COVER PAGE

Title: IMatinib for PAin in Chronic Treatment of Sickle Cell Anemia (IMPACT SCA):
A Pilot Study of Imatinib in Patients with Sickle Cell Anemia and Recurrent Vaso-occlusive Pain

NCT03997903

June 25, 2024 v10.4

IMatinib for PAin in Chronic Treatment of Sickle Cell Anemia (IMPACT SCA):
A Pilot Study of Imatinib in Patients with Sickle Cell Anemia and Recurrent Vaso-occlusive Pain

Principal Investigator Indiana University School of Medicine:
Seethal A. Jacob, MD, MS
Assistant Professor of Pediatrics
Riley Hospital for Children, Rm 4340
705 Riley Hospital Drive
Indianapolis, Indiana 46202
Email: seejacob@iu.edu
Phone: 317-944-8785
Fax: 317-948-0616

Principal Investigator Cincinnati Children's Hospital:
Charles T. Quinn, MD, MS
Professor of Pediatrics
Cincinnati Children's Hospital Medical Center
Division of Hematology
3333 Burnet Ave
Cincinnati, OH 45229
Email: charles.quinn@cchmc.org
Phone: 513-803-3086
Fax: 513-636-3549

Sub-investigators Indiana University School of Medicine:
Meghan Drayton-Jackson, DO
Assistant Professor of Pediatrics
Riley Hospital for Children

Jennifer Kaericher, NP
Riley Hospital for Children

Sub-investigators Cincinnati Children's Hospital:
Theodosia Kalfa, MD, PhD
Associate Professor of Pediatrics
Cincinnati Children's Hospital

Sub-investigators Purdue University:
Philip Low, PhD
Professor of Chemistry and Director of the Purdue Center for Drug Discovery

Faculty Biostatistician:
Susan M. Perkins, PhD
Indiana University, Indianapolis, IN

Medical and Data Coordinating Center:
Indiana University, Indianapolis, IN

Funding Sponsor:
Purdue University
CTSI University of Cincinnati

ABSTRACT

IMatinib for **PA**in in **C**hronic **T**reatment of **S**ickle **C**ell **A**nemia **(IMPACT SCA)** is a prospective, open-label Phase I/II study of imatinib to prevent pain in individuals with Sickle Cell Anemia (SCA). SCA is the most common severe inherited hematologic disorder worldwide with only one disease-modifying medication (hydroxyurea) licensed so far for its management. Although sickle hemoglobin polymerization is the initial trigger for the sickle cell shapedeformation leading to vaso-occlusive pain episodes, we have shown that increased production of reactive oxygen species (ROS) in sickle red blood cells (RBCs) and inhibition by ROS of both major RBC tyrosine phosphatases, leadsto phosphorylation of the major membrane-spanning protein, band 3. This phosphorylation results in dissociation of theRBC cytoskeleton from the lipid bilayer, release of free hemoglobin and emergence of circulating microparticles that contribute significantly to activation of the vascular endothelium and stimulation of the blood coagulation cascade, promoting the vasoocclusive events. Imatinib, a well-studied tyrosine kinase inhibitor, has been shown in our preclinicalstudies to inhibit band 3 phosphorylation in sickle RBCs and prevent the consequent microparticle release and hemolysisof these cells in vitro. We propose an early phase clinical trial of daily imatinib treatment on patients with SCA and recurrent vaso-occlusive crises to evaluate both the biochemical and clinical effects, with the goal to re-purpose this well-tolerated targeted medication for preventive management in SCA.

STUDY FLOW DIAGRAM

Study flow schema with an outline of patient visits and corresponding evaluations.

TABLE OF CONTENTS

Abbreviations

AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
CHR	complete hematologic response
CML	Chronic Myelogenous Leukemia
CRF	case report/record form
CS&E	Clinical Safety and Epidemiology
GIST	gastrointestinal stromal tumor
Hb	hemoglobin
HR	hematologic response
IEC	Independent Ethics Committee
IFN	Interferon alpha
IRB	Institutional Review Board
IDS	Investigational Drug Services
IP	Investigational Product
ITT	intention-to-treat
IULN	Institutional Upper Limit of Normal
NCI	National Cancer Institute
NIH	National Institutes for Health
od	omnia die / once a day
PDGF	platelet-derived growth factor
Ph +	Philadelphia chromosome positive
po	per os / by mouth / orally
PP	per protocol
PS	performance status
qd	quaque die / every day
RBC	red blood cell(s)
RBCM	red blood cell membrane
SAE	serious adverse event
SCF	stem cell factor
SCA	Sickle cell anemia
SCD	Sickle cell disease
SOP	Standard Operating Procedure
VOC	Vaso-occlusive crisis
WBC	White Blood Cell Count

1.0 INTRODUCTION

Sickle cell anemia (SCA) is a severe and devastating, chronic hematological disorder that affects nearly 100,000 persons in the United States and millions more worldwide.[1-3] Data from 2001-2005 reveals that annual healthcare costs related to sickle cell disease management in the U.S. exceeds $1.1 billion.[4] While supportive care and disease-modifying therapies such as hydroxyurea have mitigated patient suffering and early death from sickle cell-related complications, many patients continue to suffer from anemia, recurrent vaso-occlusive painful episodes which lead to chronic organ damage, acute chest syndrome, and strokes.[3] Although life expectancy for SCA patients in the western world has increased from 14 to approximately 50 years since 1973,[2] the quality of life for survivors remains compromised by damage to their brain, kidneys, eyes, lungs and other organs.[5-8] In some patients, the frequent requirement of opioids for pain episodes leads to opioid dependence, further limiting their quality of life.[8,9] Pain caused by vaso-occlusion is the most frequent cause of hospitalization for patients with SCA and approximately 5.2% of patients with 3-10 painful events per year make up 33% of all hospitalizations.[10] Pain rate also correlates with early death in sickle cell patients older than 20 years of age.[2]

The pathophysiology of vaso-occlusive crisis in sickle cell disease is complex and involves sickling of RBCs caused by polymerization of the deoxygenated Hemoglobin S (HbS), consequent red blood cell (RBC) rigidity, impaired microvascular blood flow, ischemia-reperfusion injury, vascular endothelial cell activation and increased blood cell-endothelium interactions.[11-13] Consequence of frequent vaso-occlussive events over time is end-organ damage.[2,14,15]

Previous research has provided solid evidence that an important contribution to vaso-occlusive events in SCA derives from RBC membrane instability that leads to intravascular hemolysis and release of membrane fragments or microparticles.[16-21] Blebbing-off of plasma membrane fragments not only releases RBC membrane-derived microparticles that are known to be prothrombotic,[19,21,22] but also reduces the cell's surface-to-volume ratio,[23,24] thereby concentrating HbS inside the cell and augmenting its propensity to polymerize. Concurrently, release of free hemoglobin from the fragmenting RBC activates the vascular endothelium to express adhesion receptors,[25] thereby increasing retention of WBC andRBC in the microvasculature[26-29] and further aggravating vaso- occlusive propensities.[30,31]

Our studies have shown that increased reactive oxygen species (ROS) in sickle RBCs inhibit both of their major tyrosine phosphatases,[32-35] leading to phosphorylation of the major membrane-spanning protein, band 3 (Figure 1).[36-38] This results in dissociation of the RBC cytoskeleton from the lipid bilayer causing membrane fragmentation, microparticle release, and the escape of free hemoglobin into the bloodstream.[38,39] This release of free hemoglobin and the circulating microparticles contribute prominently to activation of the vascular endothelium and stimulation of the blood coagulation cascade, respectively. Together, these two processes strongly promote the vaso-occlusive events that plague patients with sickle cell disease.

