedings (such as patent filings) will be honored.

8.4 Future use of biospecimens:

Following analyses of biospecimens (blood) for primary research purposes, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB or OHSRP approval, as applicable. Biospecimens may be destroyed only when permitted by the clinical director and the IRB.

Any future research use of biospecimens not defined in the protocol in which NHLBI investigators are engaged in research (e.g., they are undertaking research activities and hold the key that identifies research subjects) requires IRB review and approval. Coded biospecimens (NHLBI investigators hold the key that identifies research subjects) to be shared outside of NIH for future research use requires an executed transfer agreement. Unlinked biospecimens (no key to identify research subjects exists) to be shared outside of NIH for future research use requires an executed transfer agreement. There are a few types of biospecimens that do not require IRB or OHSRP approval for future research use outside of NIH, such as specimens from deceased individuals an executed transfer agreement is required in these special cases. Refusal of a research subject participant to allow for future use of identifiable biospecimens--other than required in the protocol or for appropriate regulatory purposes, e.g., by the FDA--will be honored by destroying the remining specimens collected under this protocol for the specific participant.

8.5 Loss or Destruction of Data:

Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

8.6 Publication Policy:

Given the research mandate of the NIH, subject data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval.

9.0 BIOSTATISTICAL CONSIDERATIONS

9.1 Primary Objective

To assess the clinical safety and tolerability of three escalating doses of AG-348, an allosteric activator of the enzyme pyruvate kinase, in subjects with stable sickle cell disease (SCD).

9.2 Endpoints

9.2.1 Primary Endpoint

- The primary endpoint of this study will be safety and tolerability of AG-348 as assessed by frequency and severity of AEs, and changes in laboratory parameters, including levels in hemoglobin, reticulocyte counts, bilirubin and lactate dehydrogenase.

9.2.2 Secondary Endpoints

- The secondary endpoints will be:
    - Dose to first achieve decrease in concentrations of 2,3-DPG from baseline.
    - Assessment of pyruvate kinase protein, 2,3-DPG, and ATP levels at different doses of AG-348 and change from baseline.
    - Change from baseline in oxygen binding p50 value at different doses of AG-348.
    - Percentage of sickled cells under normal and hypoxic ex vivo conditions at different doses of AG-348.
    - Safety endpoints, including: the type, incidence, severity, and relationship to study treatment of AEs and serious adverse events (SAEs); number of discontinuations due to AEs; results of clinical laboratory tests over time (e.g., serum chemistry, liver function test, hematology, lipids, sex steroids, urinalysis, coagulation); change from baseline in hemoglobin, reticulocyte count, LDH and serum bilirubin at week 7; physical examination findings; vital signs; 12-lead electrocardiogram (ECG) data.
    - Pharmacokinetic endpoints, including plasma concentrations over time and pharmacokinetic parameters of AG-348 (eg, area under the concentration × time curve [AUC], maximum [peak]concentration [Cmax], others as applicable).
    - Exposure-response relationship between safety parameters and AG-348 concentration

and relevant AG-348 pharmacokinetic parameters.

- Exposure-response (or pharmacokinetic-pharmacodynamic) relationship between AG-348 levels and endpoints (e.g. 2,3-DPG, oxygen sickling assay, etc) that are indicators of predisposition to HbS polymerization.

9.2.3 Exploratory Endpoints

- Measuring the glycated hemoglobin S fraction as additional supportive evidence of an increase in RBC lifespan, glycated hemoglobin is known to increase in the setting of stable glycemia.

9.3 Sample Size

This is a study designed to assess the safety and activity of escalating multiple doses of AG-348 in subjects with stable SCD. Approximately 20 - 25 patients will be enrolled to achieve 15 patients who have completed all specified dose levels. Specifically, up to 10 subjects will complete the 3 dose levels, and at least 5 subjects will complete the 4 dose levels (see Section 3.0 Study Design for details). The sample size is primarily driven by feasibility considerations and the ability to detect adverse events (Table 2).

Table 2. Probability of detecting at least one adverse event (AE) as a function of sample size and true underlying AE rate.

	True Underlying AE Rate		
Sample Size	**15%**	**10%**	**5%**
15	91%	79%	54%
20	96%	88%	64%
25	98%	93%	72%

9.4 Methods of statistical analysis:

The planned analyses will include descriptive statistics on the incidence and severity of adverse events. Of particular interest are four qualifying types of adverse events that are counted in evaluating whether a stopping rule has been met (see section 9.6). The proportion of patients with any qualifying adverse event will be summarized using sample proportion and confidence intervals for binomial distributions. In addition to this aggregate measure of any type of qualifying event, the proportions and confidence intervals of the four constituent events will be presented. Similar summaries will be produced for all treatment-emergent adverse events (TEAEs), related TEAEs (those considered by the Investigator as related to study treatment), SAEs, TEAEs leading to treatment discontinuation, TEAEs Grade ≥3 in severity, and adverse events of special interest (AESIs). Individual subject listings will be provided for any deaths, SAEs, TEAEs leading to interruption and/or reduction of study treatment dose, and TEAEs leading to treatment discontinuation. For clinical laboratory values, vital signs, and ECG

assessments, both actual values and changes from baseline will be summarized by visit using summary statistics. The number and percentage of subjects with transaminase increases of >2.5× baseline or increases to Grade ≥2 (AESI of elevated transaminase is defined in the protocol), will be summarized. Changes in hemoglobin and reticulocyte count from baseline will be summarized.

