

CLINICAL STUDY PROTOCOL

A Phase 1/2 Open-Label, Dose Escalating Study Evaluating the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of AB1 in Adult Patients with Sickle Cell Disease (SCD)

NCT05261711

Short Title:	Evaluation of safety, tolerability, PK and PD of AB1 in adult SCD patients
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2 PROTOCOL SYNOPSIS

Study Title	A Phase 1/2, Open-Label, Dose Escalating Study Evaluating the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of AB1 in Adult Patients with Sickle Cell Disease (SCD)
Short Title	Evaluation of safety, tolerability, PK, and PD of AB1 in adult SCD patients
Protocol #	2022.1.1
Study Phase	Phase 1/2
Study Sites	2
# of patients	Approximately 6 to 33 patients will be enrolled
Indication	Sickle Cell Disease (SCD)
Study Overview	Survival for Sickle Cell Disease (SCD) patients is 25–30 years less than the African-American population in general¹. The ribonucleotide reductase inhibitor drug hydroxyurea (HU) is the only drug currently approved to prevent disease organ dysfunction and prescribed prophylactically for disease modification². The cytostatic/cytotoxic mechanism of action of HU is used to indirectly reactivate fetal hemoglobin (HbF) expression. HbF interferes with sickle hemoglobin (HbS) polymerization and therefore prevents sickling^{3,4}. SCD patients with hereditary persistence of HbF, who have greater than 20% HbF as a percentage of total hemoglobin (Hb), have essentially normal lifespans⁵ (and refs therein). Induction of HbF with HU, however, is inevitably not enough to avoid complications. Patients who are compliant to HU may not have an increase in HbF levels⁶⁻⁸ and HU-responders often have minimal increases in HbF levels. The difficulty in consistently increasing HbF is significant, since there is an inverse relationship between HbF and pain crisis⁹, early death¹, and possibly stroke and pulmonary hypertension⁸. Importantly, HU is used at doses that are genotoxic, teratogenic, and testicular toxic and can compound the bone marrow damage that contributes to early death in SCD^{6,10-13}. The potential toxic damage is particularly important due to evidence of increased hematologic malignancies among patients with SCD^{14,15}. Methylation of cytosines that precede guanines mediates gene-silencing at many genes including the γ globin gene (<i>HBG</i>) that produces HbF^{16,17}. The role of DNA methylation and epigenetics in <i>HBG</i> silencing explains why decitabine, which irreversibly binds and depletes DNA methyl-transferase 1 (DNMT1) and thereby hypo-methylates the <i>HBG</i> promoter, can increase HbF by large amounts, even in severely ill and HU refractory patients^{18,19}. DNMT1 is well known to maintain methylation marks on DNA through cell division. However, high concentrations of decitabine do produce off-target anti-metabolite effects and cytotoxicity. AB1 is incorporated into DNA, where it engages the active site of DNMT1. Furthermore, DNMT1 is a co-repressor that is recruited by sequence-specific DNA-binding factors, e.g., DRED (TR2/TR4) and BCL11A, which

	direct epigenetic silencing of <i>HBG</i>^{16,18,20}. AB1 offers an improvement over traditional DNA methyltransferase inhibitors by virtue of its higher incorporation rate into DNA, at less cytotoxic levels. These properties of AB1 could aid the goal of more accessible, safer, and more efficacious DNMT1-targeted therapy. Hence, this phase 1/2 clinical trial examines the safety of AB1 and its ability to induce HbF and identifies the dose of AB1 to be used in subsequent phase 2 studies.
Study Design	This will be an open-label, dose escalating study with a starting dose of 2mg. Up to 4 additional cohorts will be enrolled at subsequently higher doses of 4mg, 8mg, 16mg, and 32mg. In each dose escalation cohort, each dose will be taken orally, once daily, for 8 weeks. • Cohort 1 – Two (2) patients will be enrolled at the 2mg dose level for 8 weeks of treatment. After both patients have received at least 4 weeks of treatment, if there are no adverse events ≥Grade 2 that are related (possibly, probably, or definitely) to the study drug, then the study will proceed to the next dose level. Note that if ≥Grade 2 toxicity is seen at this dose level, the study drug regimen may be modified (eg, twice or thrice weekly versus daily dosing). • Cohort 2 – Two (2) patients will be enrolled at the 4mg dose level for 8 weeks of treatment. After both patients have received at least 4 weeks of treatment, if there are no adverse events ≥Grade 2 that are related to the study drug, then the study will proceed to the next dose level. • Cohort 3 – Three (3) patients will be enrolled at the 8mg dose level for 8 weeks of treatment. After all patients have received at least 4 weeks of treatment, if <2 patients experience a related adverse event ≥Grade 2, then the study will proceed to the next dose level. (If ≥2 patients experience a related adverse event ≥Grade 2, the previous cohort will be expanded.) • Cohort 4 – Three (3) patients will be enrolled at the 16mg dose level for 8 weeks of treatment. After all patients have received at least 4 weeks of treatment, if <2 patients experience a related adverse event ≥Grade 2, then the study will proceed to the next and final dose level. (If ≥2 patients experience a related adverse event ≥Grade 2, the previous cohort will be expanded.) • Cohort 5 – Three (3) patients will be enrolled at the 32mg dose level for 8 weeks of treatment. • Additional cohorts may be explored at 12mg and 24mg dose levels if deemed appropriate based on safety and activity parameters. If there are any adverse events Grade ≥2 that are related (possibly, probably, or definitely) to study drug, at the 4mg cohort or in ≥2 patients in any subsequent cohort, the dose may be reduced to the previous

	cohort and an additional 3-6 patients (total of up to 9) may be enrolled into that cohort. Additionally, if Hbf levels increase >15% (expressed as a percentage of total Hb) in any cohort, the cohort can be expanded to an additional 3 to 6 patients as well as continue to the next cohort if safety parameters have been met. In the expansion cohort, patients will receive study drug treatment for an additional 4 weeks (total of 12 weeks). Approximately 6 to 33 patients will be enrolled for the entire study. Patients are eligible to enroll in a higher cohort of the study after a minimum of one month washout from AB1 dosing, the fetal hemoglobin levels return to baseline (<8.6%) and the investigator deems the patient eligible.
Study Objectives	The primary objective is to determine the safety, tolerability, and active dose range of AB1 in patients with SCD. The secondary objectives are: • To determine the clinical activity of AB1 in patients with SCD • To explore the pharmacodynamics (PD) of AB1 in patients with SCD • To characterize the pharmacokinetics (PK) of AB1 in patients with SCD The exploratory objectives are: • To evaluate time to response and duration of response • To explore exposure-response relationship between AB1 exposure, PD, and clinical outcomes • To elucidate mechanism of action, better understand PD responses, and identify potential indicators of sensitivity or response to AB1
Study Endpoints	The primary endpoint is the incidence and frequency of $\geq$Grade 2 study related adverse events over 8 weeks of multiple rising dose cohorts, with an additional primary goal to determine the dose range of biological activity. Secondary endpoints include: • To the extent the systemic drug concentration data allow, the following PK parameters will be determined or calculated from the drug concentration time data as follows: C_{max}, t_{max}, $t_{1/2}$, AUC_{0-t}, Dose Normalized AUC_{0-inf}, CL, V_z, and V_{ss} • Change in fetal hemoglobin (HbF) levels measured by HPLC (PD of AB1) and expressed as percentage of total hemoglobin (Hb) • Change in percent F-cells measured by flow cytometry • Change in percent reticulocytes Exploratory endpoints