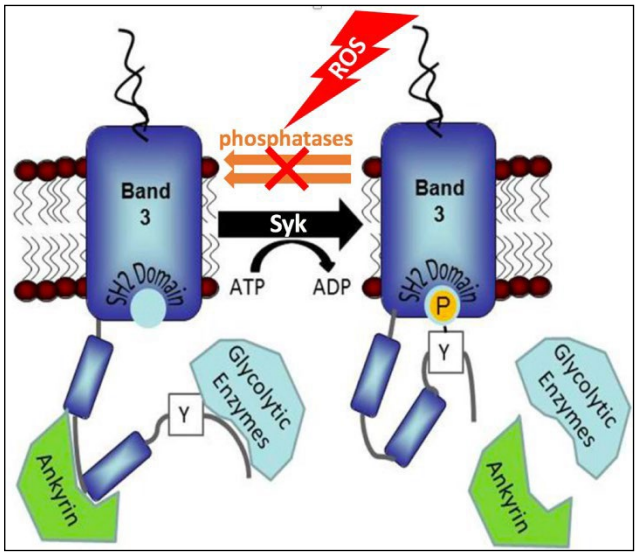


Figure 1. Band 3 in RBC membrane is regulated by tyrosine phosphorylation by Syk kinase and de-phosphorylation by RBC tyrosine phosphatases. ROS, which are increased in SCD, inhibit these phosphatases. The phosphorylation of tyrosines 8, 21, and 359 in the cytoplasmic loop of band 3 leads to displacement of the glycolytic enzyme complex, binding of the band 3 cytoplasmic loop to its SH2 domain and rupture of the band 3-ankyrin bridge to the RBC cytoskeleton.

Imatinib, a well-studied tyrosine kinase inhibitor, has been shown in our preclinical studies to suppress band 3 phosphorylation in sickle RBCs and to prevent the consequent fragmentation, microparticle release, and hemolysis of these cells in vitro. Additionally, in two published case-reports, patients with SCA, who incidentally developed chronic myelogenous leukemia (CML), experienced cessation of vaso-occlusive pain episodes upon initiation of treatment with imatinib for their CML, followed by recurrence of vaso-occlusive crises whenever imatinib therapy was inadvertently interrupted.[40,41] Therefore, **we hypothesize that imatinib, as an inhibitor of tyrosine phosphorylation of band 3, will ameliorate SCA symptoms by preventing RBC membrane destabilization and consequently intravascular hemolysis and microparticle release, which in turn would activate adhesion receptors on the endothelium and trigger the coagulation cascade, respectively.** Since imatinib is approved for use in children and can be taken for years without serious adverse events, we hypothesize that it could constitute a well-tolerated treatment for SCA that will function by a novel mechanism to inhibit vaso-occlussive crisis, pain, and chronic organ damage.

2.0 RATIONALE FOR DEVELOPMENT

Although preventive and supportive management, including prophylactic antibiotics to prevent serious infections from encapsulated organisms, opioids and non-steroidal anti-inflammatory medications to control painful episodes, blood transfusions to dilute out sickle RBCs, and hydroxyurea as a fetal Hgb inducer and disease-modifying therapy have mitigated patient suffering and extended life expectancy of patients with SCA, many patients still continue to suffer from anemia and recurrent vaso-occlusive painful episodes which lead to chronic organ damage and compromise quality of life.[1-3] Bone marrow transplant,[42,43] and in clinical trial settings gene therapy[44-46] are curative treatments, available in US and Europe, but are not feasible for all patients and not free of undesirable toxicities. New experimental therapies in the pipeline include new fetal Hb inducers, nitric oxide generators, statins and drugs that elevate the affinity of hemoglobin for oxygen.[47,48] Unfortunately, each of the well-established therapies (even hydroxyurea as monotherapy) may have limited efficacy or undesirable toxicities, while many of the experimental therapies, though promising in some cases, will likely require years before they can be routinely implemented in sub-Saharan Africa, for example, where 75% of the sickle cell patients reside.

Clearly, the need for more, safe, easily administered, inexpensive and effective SCA therapeutic options is high, in order to improve morbidity and early mortality in the 14 million patients suffering of sickle cell disease across the world. In this protocol**,** we will explore whether imatinib can inhibit *in vivo* a tyrosine kinase-mediated mechanism that induces erythrocyte membrane destabilization, an important biological factor in the clinical manifestations of sickle cell disease. Results from this study will inform a future Phase II-B trial with imatinib to assess clinical outcomes in patients with sickle cell disease.

3.0 BACKGROUND

3.1 Vaso-Occlusion and Sickle Cell Disease

Sickle cell disease is characterized by recurrent attacks of vaso-occlusive painful events that often manifests as severe pain, acute chest syndrome, or priapism. Approximately 5.2% of patients with 3-10 painful events per year make up 33% of all hospitalizations.[10] Pain rate also correlates with early death in sickle cell patients older than 20 years of age.[2]The pathophysiology of vaso-occlusion in sickle cell disease is complex, and involves not only the “sickling” of RBCs due to polymerization of deoxygenated HbS, but also increased cellular adhesiveness of sickle RBCs.[26-28] Previous research has provided solid evidence that an important contribution to vaso-occlusive events in SCD derives from the RBC membrane instability that leads to intravascular hemolysis and release of membrane fragments or microparticles.[16-

[21,49,50] Blebbing off of plasma membrane fragments not only releases RBC membrane-derived microparticles that are known to be prothrombotic,[18,21,22,31] but also reduces the cell's surface-to-volume ratio, thereby concentrating HbS inside the cell and augmenting its propensity to polymerize. Concurrently, release of hemoglobin from the fragmenting RBC increases free heme in the circulation which activates the vascular endothelium to express adhesion receptors,[15,25,30] thereby increasing retention of WBC and RBC in the microvasculature[26,27,29,51] and further aggravating vaso-occlusive propensities.

The organ failure and extreme pain commonly associated with SCD derive to a substantial degree from the above obstruction of blood vessels and associated inflammation.[1] In search for a safe and effective SCD therapy with a novel target mechanism, we have focused on finding a method to stabilize the RBC membrane against the vesiculation and intravascular hemolysis that have been shown to contribute to the development of vaso-occlusion and subsequent clinical manifestations of SCD.[30,31,52]

3.2 Preclinical Studies with Imatinib

Our preclinical studies have implicated phosphorylation of band 3 in the RBC membrane as integral to the development of vaso-occlusive events in patients with SCA (Figure 1). Band 3 is phosphorylated by the tyrosine kinase Syk, which can be inhibited by imatinib.

Some of the initial steps in the chain of molecular events leading to vascular obstruction and pain crisis in patients with sickle cell disease are the following:

1) sickle erythrocytes have elevated levels of Reactive Oxygen Species (ROS) generation due to auto-oxidation of Hb S that denatures into hemichromes (partially denatured Hb complexes preceding Heinz body formation),[53] as well as due to increased production of ROS by erythrocyte NADPH oxidase which is activated by inflammatory cytokines[36]

2) ROS inhibit both of the RBC's major tyrosine phosphatases which have active site cysteines that are highly sensitive to oxidants (Figure 1)[32-35,54]

3) in the absence of functioning tyrosine phosphatases, the major membrane-spanning protein, band 3, becomes phosphorylated on tyrosines 8, 21, and 359 by Syk tyrosine kinase[15,38,39]

4) this phosphorylation of band 3, which is absent in healthy HbA erythrocytes (Figure 2), causes dissociation of the RBC cytoskeleton from the lipid bilayer, leading to membrane weakening and fragmentation, leading to a storm of microparticle release (Figure 3) and free hemoglobin escape into the bloodstream[38,39]