Secondary endpoint analysis of dose-response modeling will employ linear mixed effects models with individual-specific random effects [76]. This is similar to a repeated measures analysis of variance but allows for a potentially more complicated correlation patterns, has more flexibility when data are missing, and allows inclusion of other covariates that may increase power (e.g. age and gender). Correlation of residuals will be explored using an unstructured correlation matrix assuming the follow-up times are relatively similar across individuals. Average outcomes for the fixed dose levels will be represented non-parametrically by coefficients for indicator variables for the dose level. As some of the dose-response outcomes may be influenced by baseline age and gender these covariates will be included in these models. Data may be transformed, or a nonlinear mixed effects model may be considered if the distribution of outcome measures suggest the assumptions of normality or linearity are inappropriate.

9.5 Criteria for Study Termination

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.
Circumstances that may warrant termination include, but are not limited to the following:

- Determination of unexpected, significant, or unacceptable risk to subjects, e.g., as determined by the Data Safety Monitoring Board [DSMB])
- Plans to modify, suspend, or discontinue the development of the study treatment
- Decisions of competent authorities or Institutional Review Board (IRB)/Independent Ethics Committee (IEC)
- Stopping rules are met
- Other administrative reasons

Should the study be closed prematurely, all study materials must be returned to the Sponsor or the Sponsor's designee

9.6 Stopping Rules:

9.6.1 Stopping Rule for Qualifying Adverse Events

A safety committee (as described in section 11.0) will evaluate safety trial data based on the below triggers and if warranted, may recommend modification or even termination of this protocol.

Stopping rules follow a Bayesian design.[77]

The stopping rule is designed to halt enrollment for further evaluation of study data by the safety committee and determination whether enrollment can continue or the study should be modified or terminated. A Bayesian posterior probability will be calculated to determine the likelihood the probability of having a qualifying adverse event is 30% or more. Qualifying AEs/SAEs are any of the following four types of events:

- Any Grade ≥3 treatment related Serious Adverse Events,
- Grade 4 anemia, unexplained by underlying disease,
- Any non-hematological AE Grade ≥ 3 assessed as related to AG-348, or
- Any grade≥2 toxicity that does not resolve to Grade ≤1 in 14 days with appropriate medical management.

When the Bayesian posterior probability reaches 85% or higher, it will trigger a meeting of the Safety Committee. The prior distribution for the probability of a qualifying adverse event is given by a beta distribution with parameters a = 1 and b = 7/3.

Table 3 summarizes the threshold numbers for the resulting boundary, which would lead to a meeting of the safety committee to evaluate stopping or modifying the study due to an excess number of qualifying AEs/SAEs.

Table 3

Number of subjects in the study	Hold enrollment for safety committee evaluation if the number of subjects who have developed a qualifying AE/SAE is
≤5	3
≤7	4
≤10	5
≤13	6
≤15	7

We investigated the performance of the above stopping rule through simulation. In each simulation we generated a set of 15 independent Bernoulli trials; each representing a patient with probability *p* for having a qualifying AE/SAE. For each simulation we determined if the stopping boundary would have been reached with consideration for halting or suspending the study. We conducted this simulation 100,000 times for each different true value of probability *p* and recorded the average number of patients treated (it may be less than 15 if the study was stopped early) and the average number of qualifying AEs/SAEs observed. In addition, we show the proportion of the 100,000 simulations in which the stopping boundary was met. Table 4

summarizes the performance of the stopping rule under a number of different values for the qualifying AE/SAE probability *p*.

Table 4: Performance of the Stopping Rule under a number of scenarios for the qualifying AE/SAE Probability *p*

Probability of a qualifying AE/SAE	0.20	0.25	0.30	0.35	0.40	0.45	0.50
Proportion of Stopped Studies	9%	17%	28%	42%	56%	69%	80%
Average number of subjects	14.2	13.6	12.7	11.7	10.4	9.3	8.1
Average number of qualifying AEs/SAEs	2.9	3.4	3.8	4.1	4.2	4.2	4.1

These simulation results suggest that our stopping rule has a low probability stopping a study when the probability of a qualifying AE/SAE is below 30%, and the probability of stopping a study is high when the true probability of a qualifying event exceeds 35%. Thus, we believe that our Bayesian stopping rule has satisfactory statistical properties.

9.6.2 Stopping Rule for mortality

A separate stopping rule is proposed for deaths that are deemed to be possibly, probably, or definitely related to study medication. Should such an incident occur entry of other patients into the study will be suspended, and patients receiving study medication will not be dose escalated and will be closely monitored for occurrences of the same even whilst assessment of the event occurs.