	• Time to HbF levels >15%, as a percentage of total Hb, and the period of time for which HbF is >15% over 8 weeks • DNA methylation (PD of AB1) in buffy-coat white blood cells • DNMT1 levels (PD of AB1) in peripheral blood mononuclear cells
Study Population	The treatment population are adult SCD patients who remain at risk of early death, as defined by published criteria²¹, and are undergoing current therapy with HU and have been on a stable dose for at least 3 months. Patients who have contraindications or are non-responsive to HU are also eligible. Since patients are continuing to have complications despite past or current therapies, this study will not interrupt standard of care.
Inclusion Criteria	1. Written, informed consent 2. Age 18 to 45 years of age, inclusive at screening 3. Confirmed SS or S-β^0-thalassemia SCD 4. Sickie crisis rate of 2-10 within the past year with no crisis in the last 28 days 5. HbF <8.6% of total Hb at screening 6. Regular compliance with comprehensive care and previous therapy
Exclusion Criteria	1. Experienced severe sepsis or septic shock within the previous 12 weeks 2. Febrile illness in the 1 week prior to baseline visit 3. Acute complications due to SCD (<i>i.e.</i>, hospitalization, acute pain, or acute chest syndrome) in the 28 days prior to screening visit 4. Plans for hospitalization, surgery, or other major procedures during the duration of the study or between screening and baseline 5. ALT $\geq 2X$ the upper limit of normal or albumin <2.0 mg/dL or direct (conjugated) bilirubin ≥ 1.5 mg/dl* 6. Estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m² at Screening. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (2021) should be used to calculate eGFR#. 7. Platelet count >800 x 10⁹/L OR <150 x 10⁹/L * 8. Absolute neutrophil count <1.5 x 10⁹/L * 9. Currently pregnant or breastfeeding

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| | <ol style="list-style-type: none"> 10. Female of active childbearing potential[§] who is unwilling or unable to adhere to the contraception requirements specified in the protocol 11. Male with female partner(s) of childbearing potential[§] who is unwilling or unable to adhere to the contraception requirements specified in the protocol 12. Altered mental status or recurrent seizures requiring anti-seizure medications 13. Moribund or any concurrent disease (e.g., hepatic, renal, cardiac, metabolic) of such severity that death within 24 weeks is likely 14. Concurrent diagnosis of malignancy including MDS, leukemia, or an abnormal karyotype 15. Known Vitamin-B12, folate, or iron deficiency 16. New York Heart Association (NYHA) class III/IV status 17. Participant is on chronic transfusion therapy (e.g., for history of TIA or stroke) and medically contraindicated to discontinue transfusions (unless multiple allo-antibodies prevent the patient from getting transfusions as scheduled) 18. Blood transfusion in the 28 days prior to screening visit or between screening and baseline visits 19. Known history of illicit drug or alcohol abuse within the past 12 months. 20. Current treatment with Oxbryta or Adakveo (must be off therapy for 30 days for Oxbryta with no plans to restart and off therapy for 3 months for Adakveo with no plans to restart) 21. If receiving HU, patient started HU within the previous 3 months; had a change in dose in the previous 3 months, or has plans to increase the dose during the study period, and if not receiving HU, patient has plans to start HU 22. Other experimental or investigational drug therapy in the past 28 days |
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*Based on screening laboratory values.

eGFR = $141 \times \min(\text{Scr}/\kappa, 1)^\alpha \times \max(\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018$ [if female] $\times 1.159$ [if African American]

where:

Scr is serum creatinine in mg/dL,
 κ is 0.7 for females and 0.9 for males,
 α is -0.329 for females and -0.411 for males,

	min indicates the minimum of Scr/k or 1, and max indicates the maximum of Scr/k or 1 \$ See Section 9 for definition of female of active child- bearing potential
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3 Background & Rationale

Hemoglobinopathies is the medical term for a group of genetic disorders that affect red blood cell (RBC) function. These disorders include sickle cell disease (SCD) and beta-thalassemia (β -thal). Both are inherited in an autosomal recessive manner. There are 3 major forms of hemoglobin (Hb), adult (HbA), fetal (HbF), and embryonic (HbE). All have two alpha chains but vary in the other globin chain. HbA has two beta chains while HbF has two gamma chains. Early after birth there is a switch from HbF to HbA. Two alpha chains plus two beta chains constitute HbA which, in normal adults, comprises about 97% of the total Hb; alpha chains combine with delta chains to constitute HbA-2 which, along with HbF, makes up the remaining 3% of Hb in adults.

SCD is caused by a mutation that replaces glutamine with valine at the sixth amino acid position in the β -globin subunit gene, resulting in formation of sickle hemoglobin (HbS). People with this form of SCD inherit two sickle cell genes ("S"), one from each parent. This is called sickle cell anemia and is usually the most severe form of the disease. HbS has two wildtype alpha chains and two mutated beta chains. About two-thirds of SCD patients are homozygous HbSS individuals but patients heterozygous for HbS and HbC (HbSC) constitute about a third of SCD cases (called HbSC disease). Generally, HbSC is less severe than HbSS. Patients with a combination of the sickle cell and beta-thalassemia mutations have a compound heterozygous condition (HbS/ β -thal, including S- β^0 -thalassemia SCD) that can range in severity from nearly asymptomatic to a severe SCD phenotype²². The sickle mutation in the beta chains predispose Hb to polymerize intracellularly under the low oxygen conditions of the microcirculation. The HbS polymer has the form of an elongated rope-like fiber, which usually aligns with other fibers, forming a fascicle that distorts red blood cells (RBCs) into crescent or sickle shape, causing a marked decrease in cell deformability. This polymerization of HbS inside RBCs, resulting in sickling, is the root cause of the disease and leads to irreversible cellular damage with hemolysis and vaso-occlusion, the two primary contributors to the pathophysiology of SCD.

During fetal life and in the newborn period, very little if any Hb beta chains are produced in humans. Instead of beta chains, gamma chains are produced. Gamma chains are specific components of HbF, which is formed by two alpha and two gamma chains. HbF ($\alpha_2\gamma_2$) accounts for up to 90% of the circulating Hb at birth. Production of HbF is mostly silenced after birth and it is replaced by HbA ($\alpha_2\beta_2$), with only a small amount of HbF persisting thereafter under normal conditions. In contrast, individuals with SCD and β -thal can have increased, although variable, amounts of HbF as a compensatory response. A relatively high capacity for HbF synthesis is a major beneficial genetic modifier of SCD severity. Different haplotypes of SCD have been defined based on their baseline levels of HbF: People with HbS who exhibit persistent pancellular persistence of high levels of HbF

(e.g. 20%–30%) are asymptomatic⁵. The highest adult levels of HbF are seen in β -thal and in hereditary persistence of fetal hemoglobin (HPFH), in which HbF can constitute up to 100% of Hb.

Thus, increasing HbF expression is a therapeutic target for SCD. The ribonucleotide reductase inhibitor drug hydroxyurea (HU) is the only drug currently approved to prevent disease organ dysfunction and prescribed prophylactically for disease modification². The cytostatic/cytotoxic mechanism of action of HU is used to indirectly reactivate HbF expression. HbF interferes with HbS polymerization and therefore prevents sickling^{3,4}. Induction of HbF with HU, however, is inevitably not enough to avoid complications. Patients who are compliant to HU may not have an increase in HbF levels⁶⁻⁸ and HU-responders often have minimal increases in HbF levels. The difficulty in consistently increasing HbF is significant, since there is an inverse relationship between HbF and pain crisis⁹, early death¹, and possibly stroke and pulmonary hypertension⁸. Importantly, HU is not only often ineffective at elevating HbF sufficiently, but is also used at doses that are genotoxic, teratogenic, and testicular toxic and can compound the bone marrow damage that contributes to early death in SCD^{6,10-13}. The potential toxic damage is particularly important due to evidence of increased hematologic malignancies among patients with SCD^{14,15}.

Methylation of cytosines that precede guanines mediates silencing of many genes including the γ globin gene (*HBG*) that produces HbF^{16,17}. The role of DNA methylation and epigenetics in *HBG* silencing explains why decitabine, which irreversibly binds and depletes DNA methyl-transferase 1 (DNMT1) and thereby hypo-methylates the *HBG* promoter, can increase HbF by large amounts, even in severely ill and HU refractory patients^{18,19}. DNMT1 is well known to maintain methylation marks on DNA through cell division. However, high concentrations of decitabine do produce off-target anti-metabolite effects and cytotoxicity. AB1 is incorporated into DNA, where it inhibits DNMT1 by engaging its active site. Furthermore, DNMT1 is a co-repressor that is recruited by sequence-specific DNA-binding factors, e.g., DRED (TR2/TR4) and BCL11A, which direct epigenetic silencing of *HBG*^{16,18,20}. AB1 offers an improvement over traditional DNA methyltransferase inhibitors by virtue of its higher incorporation rate into DNA, at less cytotoxic levels.