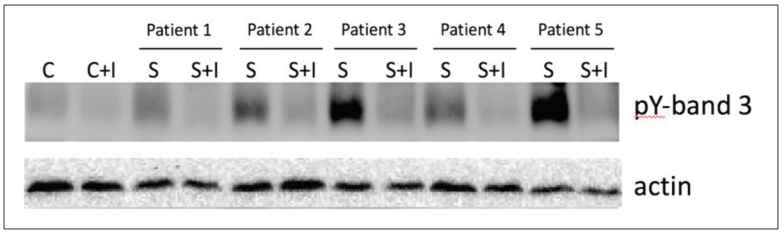


Figure 2. Anti-phosphotyrosine immunoblot of band 3 protein in control and sickle red blood cells before and after treatment in vitro with 1 μM imatinib for 3 hours. C= Control cells; C+I= Control cells + Imatinib; S= sickle cells; S+I= Sickle cells + Imatinib. Data with samples from five different patients with Sickle Cell Disease. *Note the strong tyrosine phosphorylation of band 3 in sickle cells and the paucity of phosphorylation in normal cells at baseline.*

As noted above, this release of free hemoglobin and circulating microparticles contributes prominently to activation of the vascular endothelium and stimulation of the blood coagulation cascade, respectively. Together, these two processes strongly promote the vaso-occlusive events that plague patients with SCD. Based on these considerations, we hypothesized that a tyrosine kinase inhibitor that inhibits Syk and blocks tyrosine phosphorylation of band 3 on tyrosine 8, 21, and 359 will ameliorate the symptoms of SCD by preventing RBC membrane destabilization and the consequent intravascular hemolysis, microparticle release, and decrease in RBC surface-to-volume ratio that enhances RBC sickling and vaso-occlusion.[23,24]

In search for a tyrosine kinase inhibitor that might suppress the tyrosine phosphorylation of band 3 in sickle cells, we screened Eli Lilly's tyrosine kinase library (provided by Dr. Philip Hipskind of Eli Lilly) for suppressors of band 3 tyrosine phosphorylation. Five kinase inhibitors were found to potently inhibit the aforementioned band 3 tyrosine

phosphorylation. Importantly, when the identities of the inhibitors were revealed to us at the end of the study, one inhibitor stood out from the rest because it was already approved by the FDA for use in children. This tyrosine kinase inhibitor was imatinib (Gleevec), an orally bioavailable inhibitor of a group of tyrosine kinases, including Bcr-Abl, PDGFR, and c-Kit, that is employed for treatment of chronic myelogenous leukemia (CML), gastrointestinal stromal tumors, clonal hypereosinophilic syndrome and D816V-negative mastocytosis. Most relevant to the proposed study, however, is the fact that even though imatinib must be taken by CML patients daily for the rest of the patient's life (or until resistant mutations emerge), it has an excellent safety record with limited adverse events.[55,56] Based on these considerations, imatinib was selected as a medication possible to be re-purposed for SCD, with the goal to inhibit band 3 tyrosine phosphorylation, RBC membrane vesiculation, microparticle formation and hemolysis and interrupt the chain of molecular events aggravating vaso-occlusion.

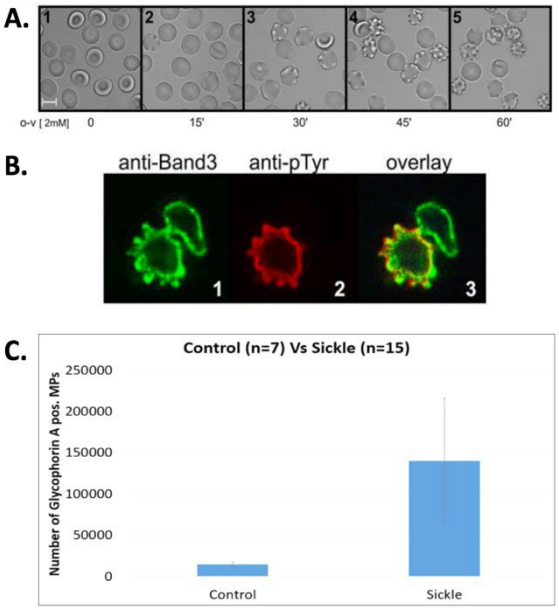


Figure 3. Incubation of RBCs with ortho-vanadate (o-v, phosphatase inhibitor) for increasing time intervals induces membrane vesiculation **(A)** associated with band 3 tyrosine phosphorylation **(B)**. **C.** Glycophorin A (GPA)-positive microparticles, derived from RBC membrane, determined by flow cytometry in the plasma of patients with sickle cell disease versus normal control (HbA) volunteers. GPA is a sialoglycoprotein that spans the RBC membrane and is easily identified by flow cytometry.

3.3 Clinical Experience with Imatinib

Two highly relevant sets of studies in which imatinib has been intentionally or unintentionally used to treat an erythrocyte-related disease should be mentioned here.

3.3.1 Imatinib to treat *P.falciparum* malaria

First, imatinib has been used to treat *P. falciparum* malaria both in human whole blood cultures of the parasitemia *in vitro* and in malaria patients in Vietnam *in vivo*.[57,58] Importantly, imatinib was selected for this application because the malaria parasite induces band 3 tyrosine phosphorylation shortly before its egress from the erythrocyte during its intra-erythrocyte life cycle.[57,58] The parasite does this in order to weaken the RBC membrane to facilitate its escape from its host RBC (i.e. the parasite exploits the same tyrosine kinase-induced RBC membrane destabilization seen in SCA). Both in *in vitro* studies and in the clinical application *in vivo*, imatinib was successful in blocking band 3 tyrosine phosphorylation and parasite egress, thereby terminating the parasitemia. Moreover, no drug related adverse events have been observed to date in malaria patients treated with imatinib.

3.3.2 Imatinib to treat SCA patients with CML

The second relevant set of studies concerns the use of imatinib to treat CML in patients who coincidentally also suffered from SCA. Anticipating that someone with SCA must have at some time contracted CML, we scrutinized the literature for case reports of SCD patients who were treated with imatinib to control their CML. Two such case reports were found. Importantly, upon treatment with imatinib, none of the patients in either of the papers experienced any further symptoms of SCA. [40,41] [40,41] In fact, in one published report, the investigator noted that if the patient missed several daysof imatinib, vaso-occlusive crises recurred, and stopped "immediately when the patient took imatinib again".[41] The authors of one of the articles speculate that imatinib may somehow suppress inflammatory cells, but they offer no data to support this hypothesis. Based on our data, we hypothesize that imatinib treatment of those patients prevented SCA- associated vaso-occlusive painful events via inhibition of Syk-induced RBC membrane destabilization.

3.4 Overview of Proposed Study

In this protocol, we propose to evaluate the biochemical effects of imatinib on sickle RBCs. Patients will be administered imatinib mesylate orally following the guidelines previously established for use of imatinib in other

disorders. The biochemical effects of imatinib on sickle RBCs will be examined, including changes in their levels of band 3 tyrosine phosphorylation and the abundances of RBC-derived microparticles in their blood. In addition, the patients will be monitored for symptoms of SCD. As noted in Fig. 2, we expect band 3 tyrosine phosphorylation to decrease dramatically in patients treated with imatinib. We also anticipate a reduction in the numbers of RBC-derived microparticles in circulation (quantitated by assaying the number of glycophorin A positive microparticles in peripheral blood samples by flow cytometry; see Fig. 1). Most importantly, we expect to see a reduction in the frequency of vaso-occlusive crises, and possibly acute chest syndrome and utilization of opioids. The study duration is planned as 6 months in order to provide adequate time for potential change in the primary endpoints (e.g. percent irreversibly sickled cells).