The IRB/DSMB and the investigators and Agios will evaluate the full circumstances of the event and determine whether to terminate the study. Should it be determined the study be terminated, enrollment in the study will be halted permanently, subjects already on the study will undergo gradual or rapid dose taper to withdraw treatment.

10.0 OFF-STUDY CRITERIA

- Subject choice
- Subject non-compliance
- Lost to follow-up
- Study completion

11.0 DATA SAFETY AND MONITORING PLAN

11.1 Safety Monitoring

***Principal Investigator*:** Accrual and safety data and conduct of the trial will be monitored by the PI and research team on an ongoing basis.

***Safety Committee*:** A safety committee will evaluate safety data based on the triggers outlined in section 9.6. and if warranted, may recommend modification or even termination of this protocol. Minutes of the safety committee's meeting will be placed in the trial master file. The safety committee contains members of the NIH study team and Agios Clinical Study team (quorum to include a minimum of Agios Clinical Development Lead, Agios Safety Lead and NIH PI).

***NIH Intramural IRB*:** Adverse event recording and reporting will be conducted in accordance with the NIH IRB policy and procedures.). Prior to implementation of this study, the protocol and the proposed subject consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to 45 CFR 46 (Protection of Human Subjects). This committee will approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or sample and/or data analysis continues.

***NHLBI Hematology DSMB*:** The NHLBI Hematology Data Safety and Monitoring Board (DSMB) will review the protocol at 6 to 12 month intervals and the interval will be determined by DSMB. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

Monitoring: As per ICH-GCP 5.18 and 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by an independent contract organization working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP) and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the

investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

12.0 ADVERSE EVENT REPORTING

12.1 Adverse Events

Each subject must be carefully monitored for the development of any AEs. This information should be obtained in the form of nonleading questions (e.g., "How are you feeling?") and from signs and symptoms detected during each examination, observations of study personnel, and spontaneous reports from subjects.

All AEs (serious and non-serious) spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded on the appropriate page of the eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings prior to initiating study drug is considered an AE and must be recorded on the appropriate pages of the eCRF. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

12.1.2 Adverse event management:

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 5.0. A copy of the criteria can be down-loaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html.

Adverse event recording will start after consent. However, prior to drug administration, only procedure-specific AEs will be captured in the database.

All AEs, including abnormal findings on laboratory evaluations, regardless of severity, will be recorded and followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion will not be followed after the 30-day period.

It should be noted that hospitalizations occurring for safety monitoring following first dose and dose escalation are not considered new inpatient hospitalizations for safety reporting and will not be reported as SAEs unless an additional Medical condition occurs which meets any serious criteria.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Exclusions to data reporting:
The following Adverse Events will be captured only in the source documents and will not be reported to the IRB.

- Laboratory values that do not meet the definition of AE.
- All grade 1 events listed as expected in the investigator's brochure.

12.1.3. Dose modifications due to Adverse Events

The Investigator will monitor all subjects for safety. It is important that, as much as possible, a subject does not abruptly discontinue study drug, due to the risk of withdrawal hemolysis or VOC. After careful consideration of the relative risk of withdrawal hemolysis or VOC when stopping study drug abruptly versus reducing the dose rapidly, the Investigator should determine if a gradual dose taper (GDT Table 6) for the management of non-acute AEs (e.g., insomnia), rapid dose taper (RDT; Table 7) for the management of acute AEs or potential SAEs (e.g., Grade 4 transaminase increase), or abrupt discontinuation of study drug to receive emergency medical treatment is necessary.

Table 5: Dose Modification for Adverse Events (Except Transaminase Increases) Considered Related to the Study Drug

Related Adverse Event(s) Severity	Dose Modification
Grade 1	• None required.
Grade 2	• None required.

Grade 3	• First Occurrence: Perform a dose taper[1] to stop the study drug until event resolution to Grade ≤1 or baseline, whichever is higher, within 21 days of suspension, and then restart AG-348 at the next lowest dose level. If event not resolved after 21 days of study drug suspension, consider discontinuation of subject from the study. • Second occurrence: Perform a dose taper[1] to discontinue the study drug
Grade 4	• Perform a dose taper[1] to discontinue the study drug

Abbreviations: BID = twice daily.
[1] All subjects who are discontinuing or reducing study drug should undergo a dose taper unless an emergency situation justifies interrupting the study drug abruptly. Dose tapers should be conducted as detailed in Section12.1.4.

12.1.4 Dose Taper Regimens

Subjects undergoing a dose taper should be monitored for signs of hemolysis and worsening of anemia. Investigators should carefully consider the relative risk of withdrawal hemolysis or VOC when rapidly reducing the dose versus gradually reducing the dose.

In subjects undergoing a gradual dose taper, the regimen detailed in Tables 6A and 6B will be followed.

Table 6A: Gradual Dose Taper Regimen for subjects escalating to maximum AG-348 dose of 50 mg BID.