These properties of AB1 could aid the goal of more accessible, safer, and more efficacious DNMT1-targeted therapy. Hence, this phase 1/2 clinical trial examines the safety of AB1 and its ability to induce Hbf and identifies the dose of AB1 to be used in subsequent phase 2 studies.

4 Study Design

This will be an open-label, dose escalating study with a starting dose of 2mg. Up to 4 additional cohorts will be enrolled at subsequently higher doses of 4mg, 8mg, 16mg, and 32mg.

- **Cohort 1** – Two (2) patients will be enrolled at the 2mg dose level for 8 weeks of treatment. After both patients have received at least 4 weeks of treatment, if there are no adverse events $\geq$ Grade 2 that are related (possibly, probably, or definitely)

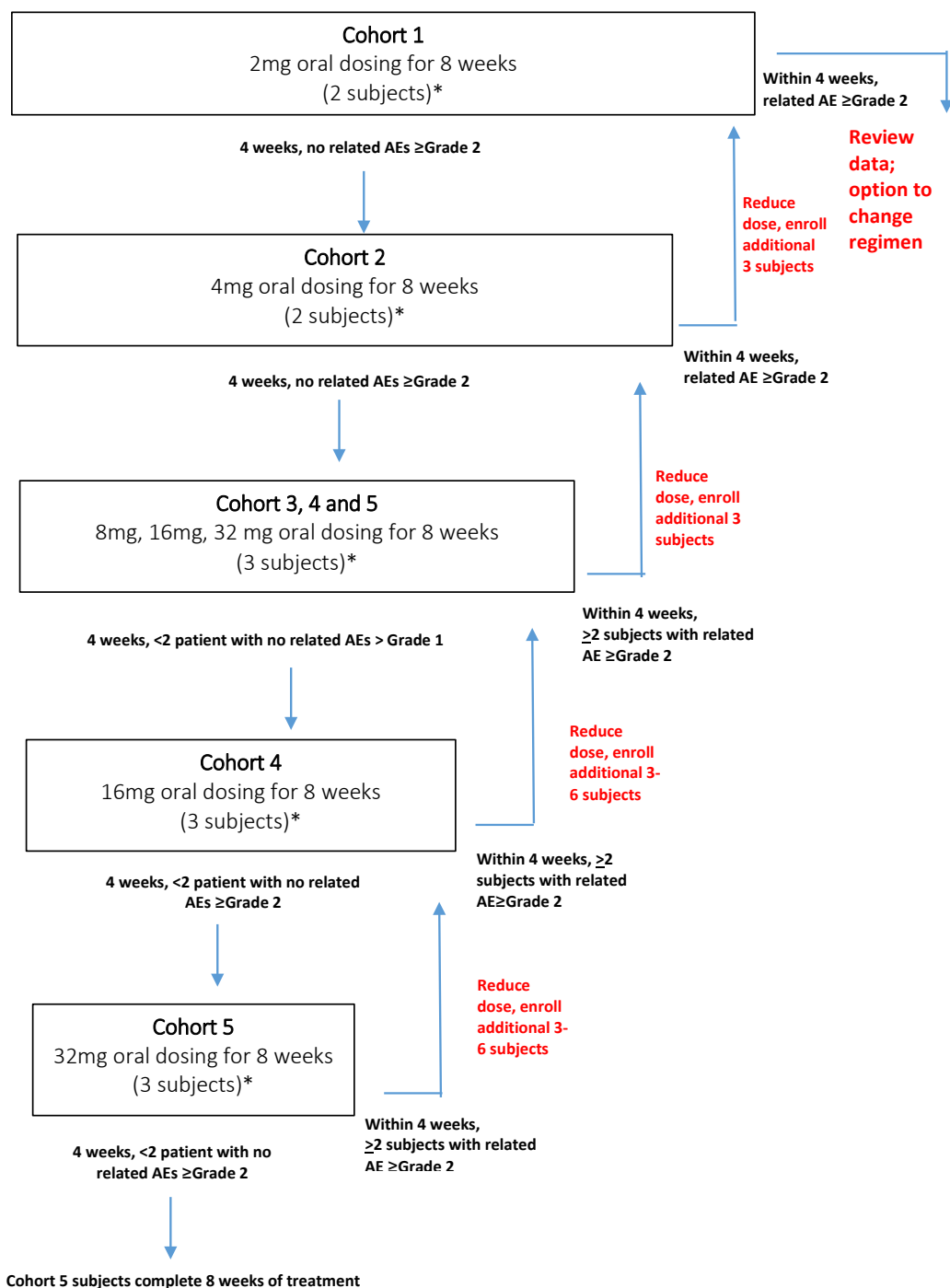
to the study drug, then the study will proceed to the next dose level. Note that if $\geq$ Grade 2 toxicity is seen at this dose level, the study drug regimen may be modified (eg, twice or thrice weekly versus daily dosing).

- **Cohort 2** – Two (2) patients will be enrolled at the 4mg dose level for 8 weeks of treatment. After both patients have received at least 4 weeks of treatment, if there are no adverse events $\geq$ Grade 2 that are related to the study drug, then the study will proceed to the next dose level.
- **Cohort 3** – Three (3) patients will be enrolled at the 8mg dose level for 8 weeks of treatment. After all patients have received at least 4 weeks of treatment, if <2 patients experience a related adverse event $\geq$ Grade 2, then the study will proceed to the next dose level. (If ≥ 2 patients experience a related adverse event $\geq$ Grade 2, the previous cohort will be expanded.)
- **Cohort 4** – Three (3) patients will be enrolled at the 16mg dose level for 8 weeks of treatment. After all patients have received at least 4 weeks of treatment, if <2 patients experience a related adverse event $\geq$ Grade 2, then the study will proceed to the next and final dose level. (If ≥ 2 patients experience a related adverse event $\geq$ Grade 2, the previous cohort will be expanded.)
- **Cohort 5** – Three (3) patients will be enrolled at the 32mg dose level for 8 weeks of treatment.
- Additional cohorts may be explored at 12 and 24mg dose levels if deemed appropriate based on safety and activity parameters.

If there are any adverse events Grade ≥ 2 that are related (possibly, probably, or definitely) to study drug, at the 4mg cohort or in ≥ 2 patients in any subsequent cohort, the dose may be reduced to the previous cohort and an additional 3-6 patients (total of up to 9) may be enrolled into that cohort. Additionally, if Hbf levels increase $>15\%$ (expressed as a percentage of total Hb) in any cohort, the cohort can be expanded to an additional 3 to 6 patients as well as continue to the next cohort if safety parameters have been met. In the expansion cohort, patients will receive study drug treatment for an additional 4 weeks (total of 12 weeks).

Approximately 6 to 33 patients may be enrolled for the entire study. Patients are eligible to enroll in a higher cohort of the study after a minimum of one month washout from AB1 dosing, if their HbF levels return to baseline ($<8.6\%$) and the investigator deems the patient eligible.

4.1 Study Schema



*If there are any adverse events Grade $\geq$ 2 that are related (possibly, probably, or definitely) to study drug, at the 4mg cohort or in $\geq$ 2 patients in any subsequent cohort, the dose may be reduced to the previous cohort and an additional 3-6 patients (total of up to 9) may be enrolled into that cohort. Additionally, if Hbf levels increase $>15\%$ (expressed as a percentage of total Hb) in any cohort, the cohort can be expanded to an additional 3 to 6 patients as well as continue to the next cohort if safety parameters have been met. In the expansion cohort, patients will receive study drug treatment for an additional 4 weeks (total of 12 weeks).

5 Study Objectives

5.1 Primary Objective

The primary objective is to determine the safety, tolerability, and active dose range of AB1 in patients with SCD.