4.0 STUDY OBJECTIVES

4.1 Primary Aims

4.11 To examine the biochemical effects of imatinib by:
   a. quantitation of band 3 phosphorylation and microparticle release from sickle RBCs
   b. functional RBC analysis

As noted in Figure 2, we expect band 3 tyrosine phosphorylation to decrease dramatically in patients treated with imatinib. We also anticipate a reduction in the numbers of RBC-derived microparticles in circulation (quantitated by assaying the number of glycophorin A positive microparticles in peripheral blood samples by flow cytometry; see Figure 3B). We will evaluate for improvement in the red cell cytoskeleton mechanics by ektacytometry (which should improve due to reduced loss of RBC membrane as a consequence of decreased band 3 phosphorylation), oxygenscan, and determination of percent irreversibly sickled cells.[59,60]

4.2 Secondary Aims

4.21 To assess the effect of imatinib on the frequency of vaso-occlusive pain events in patients with sickle cell disease
4.22 To assess the effect of imatinib on the amount of opioid use, frequency of acute chest syndrome, and number of hospitalizations
4.23 To assess the tolerability and toxicity of imatinib in patients with sickle cell disease

The clinical outcome will be a secondary aim due to the relatively short duration of the study; we expect to see a reduction in the frequency of vaso-occlusive crises, utilization of opioids, and number of hospitalizations and possibly in the incidence of acute chest syndrome and other SCA-associated complications.

4.3. Endpoints

4.31 Primary Endpoints:
   a. Biochemical Effects to RBCs (see Section 17)
      i. Band 3 Phosphorylation
      ii. Microparticle Release
   b. Functional RBC analysis (see Section 17)
      i. Percent Irreversibly Sickled Cells at Month 6
      ii. Ektacytometry
      iii. OxygenScan to evaluate for altered susceptibility to sickling

4.32 Secondary Endpoints:
   a. Clinical Outcomes

i. Vaso-occlusive Crisis (VOC)
    i. Defined as an acute episode of pain lasting greater than 24 hours, with no medically determined cause other than a vaso-occlusive event that resulted in treatment with oral or parenteral opiate agents and/or parenteral nonsteroidal anti-inflammatory agents.
    ii. To be documented in pain diary by patient/guardian and reviewed at each visit
    iii. Measured by pain scale (1-10) or Wong-Baker Faces scale based on age:
ii. Acute Chest Syndrome
    i. Defined as respiratory distress (hypoxia, shortness of breath, chest pain, tachypnea) with evidence of an infiltrate on chest x-ray
    ii. Will be reviewed at each visit, as well as extracted from the EMR by each site
iii. Opioid Use
    i. Defined as both oral and parenteral opioid use
    ii. Oral use will be documented in pain diary by patient/guardian and reviewed at each visit.
    iii. Frequency of refills and parenteral opioid use will be extracted from the EMR by each site
iv. Hospitalizations
    i. Defined as an emergency room or clinic visit resulting in an inpatient admission or observation for a sickle cell-related event (e.g. vaso-occlusive pain, acute chest syndrome, etc).
    ii. Will be reviewed at each visit, as well as extracted from the EMR by each site
v. Assessment of tolerability and evaluation for any toxicities of imatinib in patients withSCA

5.0 INVESTIGATIONAL PLAN

5.1 Study Design

This will be an open label nonrandomized pilot study to determine the effect of imatinib on band 3 phosphorylation, microparticle release from sickle RBCs, and percent irreversibly sickled cells obtained from adolescent and young adult patients with Hemoglobin SS Disease or S-Beta 0 Thalassemia before, during, and after 6 months of treatment with imatinib. We will also look at clinical outcomes during treatment (pain) and before treatment. Clinical outcomes before treatment will be collected via retrospective chart review.

5.2 Study Population

5.2.1 Inclusion criteria

1. Age: patients must be ≥18 years of age and ≤30 years of age at the time of study entry.
2. Diagnosis: Patients must have documented diagnosis of Hemoglobin SS Disease or S-Beta 0 Thalassemia by either HPLC or Hemoglobin Electrophoresis
3. Disease status: Patients must have at least 2 documented episodes of vaso-occlusive pain in the prior year as defined by an acute episode of pain lasting greater than 24 hours, with no medically determined cause other than a vaso-occlusive event that resulted in treatment with oral or parenteral opiates or with a parenteral nonsteroidal anti-inflammatory drug.
4. Performance Level: Karnofsky ≥80 for patients >10 years of age and Lansky ≥80 for patients ≤10 years of age. Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.
5. Organ function requirements:

a. Adequate bone marrow function defined as
    i. Peripheral absolute neutrophil count (ANC) ≥1000/μL
    ii. Platelet count ≥100,000/ μL (transfusion independent)
b. Adequate renal function defined as
    i. Creatinine clearance or radioisotope GFR ≥70 mL/min/1.73 m^2 or
    ii. A serum creatinine based on age/gender as follows

Table 1: Maximum serum creatinine allowed for eligibility

Age	Maximum Serum Creatinine (mg/dL)	
	Male	**Female**
2 to <6 years	0.8	0.8
6 to <10 years	1	1
10 to <13 years	1.2	1.2
13 to <16 years	1.5	1.4
≥16 years	1.7	1.4

The threshold creatinine values were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

c. Adequate Liver Function Defined As:

    i. SGPT (ALT <2.5 upper limit of normal. For the purpose of this study, the ULN for SGPT is 45 U/L
    ii. Serum albumin ≥2 g/dL
d. Adequate cardiac function defined as:
    i. Shortening fraction or ejection fraction greater than the LLN (institutional norm), and
    ii. Corrected QT interval ≤450 msec

6. Informed Consent: All patients must sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines.

5.2.2 Exclusion criteria

1. Chronic transfusion protocol.
    a. Patients currently on a chronic transfusion protocol are not eligible
2. Hydroxyurea Intolerance
    a. Patients who are ineligible for hydroxyurea due to persistent marrow suppression (e.g. thrombocytopenia, neutropenia)
3. Pregnancy or Breast-Feeding
    a. Pregnancy tests must be obtained in girls who are post-menarchal. Males or females of reproductive potential may not participate unless they have agreed to use an effective contraceptive method.
4. Concomitant Medications
    a. Investigational Drugs: Patients who are currently receiving another investigational drug.
    b. Anti-cancer agents: Patients who are currently receiving other anti-cancer agents.
    c. The following CYP3A4 inducers are prohibited 14 days before the start of imatinib and during the study with imatinib: rifampin, rifabutin, carbamazepine, Phenobarbital, phenytoin, St. John's wort, efavirenz, and tipranavir.

d. The following CYP3A4 inhibitors are prohibited 7 days before the start of imatinib and during the study with imatinib: azole antifungals (itraconazole, ketoconazole); clarithromycin, erythromycin, diltiazem, verapamil, HIV protease inhibitors (indinavir, saquinavir, ritonavir, atazanavir, nelfainavir); delavirdine.

5. Patients who have an uncontrolled infection.
6. Prior use of Imatinib: Patients who have previously received imatinib are not eligible for study.
7. Patients who in the opinion of the investigator may not be able to comply with the safety monitoring requirements of the study.
8. Patient is < 5 years free of a malignancy. Existence of any other malignant disease is not allowed.
9. Patient with Grade III/IV cardiac problems as defined by the New York Heart Association Criteria. (i.e., congestive heart failure, myocardial infarction within 6 months of study).
10. Patients with a history of QT prolongation, need for concomitant use of anti-arrhythmics or other agents known to prolong QT interval, or electrolyte derangement that cannot be corrected to within normal limits prior to initiation of study drug.
11. Patients with a family history of sudden cardiac death.
12. Patient has a severe and/or uncontrolled medical disease other than sickle cell disease (i.e., uncontrolled diabetes, chronic renal disease, or active uncontrolled infection).
13. Patient has known chronic liver disease (i.e., chronic active hepatitis, and cirrhosis).
14. Patient has a known diagnosis of human immunodeficiency virus (HIV) infection.
15. Patient had a major surgery within 2 weeks prior to study entry.

5.3. Sample Size

A total of 40 participants will be enrolled. Thirty subjects will be recruited at Riley Hospital for Children with 10 of the subjects having sickle cell anemia and up to 20 normal donor subjects enrolled for specimen shipping purposes. Ten subjects with sickle cell anemia will be recruited at Cincinnati Children's Hospital. This sample size has been determined by the primary endpoints (See Section 15 for details).