Current Dose of AG-348	**First Step ×5 days**	**Second Step ×5 days**	**Third Step x 5 days**	**Fourth Step ×5 days**
5 mg BID	5 mg QD	n/a	n/a	n/a
20 mg BID	20 mg QD	5 mg BID	5 mg QD	n/a
50 mg BID	50 mg QD	20 mg BID	20 mg QD	5 mg QD

Abbreviations: BID = twice daily; n/a = not applicable; QD = once daily.

Table 6B: Gradual Dose Taper Regimen for subjects escalating to maximum AG-348 dose of 100 mg BID.

Abbreviations: BID = twice daily; n/a = not applicable; QD = once daily; AM = daily in the morning; PM = daily at night.

Current Dose of AG-348	First Step ×5 days	Second Step ×5 days	Third Step x 5 days	Fourth Step ×5 days	Fifth Step ×5 days
5 mg BID	5 mg QD	n/a	n/a	n/a	n/a
20 mg BID	20 mg QD	5 mg BID	5 mg QD	n/a	n/a
50 mg BID	50 mg QD	20 mg BID	20 mg QD	5 mg QD	n/a
100 mg BID	50 mg BID	50 mg QD	20 mg BID	20 mg QD	5 mg QD

In subjects undergoing a Rapid Dose Taper, the regimen in Tables 7A and 7B will be followed.

Table 7A: Rapid Dose Taper Regimen for subjects escalating to maximum AG-348 dose of 50 mg BID.

Current Dose of AG-348	First Step ×3 days	Second Step ×3 days	Third Step ×3 days	Fourth Step x3 days
5 mg BID	5 mg QD	n/a	n/a	n/a
20 mg BID	20 mg QD	5 mg QD	n/a	n/a
50 mg BID	50 mg QD	20 mg BID	20 mg QD	5 mg QD

Abbreviations: BID = twice daily; n/a = not applicable; QD = once daily.

Table 7B: Rapid Dose Taper Regimen for subjects escalating to maximum AG-348 dose of 100 mg BID.

Current Dose of AG-348	First Step ×3 days	Second Step ×3 days	Third Step x3 days	Fourth Step ×3 days	Fifth Step ×3 days
5 mg BID	5 mg QD	n/a	n/a	n/a	n/a
20 mg BID	20 mg QD	5 mg BID	5 mg QD	n/a	n/a
50 mg BID	50 mg QD	20 mg BID	20 mg QD	5 mg QD	n/a
100 mg BID	100 mg AM / 50 mg PM (Day 1) 50 mg BID (Day 2-3)*	50 mg QD	20 mg BID	20 mg QD	5 mg QD

Abbreviations: BID = twice daily; n/a = not applicable; QD = once daily; AM = daily in the morning; PM = daily at night.

* For the first step of the taper, subjects will receive 100 mg of AG-348 for the first AM dose only, then reduce dose to 50 mg BID for the remainder of the 3 days.

12.1.5 Adverse Events of Special Interest

An AESI can be serious or nonserious. Ongoing monitoring and rapid communication (within 24 hours) by the Investigator to Agios is required to allow for further characterization and reporting to regulatory authorities.

Transaminase Increase

Transaminase increase is an AESI for AG-348. In the event of a transaminase increase of >2.5 × baseline or an increase in transaminase to ≥Grade 2 in severity, whichever is lower, the Investigator will report this occurrence to Agios, using the SAE form, within 24 hours of their first knowledge of the event.

An LFT panel should then be monitored weekly until the transaminases have decreased to <2.5 × baseline (defined as the mean of the Screening and Day 1 values). Additionally, the following should be taken to gain further information on the possible cause of the transaminase increase.

1. Rule out biliary causes by Liver Imaging, Liver MRI, Liver ultrasound, or Magnetic resonance cholangiopancreatography as clinically indicated.
2. Viral screen for EBV and CMV using the central laboratory.
3. Autoimmune hepatitis panel consisting of: Serum antinuclear antibody, Anti–smooth muscle antibody, Liver-kidney microsomal type 1, Antibodies to soluble liver antigen, and Anti-mitochondrial antibodies all at first alert level of transaminase increase and repeated 4 weeks later using the central laboratory, if it was negative the first time.

Table 9: Dose Modification for Transaminase Increases

Transaminase Increase Severity	Dose Modification
Grade 1 where result is not >2.5 x baseline	• None required. Follow closely.

Transaminase Increase Severity	Dose Modification
Grade 2 or Grade 1 with >2.5 x baseline	• First occurrence: none required but consider performing a dose taper[1] to stop the study drug, if deemed necessary by the Investigator and Agios. If dose is held, hold until event resolution to Grade ≤1 or baseline, whichever is higher, within 21 days of suspension, and then restart AG-348 at the same dose. • Second occurrence: perform a dose taper[1] to stop the study drug until event resolution to Grade ≤1 or baseline, whichever is higher, within 21 days of suspension, and then restart AG-348 at the next lowest dose level (ie, 50 mg BID to 20 mg BID). • Third occurrence perform a dose taper[1] to stop the study drug permanently.
Grade 3	• First occurrence: perform a dose taper[1] to stop the study drug until event resolution to Grade ≤1 or baseline, whichever is higher, within 21 days of suspension, and then restart (with confirmation from Agios) AG-348 at the next lowest dose level (ie, 50 mg BID to 20 mg BID). • Second occurrence: perform a dose taper[1] to stop the study drug permanently.
Grade 4	• Perform a dose taper[1] to stop the study drug permanently.