5.2 Secondary Objectives

The secondary objectives are:

- To determine the clinical activity of AB1 in patients with SCD
- To explore the pharmacodynamics (PD) of AB1 in patients with SCD
- To characterize the pharmacokinetics (PK) of AB1 in patients with SCD

5.3 Exploratory Objectives

The exploratory objectives are:

- To evaluate time-to-response and duration of response
- To explore exposure–response relationship between AB1 exposure, PD, and clinical outcomes
- To elucidate mechanism of action, better understand PD responses, and identify potential indicators of sensitivity or response to AB1

6 Study Endpoints

6.1 Study Endpoints and Hypothesis

The primary endpoint is the incidence and frequency of $\geq$ Grade 2 study related adverse events over 8 weeks of multiple rising dose cohorts as well as to determine the dose range of biological activity.

Secondary endpoints include:

- To the extent systemic drug concentration data allow, the following PK parameters will be determined or calculated from the drug concentration time data as follows: C_{max} , t_{max} , $t_{1/2}$, AUC_{0-t} , Dose Normalized AUC_{0-inf} , CL , V_z , V_{ss}
- Change in fetal hemoglobin (HbF) levels measured by HPLC (PD of AB1) and expressed as percent total Hb.
- Change in percent F-cells measured by fluorescence activated cell sorting (FACS)
- Change in percent reticulocytes

Exploratory endpoints include:

- Time to HbF levels $>15\%$ and the period of time for which HbF is $>15\%$ over 8 weeks
- DNA methylation (PD of AB1) in buffy-coat white blood cells
- DNMT1 levels (PD of AB1) in peripheral blood mononuclear cells

7 Study Population

7.1 Selection of Patients

The treatment population are adult SCD patients who remain at risk of early death as defined by published criteria⁵⁴, and are undergoing current therapy with HU and have been on a stable dose for at least 3 months. Patients who have contraindications or are non-responsive to HU are also eligible.

Since patients are continuing to have complications despite past or current therapies, this study will not interrupt standard of care.

7.2 Patients

Males and females between the ages of 18 and 45, inclusive at screening, with confirmed SS sickle cell disease (SCD).

7.3 Inclusion Criteria

Patients who meet all the following criteria are eligible for enrollment into the study:

1. Written, informed consent
2. Age 18 to 45 years of age, inclusive at screening
3. Confirmed SS or S- β^0 -thalassemia SCD.
4. Sickle annual crisis rate of 2–10 within the past year with no crisis in the last 28 days
5. HbF <8.6% of total Hb at screening
6. Regular compliance with comprehensive care and previous therapy

7.4 Exclusion Criteria

Patients who meet any of the following criteria are disqualified from enrollment in the study:

1. Experienced severe sepsis or septic shock within the previous 12 weeks
2. Febrile illness in the 1 week prior to baseline visit
3. Acute complication due to SCD (e.g., hospitalization, acute pain, or acute chest syndrome) in the 28 days prior to screening visit
4. Plans for hospitalization, surgery, or other major procedures during the duration of the study or between screening and baseline
5. ALT $\geq 2X$ the upper limit of normal or albumin <2.0 mg/dL or direct (conjugated) bilirubin ≥ 1.5 mg/dL*
6. Estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m² at Screening. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (2021) should be used to calculate eGFR*#
7. Platelet count >800 x 10⁹/L OR <150 x 10⁹/L*
8. Absolute neutrophil count <1.5 x 10⁹/L*

9. Currently pregnant or breastfeeding
10. Female of active childbearing potential[§] who is unwilling or unable to adhere to the contraception requirements specified in the protocol
11. Male with female partner(s) of childbearing potential[§] who is unwilling or unable to adhere to the contraception requirements specified in the protocol
12. Altered mental status or recurrent seizures requiring anti-seizure medications
13. Moribund or any concurrent disease (e.g., hepatic, renal, cardiac, metabolic) of such severity that death within 24 weeks is likely
14. Concurrent diagnosis of malignancy including MDS, leukemia, or an abnormal karyotype.
15. Known Vitamin-B12, folate, or iron deficiency
16. New York Heart Association (NYHA) class III/IV status
17. Participant is on chronic transfusion therapy (e.g., for history of TIA or stroke) and medically contraindicated to discontinue transfusions (unless multiple allo-antibodies prevent the patient from getting transfusions as scheduled)
18. Blood transfusion in the 28 days prior to screening visit or between screening and baseline visits
19. Known history of illicit drug or alcohol abuse within the past 12 months
20. Current treatment with Oxbryta or Adakveo (must be off therapy for 30 days for Oxbryta with no plans to restart and off therapy for 3 months for Adakveo with no plans to restart)
21. If receiving HU, patient started HU within the previous 3 months; had a change in dose in the previous 3 months, or has plans to increase the dose during the study period, and if not receiving HU, patient has plans to start HU
22. Other experimental or investigational drug therapy in the past 28 days

*Based on screening laboratory values.

$eGFR = 141 \times \min(Scr/k, 1)^\alpha \times \max(Scr/k, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if African American]

where:

Scr is serum creatinine in mg/dL,
 k is 0.7 for females and 0.9 for males,
 α is -0.329 for females and -0.411 for males,
 min indicates the minimum of Scr/k or 1, and
 max indicates the maximum of Scr/k or 1

[§] See [Section 9](#) for definition of female of active child- bearing potential

8 Concomitant Medications

8.1 Prohibited medications for all patients

- Any oncology drug or drug known to cause bone marrow suppression other than hydroxyurea (HU). If on HU, must have been on a stable dose for 3 months with no plans to increase dose during study period.
- Any known epigenetic modulators.
- SCD patients that are not using HU or have been taken off HU (*e.g.*, for compliance issues, poor efficacy, or other reasons) are eligible for enrollment. Restarting HU during the study or in the 3 months before screening is not allowed.
- Current therapy with either Adakveo or Oxbryta is prohibited. Patients may enroll if last dose of Oxbryta was >30 days prior or last dose of Adakveo was >90 days prior. Adakveo and Oxbryta may not be given during study period.
- Use of L-glutamine may be allowed if it is expected that there will be no change in either dose or compliance during the study.
- Use of any experimental treatments for SCD during the study is not allowed. Use prior to screening is allowed if the experimental treatment has been discontinued at least 1 month before the screening visit, if approved by Principal Investigator.

9 Contraception and Pregnancy

9.1 For female patients of reproductive potential

Female patients of reproductive potential are defined as all women physiologically capable of becoming pregnant, unless they meet one of the following conditions:

- Postmenopausal: 12 months of natural (spontaneous) amenorrhea or 6 weeks after surgical bilateral oophorectomy, with or without hysterectomy
- Post hysterectomy

All female patients of reproductive potential and their partners are required to use effective contraception during the study and for at least 90 days after their last dose as defined below:

- Using 1 or more of the following acceptable methods of contraception: surgical sterilization (*e.g.*, bilateral tubal ligation), intrauterine contraception/device, or hormonal contraception plus a barrier method, or any 2 barrier methods (a combination of male or female condom with diaphragm, sponge, or cervical cap).
- When it is the preferred and usual lifestyle of the patient, abstinence can be considered an acceptable method of contraception at the discretion of the investigator. Periodic abstinence (*e.g.*, calendar, ovulation, symptothermal, post ovulation methods) and withdrawal are not considered acceptable methods of contraception.

9.2 For male patients

Males with female partner(s) of reproductive potential are required to use effective contraception during the study and for at least 90 days after their last dose as defined below:

- Hormonal contraceptives (oral, injectable, patch, intrauterine devices) or male sterilizations.
- Female partner(s) of reproductive potential are required to follow the same guidelines as above in [Section 9.1](#).

9.3 Pregnancy

Any pregnancy should be reported to the investigator within 24 hours of awareness. If a female patient becomes pregnant, study drug will be permanently stopped, and the patient will be discontinued from the study.

10 Study Drug

AB1 will be provided as 2mg and 8mg #4 hard gelatin capsules that contain the inactive excipient microcrystalline cellulose for oral administration.

10.1 Packaging

AB1 capsules will be provided in bulk in high density polyethylene bottles with induction seals and polypropylene child resistant plastic caps. Each bottle will contain 40 capsules of either 2mg or 8mg capsules with a silica gel desiccant.

10.2 Storage and Handling

All study drug must be stored in a secure environmentally controlled and monitored area in accordance with the labeled storage conditions with access limited to the investigator and authorized study staff. AB1 should be stored refrigerated at 2°C to 8°C in the original manufacturer's container. The desiccant cannot be removed from the container.