5.4 Recruitment Strategy

Subjects will be recruited through the Pediatric Hematology/Oncology clinic at Indiana University School of Medicine, Pediatric Hematology/Oncology clinic at Cincinnati Children's Hospital and Cincinnati CTSI. Study subjects will be compensated for their participation with a $50 gift card provided at the end of each monthly visit.

6.0 TREATMENT PROGRAM

All patients with sickle cell anemia will be treated in the same group. There will not be a randomization.

Patients will receive Imatinib orally once daily for 6 months.

The starting dose for subjects will be 340 mg/m2/day with a maximum dose of 600 mg daily. Drug doses will be rounded to nearest 50 mg according to the dosing table in Appendix A. Dosing should be based on the body surface area (BSA) obtained at each clinic appointment. Drug will be dispensed as a 30-day supply at each visit. Patients will be asked to bring the prescription bottle with them to each visit to assist with monitoring compliance.

Clinic visits will occur at initiation of imatinib and then every 30 days +/- 7 days. Monthly visits will consist of interim history, assessing for adverse side effects and compliance. Participants will be asked to have blood drawn (see Section 11) 2 weeks +/- 3 days after initiation of the drug. Additionally, during the first two months of drug administration, patients will be called or texted a survey link weekly for follow-up safety assessments, except for the participant's in-clinic visits at week 2, 4 and 8. The survey link will come via an app, called Twilio, that allows REDCap the capability to send SMS text messages to survey respondents. If the patient is unable to be reached by phone for at least 3 of the 4 weekly assessments each month, they will be asked to be seen in clinic for a safety assessment. If the patient is unable to do so, they will be removed from protocol therapy for protocol non-compliance. The 1 month, 3 month, 6 month, and 1-month off-therapy visits will consist of a comprehensive history and physical

exam, as well as laboratory and diagnostic studies documented in Section 10. The other visits (2, 4 and 5-month visits post treatment initiation) will be research-nurse visits, with a provider available to sign the next month's prescription.

Patients will be given a copy of the "Patient Pain Journal" at each visit which will need to completed and returned at the next visit. This journal should be reviewed with the treating physician and/or research nurse at each clinic appointment while on study. Patients should be instructed to use the journal to record any occurrence of pain episodes (see definition in Section 5.3), the duration, and necessary interventions. They will be asked to score their pain on a scale of 1-10. Additionally, it will be used to record the date and time of each imatinib dose as well as observed side effects and supportive treatments used while on study.

Opioid use, hospitalizations, and occurrence of acute chest syndrome (see definitions in Section 5.3) will be reviewed at each study visit, as well. This information will also be extracted from the EMR at each site to confirm patient/guardian report.

6.1 Dosage:

Mylan will supply Imatinib as 100 mg tablets packaged in bottles for an exposure period of up to 6 months or so long as the patient remains on study. The starting dose for subjects will be 340 mg/m2 with a maximum dose of 600 mg daily. Tablets can be broken in half, and therefore, drug doses will be rounded to nearest 50 mg according to the dosing table in the Appendix A. Dosing should be based on the body surface area (BSA) obtained at each clinic appointment.

The prescribed dose should be administered at the same time each morning, orally, with a meal and a large glass of water. Patients should keep normal eating habits, however a low-fat (i.e. continental) breakfast is recommended and avoiding xanthine (e.g. caffeine) or grapefruit containing food or beverages. If vomiting occurs > 15 minutes after administration of the dose, no additional trial medication should be taken that day in an effort to replace the material that has been vomited.

In the absence of dose limiting toxicity (DLT), patients may receive a total of 6 months of therapy. Continuing treatment beyond this period will be at the discretion of the investigator and may become the financial responsibility of the patient if no alternative method of obtaining or paying for the drug can be identified at that time. Medication labels will comply with the legal requirements of the US and will be printed in English. The storage conditions for Imatinib will be described on the medication label. Bottles must be stored in a safe, secure location.

See Section 8 and 9 for dose-limiting toxicities and dose reductions/modifications. If the toxicity resolves to meet study parameters within 14 days of drug discontinuation, the patient may resume treatment with the dose reduced by from 340 mg/m2 to 260 mg/m2. Treatment should resume 14 days from drug discontinuation even if toxicity has resolved before 14 days in order to allow for a full 2-week rest period before resuming drug. Subjects who fail to recover to a </= grade 2 or baseline within 14 days of AE onset will permanently discontinue study drug. This does not include the following AEs: nausea and fatigue (does not require dose reduction or discontinuation). Upon restarting drug, the dose will be reduced to 260 mg/m2 (see table in Appendix A) and not re-escalated in subsequent cycles. A maximum of 2 dose reductions (340 mg/m2 to 260 mg/m2 to 200 mg/m2) will be allowed before permanent discontinuation of study drug.

6.2. Drug Accountability:

IU Health Investigational Drug Services (IDS) is a division of the Pharmacy Department whose purpose is to assist investigators in procurement, dispensing, and accountability for investigational drugs. All patients treated on this study will have investigational drug coordinated through the IDS.

The Principal Investigator will be ultimately responsible for the investigational product (IP) and the conduct of the study. The PI will delegate functions to IDS that are appropriate.

6.2.1 IDS Role and Delegated Functions

Protocols: IDS will maintain a copy of the protocol and investigator's brochure. All protocols that involve IDS must have Institutional Review Board (IRB) approval. IDS should be notified when changes have been submitted to the IRB and have access to the appropriate computer website/program to find the protocol amendments or update.

IP Inventory: IDS will provide for adequate inventory including monthly checks, arrange for IP shipments, confirm all shipments as received in good condition and maintain records of all shipments.

IP Shipments: IDS will document all IP receipt, dispensing, destruction in accordance with IDS policy and sponsor requirements. To guaranteed accurately and timely delivery and immediate shipment confirmation, IP should be shipped directly to IDS:
Investigational Drug Services
IUSCC Room C2102
550 North University Boulevard
Indianapolis, IN 46202

Storage: IDS will ensure proper storage of all IP in accordance with manufacturer or sponsor recommendations. Storage temperatures will be monitored according to IDS and IU Health monitoring systems.

Disposal: Disposal of IP will be in accordance with IDS and IUPUI procedures for Hazardous Waste Disposal. All disposal will be recorded on drug accountability records.

IP Accountability: IDS will maintain complete records on of all IP received, dispensed, disposed. Accountability Records will follow standard IDS or NCI Accountability templates unless sponsor required forms are mandated.

IP Preparation, Labeling and Administration: IDS staff will write internal standard operating procedures which include:

- Detailed preparation instructions including IV administration and expiration dating
- Proper labeling of prepared IP to comply with Pharmacy Department, Hospital, State or Federal Regulation.
- IDS will design and or approve any prescription or physician orders to be used for the IP to assure all appropriate Pharmacy and Hospital regulations are met.
-

Study Conclusion and Record Retention: At the conclusion of the study, IDS will ensure final disposition of the IP and provide all accountability records, shipping and receipt records, prescriptions and essential study correspondence to the principal investigator for final storage with all study related records.

For more complete details on IDS procedures for various functions please consult the IDS Manual of Operations.

7.0 GRADING OF ADVERSE EVENTS

An adverse event (AE) is defined as any unintended or abnormal clinical observation that is not of benefit to the patient. Either the condition was not present prior to exposure to test medication, or it has worsened in intensity or frequency following exposure to test medication. Adverse events will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria v4.03 (CTCAE):
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Any suspected or confirmed dose-limiting toxicity should be reported immediately (within 24 hours) to the principal investigator.