Abbreviations: BID = twice daily.

[1] All subjects who are discontinuing or reducing study drug should undergo a dose taper unless an emergency situation justifies interrupting the study drug abruptly. Dose tapers should be conducted as detailed in Section 12.1.4

12.2 Grading of Adverse Events

Table 10

Grade	Category	Description
1	Mild	Mild; asymptomatic; clinical or diagnostic observations only; intervention not indicated
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age- appropriate instrumental ADL

3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting selfcare ADL
4	Life threatening	Life- threatening consequences; urgent intervention indicated
5	Death	Death related to AE

12.3 Attribution of Adverse Events

Table 11

Relationship	**Attribution**	**Description**
Unrelated to investigational agent/intervention[1]	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE *is **doubtfully related*** to the intervention
Related to investigational agent/intervention[1]	Possibly	The AE ***may be related*** to the intervention
	Probably	The AE ***is likely related*** to the intervention
	Definitely	The AE ***is clearly related*** to the intervention

[1]**NOTE**: AEs listed as 'possibly, probably, or definitely' related to the investigational agent/intervention are considered to have a suspected 'reasonable causal relationship' to the investigational agent/intervention (ICH E2A).
Attribution of causality must be signed by the principal investigator

13.0 NIH INTRAMURAL IRB AND NHLBI CD REPORTING

13.1 Expedited Reporting

Events requiring expedited reporting will be submitted to the IRB per Policy 801 "Reporting Research Events".

13.2 Reports to the IRB at the time of Continuing Review:

The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements and timelines.

13.3 Reports to the CD:

The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

14.0 IND SPONSOR REPORTING CRITERIA

The PI or designee will refer to NHLBI DIR guidelines to determine CD (study sponsor) reporting requirements and timelines.

Expedited IND reporting to FDA will be performed in accordance with Guidance for Industry and Investigators Safety Reporting Requirements for INDs and BA/BE Studies December 2012.

14.1 Reporting Pregnancy

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor.

Pregnancy itself is not regarded as an SAE. However, if a patient becomes pregnant while on study, risk intrauterine exposure of the fetus to the agents which may be teratogenic. Pregnancy should be reported in an expedited manner to the Sponsor and manufacturer as Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but no later than 24 hours of when he or she becomes aware of it.

The same timelines apply when outcome information is available.

All subjects, male and female, must agree to use effective contraception during the entire study and for 28 days (female subjects) and 90 days (male subjects) following the last dose of AG-348. Abstinence is an acceptable method only

when this is in line with the normal life style of the subject, meaning that the subject plans to remain abstinent continuously throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence (e.g., calendar, symptothermal, and post-ovulation methods) and withdrawal are not acceptable methods of contraception.

14.2 Expedited Adverse Event Reporting Criteria to the IND Drug Manufacturer

Copies of all Safety Reports will be provided to Agios concurrently with their submission to the FDA, and with any other information affecting the safety of Human Subjects in research conducted under the executed CRADA with Agios Pharmaceuticals Inc.

15.0 HUMAN SUBJECT PROTECTION

15.1 Rationale for Subject Selection

Study Population: All patients 18 years old and over will be considered for this protocol. No patient will be excluded from participation based on gender, race, or ethnicity. Patients may self-refer, be recruited through the NIH office of recruitment and may include subjects participating on NIH Clinical Center Protocols.

15.2 Informed Consent Processes and Procedures

Informed consent shall be documented using the current IRB-approved consent form, which should be downloaded from the NIH Clinical Center active consent website. Each participant will receive an oral and written explanation of the goals, procedures, and risks of this study. When consent is obtained, the consent document(s) must be signed and dated by the subject, and the person obtaining consent. The original, signed informed consent document will be placed in the

medical record, and the subject will receive a signed copy of the informed consent document. Documentation of informed consent and the signed consent form will be maintained in CRIS.
The objectives of this research, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study.
At any time during participation in the protocol, should new information become available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective patient participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

15.3 Patient Advocate

A patient's rights representative is available to patients on this protocol. The representative is located in Building 10 and can be reached by phone at 301-496-2626. Patients may ask any questions about the study and may withdraw their consent at any time.

15.4 Privacy and Confidentiality

All efforts, within reason, will be made to keep subjects' private identifiable information (PII) private. Using or sharing ("disclosure") such data must follow federal privacy rules. Under certain circumstances, the United States Office of Human Research Protections (OHRP), The US Food and Drug Administration (FDA), and the NIH Institutional Review Board (IRB), will be able to inspect and copy confidential study-related records which identify participants by name. Therefore, absolute confidentiality cannot be guaranteed.