The investigator or designee must confirm appropriate temperature conditions have been maintained and any excursions should be reported to the Principal Investigator.

10.3 Accountability

Patients will be instructed to return all study drug, including empty containers, to the study center at each visit. Pill counts will be completed at each visit to document drug dispensed against drug administered.

11 Dosing and Administration

Upon signing informed consent, participants will be assigned a unique number in ascending numerical order at each study site. This number will remain consistent for the

duration of the study.

Depending on the stage of the study at the time of enrollment, the patient will be at any one of the following dose levels.

Cohort	AB1 dose level	# of patients
1	2mg	2
2	4mg	2
3	8mg	3
4	16mg	3
5	32mg	3

If there are any adverse events Grade ≥ 2 that are related (possibly, probably, or definitely) to study drug, at the 4mg cohort or in ≥ 2 patients in any subsequent cohort, the dose may be reduced to the previous cohort and an additional 3-6 patients (total of up to 9) may be enrolled into that cohort. Additionally, if HbF levels increase $>15\%$ (expressed as a percentage of total Hb) in any cohort, the cohort can be expanded to an additional 3 to 6 patients as well as continue to the next cohort if safety parameters have been met. In the expansion cohort, patients will receive study drug treatment for an additional 4 weeks (total of 12 weeks).

Patients are eligible to enroll in a higher cohort of the study after a minimum of one month washout from AB1 dosing, if HbF levels return to baseline ($<8.6\%$) and the investigator deems the patient eligible.

Additional cohorts may be explored at 12mg and 24mg dose levels if deemed appropriate, based on safety and activity parameters.

11.1 Dosing Instructions

AB1 is to be administered orally once daily with water (approximately 200mL) at approximately the same time of day (± 4 hours), with no food or antacids for 1 hour before and 2 hours after each dose. If a dose is delayed by more than 6 hours, the patient should not take that dose and should mark the dose as 'not taken'.

Capsules should not be chewed or crushed. If the dose regimen requires more than one capsule per dose, the capsules/tablets should be taken one at a time.

11.2 Dose escalation, adjustments, delays, withholding, restarting, discontinuation

11.2.1 Dose Escalation

In each cohort, once all patients have received at least 4 weeks of treatment and the following criteria have been met, then the study will proceed to the next dose level.

- For 2mg and 4mg dose levels – No adverse events $\geq$ Grade 2 that are related (possibly, probably, or definitely) to the study drug
- For 8mg, 16mg, and 32mg dose levels – Fewer than 2 patients experience a related adverse event $\geq$ Grade 2

11.2.2 Dosage Adjustments

Individual dosage adjustments should not be made without consultation with the Principal Investigator.

If there are any adverse events $\geq$ Grade 2 that are related (possibly, probably, or definitely) to study drug, at the 4mg cohort or ≥ 2 patients in any subsequent cohort, the dose for those patients may be reduced to that for the previous cohort and an additional 3-6 patients (total of up to 9) may be enrolled into that lower cohort.

If this occurs in the 2mg Cohort, the adverse events will be reviewed, and this cohort may be expanded or halted or dosing may be altered to twice or thrice weekly.

If any patient experiences a Grade 3 related adverse event drug should be withheld for up to 3 days. If the event does not resolve to Grade ≤ 2 or to baseline within 3 days, study drug should be discontinued.

11.2.3 Dosage Delays

The following dosage delay rules apply:

- If the dosing delay is ≤ 3 days, the patient should resume the treatment as soon as possible if deemed safe by the investigator.
- If the dosing delay is more than 3 days, confirm if delay is due to:
 - Treatment-related Grade 3 AEs, the patient will be removed from the study.
 - Other conditions other than treatment related AEs, the case will be reviewed by principal investigator to determine whether the patient will be allowed to resume AB1 treatment.

11.2.4 Dose Withholding

AB1 should be withheld for the following reason:

- Grade 3 related AEs

11.2.5 Restarting

AB1 dosing can be resumed if:

- The toxicities resolve to Grade ≤ 2 or return to patients' baseline values within 3 days
- Restarting of therapy is deemed safe by the principal investigator

11.2.6 Permanent Discontinuation

Patients may decide to discontinue participation at any time during the study. In addition, patients will permanently discontinue receiving the investigational product if:

- Patients experience adverse events meeting the DLT criteria (Grade 4 pancytopenia or leukopenia) at any time. Patients will be followed until the DLT is resolved, returns to baseline value, or is considered stable. Patients will be withdrawn from AB1 treatment and will be treated as deemed appropriate by the investigator or treating physician.

- Any life-threatening adverse event
- Intolerability of the study treatment
- The dosing is delayed >3 days due to AB1-related Grade 3 AEs
- Clinically significant deterioration of health status
- Withdrawal of informed consent
- Patient becomes pregnant or is breastfeeding
- Initiation of new therapy for SCD
- Study is terminated by drug manufacturer or Investigator

12 Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

Discontinuation from study intervention (*i.e.*, the study drug) does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to, changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any such new clinically relevant finding will be reported as an adverse event (AE).

Upon treatment discontinuation all end of treatment evaluations and tests will be conducted. All participants who discontinue due to an AE must be followed until the event resolves or stabilizes.

Appropriate medical care should be provided until signs/symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status on the AE will be reported in the participants' medical records and the appropriate eCRF.

12.1 Discontinuation of Study

Reasons for study discontinuation may include, but are not limited to:

- Safety concerns as indicated by the Investigator
- Request to discontinue the trial by a regulatory or health authority or an IRB
- Manufacturing difficulties / concerns

12.2 Discontinuation of Treatment

Reasons for patient discontinuation of study treatment may include, but are not limited to:

1. Toxicity that in the opinion of the Investigator is not adequately controlled and/or recurrent despite necessary dose modifications
2. Discontinuation of patient participation in the study (see next section)

12.3 Participant Discontinuation/Withdrawal from the Study

An Investigator may discontinue or withdraw a participant from the study for the following

reasons:

1. Pregnancy
2. Death
3. Toxicity: treatment related or unrelated
4. Investigator judgment: the investigator may discontinue a participant if, in his/her judgment, it is in the best interest of the participant to do so
5. Noncompliance
6. Participant unable to receive study drug for 3 days or more every 4 weeks
7. Investigator decision

Participants are free to withdraw from participation in the study at any time upon request. The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF).

Patients who sign the informed consent form but do not receive treatment may be replaced.

Patients who sign the informed consent form and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study: a) will not be replaced if the reason for discontinuation/withdrawal is DLT/toxicity related to the study drug, or b) will be replaced if the reason for discontinuation/withdrawal is not toxicity related and deemed appropriate otherwise by the study Principal Investigator.

12.4 Lost to Follow Up

A participant will be considered lost to follow-up if he or she fails to return for more than 25% of the scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

1. The site will attempt to contact the participant and reschedule the missed visit at the earliest appropriate time and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
2. Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's medical record or study file.
3. Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

13 Study Assessments

Study Procedures and their timings are summarized in the Schedule of Assessments

(SoA) ([Section 14](#) [Dose Escalation Cohorts] and [Section 15](#) [Dose Expansion Cohort]).

1. Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.
2. All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
3. Procedures conducted as part of the participant's routine clinical management (e.g., CMP) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA.
4. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.
5. The maximum amount of blood to be collected will not exceed 550ml over the study period if receiving treatment for 8 weeks or 600ml if receiving treatment for 12 weeks.

13.1 Screening (Pre-Treatment) Evaluations

Patients must sign the informed consent form prior to any study-related procedures.

13.1.1 Screening Visit (Week -2 or Week -1)

The following assessments will be completed at the Screening Visit:

- Complete medical history from the preceding year, major complications of SCD from birth (for example, stroke, acute chest syndrome etc.), and major non-sickle cell related diagnoses from birth (for example, cancer diagnoses)
- For females: last menstrual period, history of hysterectomy and/or oophorectomy, history of bilateral tubal ligation, history of oral contraceptive or Depo-Provera (medroxyprogesterone acetate) use.
- Determination of birth control measures, if applicable
- Crisis frequency and descriptions (over the preceding year) – per patient report and medical record review
- Physical examination
- Vital signs
- Blood will be collected for various laboratory studies (see SoA; [Section 14](#) [Dose Escalation Cohorts] and [Section 15](#) [Dose Expansion Cohort])
- Concomitant medications (started within 30 days of first dose of study drug).