Principal investigators (PI) must report to the IRB as soon as possible, but in all cases within 5 working days from notification of any event that appears on the **List of Events that Require Prompt Reporting to the IU IRB.**

7.1 List of Events that Require Prompt Reporting to the IUPUI IRB. Any of the following:

1.1.1 Event (including adverse events, injuries, side effects during the research study), which in the opinion of the PI

1.1.1.1 caused harm to one or more subjects or others, or placed one or more subjects or others at increased risk of harm; AND
1.1.1.2 was unexpected; AND
1.1.1.3 was related to the research procedures

Note: After the study is closed with the IRB, these events should only be reported if they are profound or they demonstrate long-term risks that would necessitate notifying subjects.

1.1.2 Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject (e.g. purposeful and for subject safety) (on-site only)

1.1.3 Complaint of a subject that indicates unexpected risks, or complaint that cannot be resolved by the research team (on-site only)

1.1.4 Interim findings and safety monitoring reports that indicate an unexpected change to the risks or potential benefits of the research, in terms of severity or frequency

1.1.5 Publication in the literature that indicates an unexpected change to the risks or potential benefits of the research

1.1.6 Change in FDA labeling or withdrawal from marketing of a drug, device, or biologic used in a research study

1.1.7 Noncompliance (as defined in this policy and on-site only)

Serious Adverse Events (SAE) and unanticipated problems involving risk to subjects or others (UPIRSOs)
An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:
• death
• a life-threatening adverse experience
• inpatient hospitalization or prolongation of existing hospitalization++
• a persistent or significant disability/incapacity
• a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

++ Note that hospitalizations for the following reasons should not be reported as serious adverse events:
• Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
• Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
• Social reasons and respite care in the absence of any deterioration in the patient's general condition

• Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event

Any serious or immediately life-threatening adverse experience, including those resulting in death, occurring while the subject is receiving study drug, or within 30 days of the subject's last dose of protocol therapy, regardless of the treating physician's opinion regarding drug relationship, will be reported by telephone and/or e-mail (within 24 hours of the event) to the Study PI and appropriate parties.

Serious adverse events are to be reported via Oncore on the SAE Form within 5 business days of event notification.

Study principal investigators are be notified of serious adverse events via email within 5 business days of eventnotification.

Instructions for rapid notification of unexpected serious adverse events reporting procedures

When an unexpected serious adverse event occurs, the investigator (study chair) must complete the FDA MedWatch 3500a form in English and assess the relationship to study treatment.

Follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or discontinued study participation. The MedWatch form and fax confirmation sheet must be retained. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, and the presence or absence of any congenital abnormalities or birth defects.

8.0 DEFINITION OF DOSE-LIMITING TOXICITY (DLT)

DLT will be defined as any of the following events that are possibly, probably, or definitely attributable to imatinib.

1. Non-hematologic dose-limiting toxicity
    - Any Grade 4 non-hematologic toxicity
    - Any Grade 3 non-hematologic toxicity with the specific exception of:
        - Grade 3 ALT that return to levels that meet initial eligibility criteria within 7 days of study drug interruption and that do not recur upon rechallenge with study drug. Note: For the purposes of this trial the ULN for ALT is defined as 45 U/L.
        - Grade 3 AST
        - Grade 3 fever or infection <5 days duration
        - Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation within 14 days of initiating supplementation
        - Asymptomatic Grade 3 elevations in amylase or lipase resolving to <Grade 1 within 7 days of study drug interruption and that do not recur upon re-challenge with study drug
    - Grade 2 or greater left ventricular systolic dysfunction OR an absolute decrease in shortening fraction of 8 percentage points from baseline.
    - Grade 2 allergic reactions that necessitate discontinuation of study drug will not be considered a dose limiting toxicity.
    - Any Grade 2 non-hematological toxicity that persists for ≥7 days and is considered sufficiently medically significant or sufficiently intolerable by patients that it requires treatment interruption.
    - Any adverse event requiring interruption of study drug for >7 days or which recurs upon drug re-challenge.

2. Hematologic dose limiting toxicity

- Grade 3 thrombocytopenia with Grade 3 or 4 hemorrhage
- Grade 4 thrombocytopenia (platelet count <25,000/mm^3) or
- Grade 4 neutropenia.

9.0 DOSE MODIFICATIONS FOR ADVERSE EVENTS

The study PIs must be notified of any dosage modification.

1. **Dose modifications for hematological toxicity** (Of note, such adverse events have been observed in patients with CML (chronic myelogenous leukemia), likely contributed by concurrent morbidities).
   - **a.** If a patient experiences Grade 3 neutropenia (ANC<1000) or Grade 3 thrombocytopenia (platelets <50,000/mm^3) with or without Grade 1 or 2 non-CNS hemorrhage, the treatment will be withheld. Counts should be checked twice weekly during this time. If the toxicity resolves to meet on study parameters within 14 days of drug discontinuation, the patient may resume treatment with the dose reduced as mentioned in Section 6.1. Treatment should resume 14 days from drug discontinuation even if toxicity has resolved before 14 days in order to allow for a full 2-week rest period before resuming drug.
   - **b.** If a patient experiences Grade 1 CNS hemorrhage or Grade 3 or > non-CNS hemorrhage, the treatment will be withheld. Treatment will not be resumed and the participant will discontinue study drug permanently.
   - **c.** If toxicity does not resolve to meet on study parameters within 14 days of drug discontinuation, the patient must be removed from protocol therapy.
   - **d.** If dose-limiting toxicity recurs in a patient who has resumed treatment at the reduced dose (maximum of 2 dose reductions allowed), the patient must be removed from protocol therapy.
   - **e.** Myeloid growth factors will not be used on study.

2. **Dose modifications for non-hematological toxicity.**
   - a. If a patient experiences non-hematologic DLT as defined in Section 8, the treatment will be withheld. If the toxicity resolves to meet on study parameters within 14 days of drug discontinuation, the patient may resume treatment with the dose reduced as mentioned in Section 6.1 (see Appendix A). Treatment should resume 14 days from drug discontinuation even if toxicity has resolved before 14 days. Doses reduced for toxicity will not be re-escalated, even if there is minimal or no toxicity with the reduced dose.
   - b. If toxicity does not resolve to meet on study parameters within 14 days of drug discontinuation, the patient must be removed from study.
   - c. If dose-limiting toxicity recurs in a patient who has resumed treatment at the reduced dose level, the patient must be removed from protocol therapy.
   - d. Dose modifications for left ventricular systolic dysfunction: Left ventricular systolic dysfunction will be graded according to CTCAE criteria but will also take into account absolute changes in shortening fraction.

     Grade 1 left ventricular systolic dysfunction: Continue imatinib at current dose level and repeat echocardiogram in 30 days.

     Grade 2 left ventricular systolic dysfunction OR >8 absolute percentage point decrease in shortening fraction: hold study drug and repeat echocardiogram in 7 days. If repeat echocardiogram confirms toxicity, patient will be taken off-protocol therapy and should be referred to a cardiologist. If repeat echocardiogram does not confirm toxicity, patient may restart therapy with repeat echocardiogram 14 days after restart. If any two echocardiograms demonstrate Grade 2 dysfunction OR >8 absolute

percentage point decrease in shortening fraction, patients will be taken off-protocol therapy and should be referred to a cardiologist.

≥Grade 3 left ventricular systolic dysfunction: Off-protocol therapy. Referral to cardiologist.

10.0 SUPPORITIVE CARE AND OTHER CONCOMITANT THERAPY

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed, provided their use is documented in the patient records and on the appropriate case report form. Concomitant medication use will be reviewed at each visit to assess for potential interaction with Imatinib. If a medication that has the potential to interact with imatinib is identified at initial screening, the investigator will determine whether it is in the best interest of the subject to continue with participation in the trial. If the potential interaction poses a risk that is deemed unacceptable, the subject will either stop the concomitant medication and wait an appropriate amount of time to allow clearance of this medication prior to taking Imatinib or, if the concomitant medication is unable to be discontinued, then the subject will be excluded from participation in the trial. In the event a subject is prescribed a medication with the potential for interaction with Imatinib during the course of participation in the trial and the potential risk is deemed too high then the subject will be instructed not to start this medication or will have to be withdrawn from study. All subjects will be instructed to discuss any new medications with the investigator or study personnel prior to taking in order to reduce the possibility of serious drug interactions.