15.5 Risks and Discomforts

15.5.1 Summary of safety findings from clinical trials of AG-348

Overall, AG-348 has been generally well tolerated among healthy adult subjects and adult subjects with PK deficiency. Safety data are available as of 27 March 2017 from 36 healthy subjects treated with AG-348 at single doses ranging from 30 to 2,500 mg (Study AG348-C-001) [78], 36 healthy adult subjects treated with multiple doses ranging from 15 to 700 mg BID or 120 mg QD for 14 days (Study AG348-C-002), and 52 subjects with PK deficiency randomized to initial treatment with 50 or 300 mg BID (Study AG348-C-003). Many of the adult subjects with PK deficiency have actually received doses lower than 50 or 300 mg, as per protocol-allowed dose reductions.
After a single AG-348 dose in healthy adult subjects, AEs reported by >1 subject at any time on study (either under fasted or fed conditions) included headache (22%), nausea (14%), and contact dermatitis and vomiting (each 6%). After repeated dosing of AG-348 for 14 days in healthy subjects, AEs that occurred in >5% of all AG-348-treated subjects across all cohorts (i.e., >1 subject) included headache and nausea (13.9% each); vomiting, decreased appetite, feeling

hot, and restlessness (8.3% each); and dizziness, fatigue, vessel puncture site bruise, hyperhidrosis, dermatitis allergic, and drug eruption (5.6% each).
A dose-relationship was apparent with regard to the incidence of gastrointestinal events, primarily nausea and vomiting, with the incidence of such events increasing with increasing dose.
All but 1 AE reported in healthy adult subjects was Grade 1 or 2 in severity. The only Grade 3 AE, occurring in 1 subject treated with AG-348 700 mg in the MAD study, was elevated liver function tests (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), which was considered to be study drug-related and led to study drug discontinuation. Overall, 4 subjects discontinued Study AG348-C-002 prematurely; 2 subjects discontinued due to withdrawal by subject (each experienced vomiting) and 2 subjects were discontinued due to AEs (vomiting, liver function test abnormal, and decreased appetite in 1 subject and drug eruption in the other subject).
No deaths or other serious adverse events (SAEs) were reported in healthy adult subjects. Only 1 DLT was documented in Study AG348-C-002 for the event of Grade 3 elevated liver function tests described above.
In Study AG348-C-003, of the 52 adult subjects with PK deficiency who received AG-348, 52 subjects (100%) experienced at least 1 TEAE. The AEs that occurred in >10% of AG-348-treated subjects across both cohorts included headache (24 [46.2%] subjects); insomnia (22 [42.3%] subjects); nausea (21 [40.4%] subjects); viral upper respiratory tract infection (16 [30.8%] subjects); influenza, arthralgia, fatigue, hot flush, vomiting, diarrhoea (89 [17.3%] subjects each); cough, oropharyngeal pain, dizziness, pyrexia (8 [15.4%] subjects each); gastroenteritis, back pain, dysmenorrhoea (7 [13.5%] subjects each) and dyspepsia (6 [11.5%] subjects each).

There were no deaths in Study AG348-C-003, and 20 SAEs were reported in 16 subjects, including pharyngitis and hemolytic anemia (2 subjects); and cellulitis, gastroenteritis, influenza, hemolysis, colitis, enteritis, inguinal hernia, mesenteric vein thrombosis, cholelithiasis, post procedural hemorrhage, increased alanine aminotransferase, hypertriglyceridemia, osteoporosis, renal cyst, ovarian cyst and unintended pregnancy (1 subject each). The majority of these SAEs were assessed as unrelated to AG-348 treatment.
Important identified risks associated with administration of AG-348 in clinical studies include bone mineral density decrease (including osteoporosis and osteopenia due to aromatase inhibition), withdrawal hemolysis, and insomnia (not clinically serious, i.e., not Grade 3 or Grade 4). Potential risks associated with AG-348 administration include anaphylactoid reaction, aromatase inhibition, gastrointestinal disturbances, photosensitivity, transaminase increases, and triglyceride increase. Transaminase elevations are adverse events of special interest (AESIs) for AG-348.

<u>The following risks are assessed by Agios as being related to AG-348</u>

Risk of Withdrawal Hemolysis

In Study AG348-C-003 conducted in adult subjects with PK deficiency, 2 of 52 subjects (3.8%) experienced hemolysis upon sudden withdrawal of the drug, including 1 SAE of withdrawal hemolysis. In both subjects, a rapid Hb increase during AG-348 treatment was followed by a sudden discontinuation of AG-348 without taper, resulting in withdrawal hemolysis and anaemia. By contrast, subjects who missed only a few doses of AG-348, or for whom the dose was reduced, did not experience TEAEs indicative of hemolysis, and their Hb levels were either not recorded immediately after the short interruption or decreased gradually after the dose reduction. Refer to the study protocol for specific procedures for dose modifications to prevent withdrawal hemolysis in subjects with PK deficiency. When subjects experience an AE, the Investigator should consider the relative risk of a potential withdrawal hemolysis versus maintaining the subject on study treatment. The Investigator should consider the severity of the AE and the need to remove the subject from the drug.