13.1.2 Baseline / Enrollment Visit – Day 1 (prior to starting study drug)

The maximum time interval between Screening Visit and initiation of study drug (Day 1) should be no longer than 2 weeks.

The following assessments will be completed at the Baseline Visit (Day 1), prior to starting study drug:

- Interim medical history, directed physical examination
- Vital signs, including weight
 - Blood will be collected for various laboratory studies (see SoA; [Section 14](#) [Dose Escalation Cohorts] and [Section 15](#) [Dose Expansion Cohort])
 - Blood for PK analysis

13.2 On-Study Visits and Follow-up Evaluations

Patients will start study drug on Day 1. Evaluations to be performed after administration of first dose of AB1:

- Blood for PK analysis at 30 minutes, 1 hour, 2 hours, 4-5 hours, and 24 hours post-dose
- Day 2 is onsite and includes the 24- hour post-dose blood draw and evaluation of any adverse events and/or changes in concomitant medications

13.2.1 Weeks 4, 6, 8 (and in Expansion Cohort, Weeks 10 and 12)

Week 2, Week 4, Week 6, Week 8, and, as applicable, Week 10 and 12, will be on-site visits while on treatment. Evaluations to be performed are:

- Interim medical history, directed physical examination
- Vital signs
- Crisis occurrence
- Adverse events
- Concomitant medication updates
- Blood will be collected for various laboratory studies (see SoA; [Section 14](#) [Dose Escalation Cohorts] and [Section 15](#) [Dose Expansion Cohort])
- Study drug accountability

13.2.2 Weeks 3, 5, 7

Week 3, Week 5, and Week 7 will be telephone visits while on treatment. Crisis occurrence, adverse event and concomitant medication reviews will be recorded.

13.2.3 Follow-up Visit (Week 10 in Dose Escalation Cohorts; Week 14 in Expansion Cohort)

The follow-up visit should occur with 14 days (± 3 days) of the last day of study drug. This will be an on-site visit. Evaluations to be performed are:

- Interim medical history, directed physical examination

- Vital signs, including weight
- Crisis occurrence
- Adverse events
- Concomitant medication updates
- Blood will be collected for various laboratory studies (see SoA; [Section 14](#) [Dose Escalation Cohorts] and [Section 15](#) [Dose Expansion Cohort])

13.2.4 Early Termination

Patients who prematurely discontinue the study for any reason will be asked to return for an Early Termination visit within 3 days following the last dose of study drug. If it is determined that the patient will discontinue study drug while at the study site, the Early Termination visit should be conducted at that time.

Evaluations to be performed are:

- Interim medical history, directed physical examination
- Vital signs, including weight
- Crisis occurrence
- Adverse events
- Concomitant medication updates
- Blood will be collected for various laboratory studies (see SoA; [Section 14](#) [Dose Escalation Cohorts] and [Section 15](#) [Dose Expansion Cohort])

14 Schedule of Assessments: Dose Escalation Cohorts

Assessment	Screen	Treatment Period Follow-Up											Early Term
	Screening Visit	Base-line (Day 1)	Day 2	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 10	Early Termination
	Within 2 weeks of Day 1	D1	D2	D7 (±3) 	D14 (±3)	D21 (±3) 	D28 (±3)	D35 (±3) 	D42 (±3)	D49 (±3) 	D56 (±3)	Within 14 (±3) days of last dose	Within 3 days of last dose
Informed Consent	X												
Medical History	X												
PE	X												
Vitals	X	X	X		X		X		X		X	X	X
Targeted PE		X			X		X		X		X	X	X
Crisis & AE Review	X	X	X	X	X	X	X	X	X	X	X	X	X
Con Meds	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test (females)	X										X		
Erythropoietin level	X										X		
CBC with differential, reticulocytes	X	X			X		X		X		X	X	X
Complete metabolic panel	X	X			X		X		X		X	X	X
LDH	X	X			X		X		X		X	X	X
%HbF	X	X			X		X		X		X	X	X
F-cells	X	X			X		X		X		X	X	X
DNMT1 and DNA methylation analysis		X					X				X		X
fHG gene expression		X					X				X		X
Study Drug Administration		X					DAILY (once a day)		X				
Study Drug Reconciliation					X		X		X		X		
Plasma AB1 levels		X*	X				X				X		

*Plasma AB1 levels to be collected pre-dose and at 30 minutes and 1, 2, 4-5, and 24 hours post-dose on Day 1. The 24-hour post-dose sample will be collected prior to dosing the next day. Week 4 will be drawn pre-dose. Week 8 will include pre-dose, 30 mins, 1, 2, and 4-5 hours post-dose.

The amount of blood drawn will not exceed 550ml over 8 weeks of treatment.

15 Schedule of Assessments: Dose Expansion Cohort

Assessment	Screen Screening Visit	Treatment Period Follow-Up													Early Term	
		Baseline (Day 1)	Day 2	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 10	Week 12	Week 14	Early Termination	
				D7 (±3)	D14 (±3)	D21 (±3)	D28 (±3)	D35 (±3)	D42 (±3)	D49 (±3)	D56 (±3)	D70 (±3)	D84 (±3)	Within 14 (±3) days of last dose	Within 3 days of last dose	
Informed Consent	X															
Medical History	X															
PE	X															
Vitals	X	X	X		X		X		X		X	X	X	X	X	X
Interim MH		X			X		X		X		X	X	X	X	X	X
Targeted PE		X			X		X		X		X	X	X	X	X	X
Crisis & AE Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Con Meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test (females)	X												X			
Erythropoietin level	X										X					
CBC with differential, reticulocytes	X	X			X		X		X		X		X	X	X	X
Complete metabolic panel	X	X			X		X		X		X		X	X	X	X
LDH	X	X			X		X		X		X		X	X	X	X
%HbF	X	X			X		X		X		X		X	X	X	X
F-cells	X	X			X		X		X		X		X	X	X	X
DNMT1 and DNA methylation analysis		X					X				X		X		X	X
fHG gene expression		X					X				X		X		X	X
Study Drug Administration		X	DAILY (once a day)													
Study Drug Reconciliation					X		X		X		X		X			
Plasma AB1 levels		X*	X				X				X		X			

*Plasma AB1 levels to be collected pre-dose and at 30 minutes and 1, 2, 4-5, and 24 hours post-dose on Day 1. Week 4 and Week 8 will be pre-dose. Week 12 will be pre-dose and 30 mins, 1, 2, and 4-5 hours post-dose.

The amount of blood drawn will not exceed 600ml over 12 weeks of treatment.

16 Efficacy, Pharmacodynamic (PD), and Scientific Evaluations

16.1 SCD-Related Crises

SCD-related crises will be measured at the Screening Visit (retrospective estimation for the 1-year period preceding enrollment) and documented every week.

Definitions:

- *Crisis frequency* is defined as the number of crises divided by the length of time in years over which the number of crises is calculated.
- *Crisis*, for purposes of analysis, is defined as a pain crisis, pneumonia, acute chest syndrome, priapism, hepatic sequestration, stroke, or initiation of chronic transfusion therapy (as defined below). Pain due to chronic complications of SCD, such as aseptic necrosis, osteomyelitis, or leg ulcers will not be considered as crises.
- A *pain crisis* is defined as the occurrence of pain in the extremities, back, abdomen, chest, or head that lasts at least 2 hours; requires a visit to a medical facility; and that has no evident cause other than SCD-related VO. Pain due to chronic complications of SCD, such as aseptic necrosis, osteomyelitis, or leg ulcers will not be considered as crises.
- *Pneumonia* is defined as chest infiltrates on chest X-ray or chest CT scan associated with fever and an identified infectious etiology.
- *Acute Chest Syndrome* is defined as a new infiltrate on chest X-ray or chest CT scan associated with one or more new symptoms: fever, chest pain, cough, sputum production, dyspnea, or hypoxia.
- *Hepatic sequestration* is defined as a sudden increase in liver size of ≤ 2 cm for children and ≤ 5 cm for adults associated with pain in the right upper quadrant, a decrease in the Hb concentration of at least 2 g/dL with reticulocytosis, and increased abnormality in liver function tests that cannot be attributed to gall stone disease.
- *Priapism* is defined as a painful persistent penile erection lasting 30 minutes or more.
- *Stroke is defined as a thrombotic or hemorrhagic event involving the central nervous system* (as demonstrated by hemorrhage or infarction on CT scan or MRI of the brain or persistently hemorrhagic spinal fluid on lumbar puncture) *that results in focal neurologic deficits*.
- *Chronic transfusion therapy* is defined as blood transfusions occurring 1 or more times per month for 3 consecutive months.