10.1 Investigational Agents: no other investigational agents may be given while the patient is on study.

10.2 Concomitant Medications

Drugs that may increase imatinib plasma concentrations

Caution is recommended when administering imatinib with inhibitors of the cytochrome P450 isoenzyme CYP3A4 family (e.g., ketoconazole, itraconazole, erythromycin, and clarithromycin). Substances that inhibit CYP3A4 activity may decrease metabolism and increase imatinib concentrations. There was a significant increase in exposure to imatinib when the compound was co-administered with ketoconazole, a CYP3A4 inhibitor.

Drugs that may decrease imatinib plasma concentrations

Substances that are inducers of CYP3A4 activity may increase metabolism and decrease imatinib plasma concentrations. Co-medications that induce CYP3A4 (e.g., dexamethasone, phenytoin, carbamazepine, rifampicin, phenobarbital or St. John's Wort) may reduce exposure to imatinib. There was a significant decrease in exposure to imatinib when imatinib was co-administered with rifampicin, an inducer of CYP3A4.

Drugs that may have their plasma concentration altered by imatinib

Imatinib increases the mean C_{max} and AUC of simvastatin (a CYP3A4 substrate) 2- and 3.5-fold, respectively, indicating inhibition of CYP3A4 by imatinib. Particular caution is recommended when administering imatinib with CYP3A4/5 substrates with a narrow therapeutic window (e.g., cyclosporine or pimozide). Imatinib will increase plasma concentrations of other CYP3A4 metabolized drugs (e.g., triazolo-benzodiazepines, dihydropyridine calcium channel blockers, certain HMG-CoA reductase inhibitors, etc.).

In vitro, imatinib inhibits the activity of cytochrome P450 isoenzyme CYP2D6 at concentrations similar to those that affect CYP3A4/5 activity. Systemic exposure to substrates of CYP2D6 is expected to be increased when co-administered with imatinib. No specific studies have been performed and caution is recommended.

Corticosteroids may induce CYP3A4 and are not routinely recommended on-study unless deemed absolutely necessary. Discussion with the principal investigator is not required prior to initiating corticosteroids.

A list of drugs metabolized by CYP3A4/5 and CYP2D6 is provided in Appendix B.

Concomitant treatment with dysrhythmic drugs, i.e., terfenadine, quinidine, procainamide, sotalol, probucol, bepridil, haloperidol, risperdone, and indapamide, is not recommended.

10.3 Concurrent Anticancer Therapy: Concurrent cancer therapy, including chemotherapy, radiation therapy immunotherapy, or biologic therapy may NOT be administered to patients receiving study drug. If these treatments are administered the patient will be removed from protocol therapy.

11.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

Subjects will be prescreened for potential eligibility prior to initial clinic visit. All entry/eligibility laboratory and imaging studies will be performed at or within 2 weeks of the initial visit. Baseline EKG to rule-out prolonged QTc, and if needed, echocardiogram, is required within 2 weeks of starting protocol therapy. If eligibility studies are drawn but the participant was not able to be enrolled/initiate study drug (e.g. hospitalized) in this timeframe, all eligibility laboratory studies will need to be repeated. EKG and ECHO do not need repeated unless there are new clinical concerns. Each study visit will occur approximately 4 weeks (+/- 1 week) after the previous.

STUDIES TO BE OBTAINED	Baseline	2 Weeks	1 Month	2 Months	3 Months	4 Months	5 Months	6 Months	1 month Off Study
Phone Assessment (Weekly)			X	X					
History	X		X	X	X	X	X	X	X
Physical exam with vital signs	X		X		X			X	X
Height, weight, BSA	X		X	X	X	X	X	X	X
Performance Status	X		X	X		X	X		
CBC/platelet with differential, Reticulocyte Count	X	X	X	X	X	X	X	X	X
HPLC or Hemoglobin EP	X								
Ektacytometry, OxygenScan, F% correlation, Blood Viscosity#	X		X		X			X	X
% ISCs (irreversibly sickled cells)	X		X		X			X	X
D-dimer	X		X		X			X	X
Urine Pregnancy test (female)	X		X		X			X	
CMP^, Mag, Phos, Uric Acid, Amylase, Lipase and tryptase	X		X		X			X	X
Biochemical Analysis Sample	X		X		X			X	X
EKG*	X								X
Echocardiogram*	X								
Patient Pain Journal			X	X	X	X	X	X	X
Concomitant Medication Assessment	X	X	X	X	X	X	X	X	X

Table 2. Studies to confirm eligibility and monitor safety and efficacy on imatinib

*During study treatment, monitor for signs and symptoms of arrhythmia and for edema and fluid retention that may cause congestive heart failure. Obtain EKG/echocardiogram and cardiology consult as clinically indicated.

#Blood samples for these studies to be sent to Cincinnati

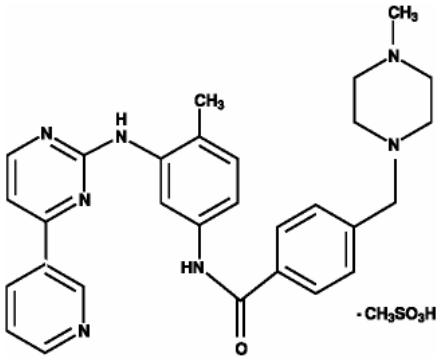
^ CMP should include Albumin, Alkaline phosphatase, ALT (SGPT), AST (SGOT), BUN, Calcium, Chloride, Carbon Dioxide, Creatinine, Glucose, Potassium, Sodium, Total Bilirubin, Total Protein, and a calculation of eGFR.

12.0 AGENT INFORMATION

Imatinib Mesylate

1. **Chemistry**

   Imatinib mesylate, a phenylaminopyrimidine derivative, is designated chemically as 4-[(4-12 Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-13 phenyl]benzamide methanesulfonate and its structural formula is:



   It was originally found as an inhibitor of the tyrosine kinase activity of the *BCR-ABL1* fusion gene (oncoprotein), the product of the Philadelphia chromosome. Imatinib mesylate has also shown activity in blocking the tyrosine kinase activity of c-kit, the stem-cell factor receptor, and of PDGFR, the platelet-derived growth factor receptor and cellular events mediated by PDGF and SCF. Our studies have demonstrated that it also has significant blocking activity of Syk, the tyrosine kinase that phosphorylates band 3 in erythrocytes.

2. **Mechanism of Action**

   Imatinib (STI571), a phenylaminopyrimidine derivative, is a selective inhibitor of the tyrosine kinase activity of the *BCR-ABL1* fusion gene (oncoprotein), the product of the Philadelphia chromosome. Imatinib mesylate has also shown high activity in blocking the tyrosine kinase activity of stem-cell factor receptor (SCF), c-kit and platelet-derived growth factor PDGF and cellular events mediated by PDGF and SCF. The ability of imatinib to inhibit *BCR-ABL1* tyrosine kinase activity is related to its occupancy of the kinase pocket of the protein, which blocks access to ATP and prevents substrate phosphorylation inhibiting BCR- ABL dependent cellular proliferation. Imatinib has caused apoptosis or arrest of growth in hematopoietic cells expressing *BCR-ABL1*.