Risk of Insomnia

In Study AG348-C-003 conducted in adult subjects with PK deficiency, insomnia was reported in 22 of 52 subjects (42.3%). In the AG-348 50 mg BID initial treatment group, insomnia was reported in 6 of 27 subjects (22.2%) (5 subjects with TEAEs of insomnia and 1 subject with a TEAE of initial insomnia), and 2 of the TEAEs of insomnia required dose modification. In the AG-348 300 mg BID initial treatment group, insomnia was reported in 16 of 25 subjects (64%). Dose reduction was required in 5 subjects in the 300 mg BID initial treatment group due to TEAEs of insomnia. One of these subjects and 1 additional subject had a temporary dose interruption of AG-348 because of insomnia. None of the insomnia TEAEs were SAEs. Two TEAEs of insomnia (1 TEAE each in the initial AG-348 50 mg and 300 mg BID initial treatment groups) were Grade 3 in severity.

Risk of Bone Mineral Density Decrease

AG-348 inhibits human aromatase activity based on studies in human placental microsomes and rat ovarian microsomes. In Study AG348-C-003, 1 subject from 52 treated subjects in Study AG348-C-003 experienced a medically important event of osteoporosis (Grade 2), assessed as likely due to aromatase
inhibition. Subjects in clinical studies are monitored for potential aromatase inhibition by serial assessments of serum hormone levels and bone density (DXA) scans. As this has occurred in a single subject, this event is not considered expected for reporting purposes.

15.5.2 Related to blood draws & IV

No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws, vasovagal reactions or infections may rarely occur.

15.5.3 Related to cardiac monitoring (during screening)

Echocardiogram (ECHO): An echocardiogram uses sound waves to visualize and evaluate the function of the heart. There are no risks associated with obtaining an echocardiogram.

Electrocardiogram (ECG): An electrocardiogram is a test that measures the electrical activity of the heartbeat. A technician will place electrodes on the chest, arms, and legs. The electrodes are soft and do not cause discomfort when placed or removed by the technician. There are no risks associated with obtaining an electrocardiogram.

15.6 Conflict of Interest

A Guide to Avoiding Financial and Non-Financial Conflicts of Interest or Perceived Conflicts of Interest in Clinical Research at NIH (September 2015) has been distributed to all NIH investigators who have reviewed the guide and indicated no conflicts of interest exist. No investigators hold patents or patents pending, or have any financial conflict of interest associated with this study.
This protocol has no associated patents or CTAs.

A CRADA between Agios and the NHLBI is in the process of being established.

16.0 COMPENSATION & REIMBURSEMENT

Reimbursement for local travel, US travel, food, and lodging will be in accordance with NHLBI travel policy reimbursement will be consistent with NIH and NHLBI guidelines.

Financial Compensation

Subjects will receive monetary compensation for their time and inconvenience in accordance with present NIH guidelines.

Table 12A: Compensation for subjects escalating to maximum dose of 50 mg BID.

Procedure(s)/Test(s)	IU*	Amount	Frequency	Total amount
EKG	**1**	**$10**	**2**	**$20**
Oxygen monitoring during inpatient stay	**1**	**$10**	**4**	**$40**
Medical History and Physical Exam	**2.5**	**$25**	**8**	**$200**
Urinalysis	**1**	**$10**	**2**	**$20**
Blood Draw	**1**	**$10**	**8**	**$80**
Blood Draw through an IV	**1**	**$10**	**20**	**$200**
IV placement	**1**	**$10**	**4**	**$40**
Diary	**1**	**$10**	**6**	**$60**
Drug Administration, General	**2**	**$20**	**4**	**$80**
Inpatient visit $40 per night	**N/A**	**$40**	**4**	**$160**
OUTPATIENT- 1st HOUR	**NA**	**$20**	**4**	**$80**
OUTPATIENT TIME- Not to Exceed More than 4 Hours	**3**	**$30**	**3**	**$90**
TOTAL				**$1,070**

Table 12B: Compensation for subjects escalating to maximum dose of 100 mg BID.

Procedure(s)/Test(s)	IU*	Amount	Frequency	Total amount
EKG	**1**	**$10**	**2**	**$20**
Oxygen monitoring during inpatient stay	**1**	**$10**	**5**	**$50**
Medical History and Physical Exam	**2.5**	**$25**	**9**	**$225**
Urinalysis	**1**	**$10**	**2**	**$20**
Blood Draw	**1**	**$10**	**9**	**$90**
Blood Draw through an IV	**1**	**$10**	**25**	**$250**
IV placement	**1**	**$10**	**5**	**$50**
Diary	**1**	**$10**	**7**	**$70**
Drug Administration, General	**2**	**$20**	**5**	**$100**
Inpatient visit $40 per night	**N/A**	**$40**	**5**	**$200**
OUTPATIENT- 1st HOUR	**NA**	**$20**	**4**	**$80**
OUTPATIENT TIME- Not to Exceed More than 4 Hours	**3**	**$30**	**3**	**$90**
TOTAL				**$1245**