Information on the occurrence each of these sickle cell related complications will be collected every study visit and site personnel will be instructed to report each event as an adverse event or serious adverse event, as appropriate.

16.2 Percentage of HbF

The percentage of HbF as a proportion of total hemoglobin (also denoted as %HbF) will be measured by high performance liquid chromatography. HbF level, which is expressed as percent total Hb unless otherwise indicated, is measured at the timepoints described in the SoA.

16.3 Global DNA methylation assay

The anticipated effect of this regimen is to produce a plateau (not progressive) level of DNA hypomethylation. This will be confirmed by pyrosequencing of repetitive DNA elements in bisulfite treated DNA extracted from buffy-coat white blood cells in the patient samples. A beta globin locus is used for assessment of DNA methylation changes in LCR and globin genes. Bisulfite modification of DNA and pyrosequencing of repetitive element DNA is performed as per standard protocols²³ in the laboratory of Augusta University.

16.4 DNMT1 protein level assay

The proximal PD effect of decitabine that precedes DNA hypomethylation is depletion of the DNA methylating enzyme DNMT1. DNMT1 not only methylates DNA but is also an essential component of histone modifying multi-protein complexes. Therefore, the epigenetic consequences of DNMT1 depletion include removal of histone marks (e.g., H3K27 trimethylation) associated with transcription repression. Since a spectrum of epigenetic effects are produced downstream of DNMT1 depletion, DNMT1 levels may be a more reliable pharmacodynamic index of the biological consequences of decitabine on transcription regulation than DNA methylation analysis. Therefore, DNMT1 levels (measured by a quantitative immuno-fluorescence assay of peripheral blood nucleated cells) will be correlated with global DNA methylation, HbF levels, and platelet counts.

17 Safety Monitoring

17.1 Safety Assessments Overview

Patients enrolled in this trial will receive all therapy and monitoring that is considered standard of care for patients with SCD, in addition to the study specific monitoring designed to identify changes in laboratory parameters that can trigger dose modifications to decrease the possibility of clinical adverse events. Patients will be asked about signs and symptoms at each clinical encounter. Study personnel will collect information on ER visits and hospitalizations, clinic visits for pain, sickle cell related complications, and pain medication use. Information on the occurrence of sickle cell related complications will be collected at every visit and site personnel will be instructed to report each event as an adverse event or serious adverse event, as appropriate. All clinical information will be collected using CRFs.

17.2 Adverse Events Definitions

All adverse events will be reported from the time of consent to Week 12 (or 16 for expansion) visit and classified relative to their seriousness, severity, and relatedness. Adverse events reported from time of consent until post first dose will be recorded as medical history. Any related serious adverse should be reported up to 30 days post-dose.

The following definitions have been adapted from FDA regulations and ICH guidelines for use in this study:

- An adverse event (AE) is defined as any untoward medical occurrence in a study patient that does not necessarily have a causal relationship with treatment administered in the study. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the study. Adverse Event data are recorded on the Case Report Form (CRF).
- A *serious* AE (SAE) is defined as an AE that results in death, is life-threatening (*i.e.*, an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe), requires inpatient hospitalization or prolongation of existing hospitalization, or results in persistent or significant disability/incapacity; results in a congenital anomaly/birth defect. In the opinion of the investigator, important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above, may be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.
- An *adverse clinical laboratory trend* is a shift, in an adverse direction, in the mean (or median) change from baseline of a clinical laboratory parameter that is more adverse in an active treatment group than in a control group. For example: mean ALT change from baseline increases significantly more in the active treatment group than in the control group.
- For the purposes of this study, an expected SAE is an AE that is serious, expected, and likely related to the patient's underlying disease process. For this study, the AEs listed in [Table 1](#) are expected SAEs.
- For the purposes of this study, an unexpected SAE is an AE that is serious, unexpected, and potentially related to the study drug / therapy. For this study, unexpected SAEs are AEs that are not listed in [Table 1](#). This also includes events that are anticipated within the drug class but not specifically mentioned as occurring with the particular drug under investigation.

Table 1: List of Expected Adverse Events (AEs)

Acute chest syndrome (ACS)	Elevated urinary urobilinogen	Pain, abdominal
Anemia	Fatigue	Priapism
Aplastic crisis/anemia	Fever	Proteinuria/albuminuria
Arthralgia	Hand-foot syndrome	Pulmonary embolus
	Hematuria	Pulmonary hypertension
Avascular necrosis of femoral head, hip, or shoulder	Hemiplegia	Pulmonary parenchymal infiltrates on chest x-ray
	Hemolysis	Pyelonephritis
Bone infarction	Hepatic sequestration	Renal insufficiency
Cardiomegaly	Hepatosplenomegaly	Renal failure
Cerebrovascular accident	Hyperplastic bone marrow	Renal papillary necrosis
Cholecystitis	Hyposthenuria	Reticulocytosis (>10%)
Cranial nerve palsy	Hypoxemia (pO ₂ <65 mmHg)	Retinal hemorrhage or retinal disease
Constipation	Infection	Rhabdomyolysis
Cough	Jaundice/Liver function test abnormalities	Sepsis
Congestive heart failure	Leukocytosis or Leukopenia	Skin ulcers
Decreased kidney function	Meningitis	Splenic sequestration
Decreased lung function	Nausea	Thrombocytopenia
Diarrhea	Neutropenia	Thrombosis
	Pain, bone or joint	

17.3 Adverse Event Severity and Relatedness to Treatment

Investigators will “grade” the severity of all AEs using the following scale found in CTCAE v 4.0 ([Table 2](#)); the results will be recorded on the CRF and the SAE form, if necessary.

Table 2: Severity Grading of AEs

Severity Grade	Description
1	Mild. Awareness of sign, symptom, or event, but easily tolerated; does not interfere with usual daily activities or tasks.
2	Moderate. Discomfort enough to cause interference with usual daily activity; may warrant therapeutic intervention.
3	Severe. Incapacitating: inability to perform usual activities and daily tasks; significantly affects clinical status; requires therapeutic intervention.
4	Life-threatening. Adverse event is life-threatening.
5	Death. Adverse event causes death.

The nomenclature for assessing the causal relationship between an AE and the study drug is listed in Table 3. The investigator will determine the category that overall best “fits” the relationship between the AE and the study drug and record the evaluation on the CRF and the SAE form, if necessary.

Table 3: Adverse Event Relatedness

Relatedness	Description
Unrelated	• No temporal association to study drug. • An alternate etiology has been established. • The event does not follow the known pattern of response to study drug. • The event does not reappear or worsen with re-challenge.
Probably not related / remotely related	• No temporal association to study drug. • Event could readily be produced by clinical state, environmental or other interventions. • The event does not follow the known pattern of response to study drug. • The event does not reappear or worsen with re-challenge.
Possibly related	• Reasonable temporal relationship to study drug. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study drug <u>or</u> as yet unknown pattern of response.
Probably related	• There is a reasonable temporal association with the study drug. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study drug. • The event decreases with de-challenge.
Definitely related	• There is a reasonable temporal relationship to the study drug. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study drug. • The event decreases with de-challenge and recurs with re-challenge.

17.4 Assessment of Adverse Event Outcome

The clinical investigator will follow every AE to a satisfactory outcome or stabilization of the event, even when this requires a time period beyond the scope of the study. This is particularly applicable to SAEs.