17.0 PHARMACEUTICALS – AG-348

Supply: AG-348 will be supplied by Agios Pharmaceuticals, Inc. 88 Sidney Street Cambridge, MA 02139-4169

Pharmacology: Investigational drug AG-348 (formerly known as AGI-1480 and AGX-0841) is a

potent, broad-spectrum activator of alleles of the red blood cell (RBC)-specific form of pyruvate kinase (PKR). PKR is 1 of 4 pyruvate kinase isoenzymes expressed in human tissues from 2 distinct genes. PKR and liver-type pyruvate kinase (PKL) are generated from the PKLR gene by 2 separate tissue-specific promoters, while PKM1 and PKM2 are from the PKM gene via differential splicing of the ribonucleic acid. AG-348 is an allosteric activator of PKR, PKL, and PKM2, with similar potency against each.
In clinical studies, AG-348 has been administered orally at the dose and regimen prescribed in the study protocols.

Product description: AG-348 is currently supplied for oral administration as tablets to all subjects. Tablets are supplied in dose strengths of 5, 20, or 50 mg of mitapivat. The tablets contain the following inactive ingredients: microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, and mannitol. Tablets are coated with a nonfunctional film coat composed of generally recognized as safe (GRAS) excipients.

Storage and Stability: The recommended storage condition is stated on the product label to ensure the stability and proper product identification of the mitapivat tablets, the drug should be dispensed from the packaging in which it is supplied.

Route of Administration: Oral tablets.

Administration: AG-348 should be taken orally and swallowed whole with water. The tablets are not to be crushed, chewed, or dissolved in water. Doses of study drug may be taken with or without food.

If a subject misses a scheduled dose by 4 hours or less, they should still take that dose. If a subject misses a scheduled dose by more than 4 hours, they should skip that dose. If a dose is skipped, the next dose should then be taken approximately 24 hours from the previous dose taken. If a subject experiences a dose interruption for non-safety reasons, they should be restarted on the prescribed dose as soon as possible.

Toxicities: The most common adverse events experienced by subjects receiving study drug are:

- Headache (44.3% of patients)
- Nausea (34.2% of patients)
- Insomnia (34.2% of patients)
- Nasopharyngitis (The common cold) (27.8% of patients)
- Fatigue (24.1% of patients)
- Vomiting (17.7% of patients)
- Alanine aminotransferase increased (16.5% of patients)
- Oropharyngeal pain (15.2% of patients)
- Back pain (15.2% of patients)
- Diarrhea (13.9% of patients)
- Influenza (13.9% of patients)
- Cough (12.7% of patients)

- Hot flush (12.7% of patients)
- Dizziness (12.7% of patients)
- Upper respiratory tract infection (12.7% of patients)
- Pyrexia (11.4% of patients)
- Arthralgia (11.4% of patients)
- Hypertriglyceridemia (11.4% of patients)
- Dyspepsia (11.4% of patients)
- Asthenia (10.1% of patients)
- Gastroenteritis (10.1% of patients)
- Dysmenorrhea (10.1% of patients)

Potential Drug Interactions

In vitro studies using human liver microsomes and recombinant CYP enzymes have shown that AG-348 is primarily metabolized by CYP3A4 (>70%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. In addition, AG-348 has been shown to be a weak time-dependent CYP3A4 inhibitor and a potential inducer of CYP3A4 and CYP2B6 in vitro. In vitro transporter studies have shown that AG-348 is a substrate and inhibitor of P-gp. Based on these results, below is a list of concomitant therapy to be avoided and concomitant therapy requiring careful monitoring (see also Appendix 1- Investigator's Brochure detached from this protocol) for lists of medications to be avoided or carefully monitored).

Since AG-348 exhibits pH-dependent solubility, proton-pump inhibitors and H2-receptor antagonists may decrease the absorption of AG-348.

The following therapies are contraindicated during the study:

- Strong inhibitors of CYP3A4
- Products known to inhibit CYP3A4 such as grapefruit or grapefruit juice
- Strong inducers of CYP3A4

The following therapies should be avoided and replaced with alternative treatments. If this is not possible, subjects receiving these drugs should be adequately monitored.

- Moderate inhibitors of CYP3A4
- Moderate inducers of CYP3A4
- Corticosteroids (sensitive substrates of CYP3A4 and weak CYP3A4 inducers)
- Sensitive substrates of CYP3A4.
- Proton-pump inhibitors and H2-receptor antagonists

AG-348, as a potential CYP3A4 inducer, has the potential to reduce the effectiveness of hormonal contraceptives. Therefore, female subjects using hormonal contraceptives must also utilize a barrier method while enrolled in the study and until at least 30 days after their last dose of study drug.

Oral penicillin are not expected to interact with AG-348.

18.0 Reference

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