The investigator will record each AE outcome on the CRF. The terms used to describe AE outcome (i.e., outcome of reaction/event at the time of last observation) are as follows:

- Ongoing,
- Resolved without sequelae,
- Resolved with sequelae, or
- Death.

Actions taken in response to an AE and follow-up results (including laboratory results) will be recorded in the patient's medical record, in accordance with local procedure. Any

treatment administered for the AE must be recorded in the patient's CRF. When patients are discontinued from the study due to an AE, relevant clinical assessments and laboratory tests will be repeated as necessary until final resolution or stabilization occurs.

17.5 Serious Adverse Event Reporting; Suspension Guidelines

The safety data for this study will be reviewed by Dr. Nirmish Shah (principal investigator). Serious safety issues that arise in this study will be brought to the attention Dr. Shah and he will make recommendations to the Institutional Review Board (IRB). The IRB will consider the recommendations, determine an appropriate action, and may notify the drug manufacturer. Dr. Shah will notify all participating investigators. This section defines "serious safety issues" and describes procedures for bringing them to the attention of the IRB.

This study is being conducted under an Investigational New Drug (IND) Application. Consequently, U.S. Government regulations require that all "unexpected SAEs" (defined in Section 17.2) must be reported to the FDA within either 7 calendar days (fatal or life-threatening events) or 15 calendar days (non-fatal or non-life-threatening). The FDA has the authority to direct the investigators to suspend or terminate the study or take other actions.

The PI and study investigators will make the following types of reports that can alert the IRB to a potential safety issue:

- Ad hoc reports of unexpected SAEs that are made within 7 or 15 calendar days, as specified in subsequent paragraphs.

17.6 Reporting of Unexpected Serious Adverse Events

SAEs that arise after the Informed Consent is signed until 30 days after the last dose of study drug must be reported. [Table 4](#) summarizes the procedures and timing for alerting the principal investigator of unexpected SAEs. Within 24 hours of being informed of the occurrence of an unexpected SAE the investigator must make an initial report to:

1. the drug manufacturer
2. the institution regulatory specialist

All SAEs must be reported to the Principal Investigator and drug manufacturer on the Crises and Adverse Event Report Form; this report form should be completed for both initial and follow up SAEs.

The initial SAE report received should include the following minimum information: patient initials, patient study ID; study drug; an identifiable reporting source; and an event or outcome that can be identified as serious.

The investigator must also report the unexpected SAE to the site's Institutional Review Board (IRB) in accordance with that IRB's regulations or procedures.

The investigator, the study coordinator and the institution regulatory specialist will collaborate to prepare a FDA Form 3500A (MEDWATCH) for the unexpected SAE. This report should describe the event as fully as possible.

Supporting documentation (e.g., CRF pages, lab reports, summary notes, autopsy reports) should accompany the report.

A fatal or life-threatening unexpected SAE must be reported to the FDA no later than 7 calendar days after the Sponsor's/regulatory specialist's receipt of information. A non-fatal, non-life-threatening unexpected SAE must be reported to the FDA No later than 15 calendar days after the Sponsor's/ regulatory specialist's receipt of information.

The investigator will follow the progress of a patient who experiences an unexpected SAE until the SAE is resolved or considered stable. When the unexpected SAE has not resolved by the report deadline, the investigator will make follow-up reports in accordance with directions from the principal investigator, the FDA, and/or the site's IRB.

Table 4: Summary of Procedures and Timing for Alerting the IRB and NHLBI Project Officer of Unexpected SAEs

Situation or Event	Summary of Procedure (See text for details.)	Critical Value for IRB "Alert"
Unexpected SAEs	- Investigator notifies Dr. Nirmish Shah within 24 hours. - Site investigator, Regulatory Specialist, and Dr Nirmish Shah prepare report using FDA forms and submit report to NHLBI Project Officer, IRBs, study investigators. Report: - Fatal or life-threatening events: within 7 calendar days. - Otherwise: within 15 calendar days.	Alert all cases.

17.7 Reporting of Expected Serious Adverse Events

Investigators will report all expected SAEs within 3 business days to Dr. Nirmish Shah (telephone/text-252 258 5628; email- Nirmish.shah@duke.edu). The study coordinator and the Institution Regulatory Specialist will collaborate to prepare a report of the expected SAEs that meet the following criteria (applicable FDA reporting format will be used).

Events occurring at a higher rate than anticipated that meet the following

- Serious
- Expected (known consequences of the disease under investigation, commonly known events in the study population, or listed in the protocol / investigator brochure)
- Suspected to be related to the investigational product

Site PIs are responsible for reporting all SAEs to the appropriate IRB or ethics committee in accordance with local laws and regulations.

18 Endpoints

18.1 Tolerability / Safety Endpoints

Safety and tolerability, based on the assessment of adverse events (AEs), serious AEs

(SAEs), clinically significant laboratory test result, crises, and vital signs.

18.2 Pharmacokinetic Endpoints

PK parameter estimates of AB1 and metabolites derived from plasma concentration-time data as outlined in the SoA.

18.3 Pharmacodynamic target engagement endpoints (TEE)

HbS and HbF, as percent of total Hb, as measured by HPLC, F-cell percentage as measured by FACS, reticulocyte percentage as measured by FACS, DNA methylation and DNMT1 levels

18.4 Potential Other Exploratory endpoints

Additional exploratory endpoints will be pursued, if feasible:

- Point of sickling, elongation max, and elongation min by Lorca
- %F-reticulocyte by FACS
- Blood samples may be collected to aid the discovery of other potential biomarkers to measure SCD activity.

19 Statistics

19.1 Sample Size Justification

Sample sizes are not based on statistical considerations, however, are considered sufficient to obtain initial estimates of the safety, tolerability, PK, and target engagement properties of AB1 in SCD patients.

This sample size is also considered sufficient to investigate, for the first-time, the potential early changes that may occur in patients with SCD. These include disease relevant biomarkers during repeated treatment with AB1, including increases from baseline in F cells and HbF levels and decreases in reticulocytes and sickle RBCs. Therefore, results will include descriptive statistics and not be analyzed based on *p* values, including analysis of dose-response and effect size.

19.2 Statistical Methodology

A statistical analysis plan (SAP) will be generated and approved prior to database lock. All planned statistical analysis will be detailed in the SAP. The final clinical study report (CSR) will describe and justify any deviations from the planned analysis.

In general, all study endpoints will be summarized using descriptive statistics. Descriptive statistics for continuous data will include number of patients (n), mean, standard deviation (SD), median, minimum, and maximum. Summaries of change from baseline variables will include only patients who have both a baseline value and corresponding value at the time point of interest. Baseline will be defined as the last value prior to initiation of blinded treatment. Descriptive statistics for categorical data will include frequency and percentage. Where appropriate, descriptive statistics may be presented with 95% confidence intervals (CIs).

Listings will be provided for all collected study data.

19.3 Analysis set

The safety analysis set is defined as all patients who are randomized and receive at least one dose of study drug. The safety analysis set will be used for all safety analyses. The PK analysis set is defined as all patients who receive study drug and have at least one evaluable PK parameter.

The PK analysis set will be used for all plasma concentration and PK parameter analyses.

The efficacy analysis set is defined as all patients who receive study drug and have at least one pharmacodynamic marker or efficacy evaluation.

Demographics and baseline characteristics

Demographics and baseline characteristics will be summarized using descriptive statistics by dose and treatment and overall.

19.3.1 Safety analysis

The latest version of Medical Dictionary for Regulatory Activities (MedDRA) will be used for the coding of AEs. The overall incidence of AEs will be displayed by MedDRA system organ class, preferred term, and treatment group. Vital signs, ECG, and clinical laboratory test results and change from baseline values will be summarized using descriptive statistics by treatment group and timepoint.

19.3.2 Pharmacokinetic analysis

Plasma concentrations of AB1 and metabolite(s) will be summarized using descriptive statistics by treatment group and sample nominal timepoint. For each treatment group, PK parameters will be estimated using standard noncompartmental methods. PK parameters to be estimated may include, but are not limited to, the following: AUC_{0-12} , AUC_{0-24} , AUC_{0-last} , AUC_{tau} , C_{max} , T_{max} , and $T_{1/2}$.

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