   Imatinib is well-absorbed after oral administration; maximum plasma concentrations are achieved 2 to 4 hours after administration. The elimination half-lives of imatinib and its major active metabolite, the N-desmethyl derivative, are approximately 18 and 40 hours, respectively. The elimination half-life of the parent drug in children is approximately 15 hours. Imatinib is approximately 95% protein-bound, mostly to albumin and alpha-1- acid glycoprotein. CYP3A4 is the major enzyme responsible for metabolism. CYP1A2, CYP2D6, CYP2C9, and CYP2C19 play minor roles in metabolism. Drugs metabolized by these same enzymes should be avoided or used with caution to avoid unwanted drug interactions. Severe hepatic impairment (bilirubin >3-10 times ULN) increases AUC by 45% to 55% for imatinib and its active metabolite, respectively. Elimination is predominately in the feces, mostly as metabolites.

3. **Supplier**

   Mylan Pharmaceuticals

13.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY

1. Adverse Events requiring removal from study
2. Refusal of further protocol therapy by patient/parent/guardian.
3. Non-compliance that in the opinion of the investigator does not allow for ongoing participation, including <70% of drug prescribed being taken. Compliance will not be contingent on completion of the pain journal as this not the primary endpoint of the study.
4. Physician determines continued protocol therapy is not in the patient's best interest.

Patients who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Ongoing adverse events, or adverse events that emerge after the patient is removed from protocol therapy, but within 30 days of the last dose of investigational agent, must be followed and reported via RDE and AERS (if applicable). Follow-up data will be required unless consent is withdrawn.

14.0 OFF STUDY CRITERIA

1. Thirty days after the last dose of the investigational agent.
2. Death
3. Lost to follow-up
4. Withdrawal of consent for any further data submission.
5. Enrollment onto another therapeutic study

15.0 STATISTICAL CONSIDERATIONS

15.1 Sample Size Justification

This sample size has been determined by the primary endpoint of microparticle released at 6 months with an adjustment to the alpha level to account for the fact there are five primary endpoints. From Figure 1, the mean (standard deviation) of number of microparticles released is 140,000 (75,000) and it is anticipated that treatment with imatinib will decrease that amount at least 50% to 70,000. A two-sided paired t-test with alpha level 0.05/5 = 0.01 will have 89% power to detect this difference when the sample size is 20 subjects (assuming the pre/post treatment correlation is 0.5). We estimate a sample size of 20 patients total (10 patients from each site) would be feasible to recruit and would meet these statistical criteria to provide adequate data to assess effect.

15.2 Safety evaluation

The assessment of safety will be based mainly on the frequency of adverse events, particularly adverse events leading to discontinuation of treatment and on the number of significant laboratory abnormalities.

Adverse events will be summarized by presenting the number and percentage (as appropriate) of patients having any adverse event by body system, type of adverse event, and maximum severity according to CTCAE grade. Those adverse events which result in death, discontinuation or are otherwise classified as dose limiting will be presented separately.

Laboratory data will be summarized using the NCI CTCAE version 4.03 criteria.

15.3 Safety Monitoring

DLTs (see Section 8 for definition) will be continuously monitored. If the stopping rule is met, enrollment will be halted, and a data safety meeting will be convened to determine whether or not the study should be continued. Sequential boundaries will be used to monitor dose-limiting toxicity rate. The accrual will be halted if excessive numbers of dose-limiting toxicities are seen, that is, if the number of dose-limiting toxicities is equal to or exceeds b_n out of n patients with full follow-up (see table below). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most [probability of early stopping] when the rate of dose-limiting toxicity is equal to the acceptable rate [event probability θ].

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	-	3	4	4	4	5	5	5	6	6	6	7	7	7	8	8	8	9	9

This boundary is equivalent to testing the null hypothesis, after each patient, that the DLT rate is equal to 0.2, using a one-sided level 0.021430 test[61].

15.4 Efficacy evaluation

Descriptive statistics will be calculated for all endpoints before, during, and after treatment. Changes before and 6 months after treatment in biochemical endpoints will be tested with two-sided paired t-tests. This analysis will be supplemented with single group repeated measures ANOVA to test for effects over time since there are 3 measures on treatment. Many secondary endpoints are counts (e.g. frequency of refills of opioids, number of hospitalizations). Depending on the distributions of these outcomes, we will either: 1) dichotomize and compare with McNemar's test; 2) treat as ordinal and compare with a Cochran-Mantel-Hazensel type test; or 3) treat as continuous and conduct either paired t-test or Wilcoxon signed rank tests. Since the pain measure will depend on age, we will only statistically analyze when there are at least 6 participants using a single-group repeated measures ANOVA model. Otherwise, only graphical descriptions will be used.

16.0 INCLUSION OF CHILDREN, WOMEN, AND MINORITIES

The study is open to all participants regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population. If differences in outcome that correlate to gender, racial, or ethnic identity that are outside of the expected distribution for this patient population are noted, accrual may be expanded or additional studies may be performed to investigate those difference more fully.

17.0 CORRELATIVE STUDIES (Please see Lab Manual)

18.0 DATA MANAGEMENT AND DATA SAFETY MONITORING

18.1 Monitoring and Reporting Guidelines

Investigators will conduct continuous review of data and patient safety. As accrual warrants, review meetings will take place monthly and will include principal investigators Seethal Jacob, MD, MS; Charles Quinn, MD, Theodosia Kalfa, MD, PhD, study coordinators and clinical research nurses. Monthly meetings will include review of data, the number of patients, significant toxicities as described in the protocol, and responses observed.

18.21. Early Study Closure

At any time during the conduct of the trial, if it is the opinion of the investigators that the risks (or benefits) to the subject warrant early closure of the study, the Compliance Officer must be notified within 1 business day via email, and the IRB must be notified within 5 business days. Alternatively, the investigators may initiate suspension or early closure of the study based on their monthly reviews.

18.3 Oversight of Study Progress

Data collection and data management for this study will be conducted with the support of the Center for Cancer and Blood Disorders Clinical Research Office and the Data Management Core at Indiana University School of Medicine. This team will be responsible for assisting the PI in assuring protocol compliance as well as reviewing, transcribing and tracking of all clinical and safety related data.

18.31. Continuing Review
All Continuing Reviews will be reviewed annually or as dictated by the Institutional Review Board.

18.4 Study Data Management
Primary data will be collected from treatment or test results included in the subject's medical record or source documentation given by the subject (i.e, patient diary). Patients will be registered in OnCore. Data will be entered on the Case Report Forms (CRFs) developed for this study stored electronically in REDCap. This database will be backed up automatically every week. Quality assurance steps will include: 1) built-in range checks; 2) testing of the database by the study team prior to moving to production mode; and 3) review and verification of all by the principal investigator and the research manager. Specific data questions will be given to the appropriate personnel who will then investigate and resolve the questions with the help of the investigator as necessary. Additionally, the following quality control methods will be used: 1) single entry of data with random checks of accuracy; and 2) extraction and cleaning of data that will be used for analysis every 6 months.

Data will be recorded on the CRFs and will be kept confidential with only the principal investigator, co-investigators, statisticians, IRB and the research personnel having access to individually identifiable data. The CRFs will be locked in a cabinet in a secure area with limited access to the building.

Data from this study will be retained for a minimum of 7 years for health data per Indiana State law. Following this period, paper will be shredded and permanently deleted from any computers.

Identifiable data will be shared only between research team members approved on the "Investigator List" in any of the following ways: encrypted email, US postal service or trackable courier services (i.e. FedEX), fax in a secured area, shared drive with password protection, personal delivery by authorized research personnel and/or private telephone conversations to authorized personnel.

19.0 PROCEDURES AND INSTRUCTIONS

19.1 Administrative Procedures

19.1.1 Changes to the Protocol
Any change or addition to this protocol requires a written protocol amendment that must be approved by all the principal investigators before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study, require additional approval by the IRB.

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB of each center must be kept informed of such administrative changes.

19.2 Ethics and Good Clinical Practice
This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

21.21. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
21.22. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
21.23. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
21.24. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

19.3 Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC/REB).

19.4 Informed Consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

REFERENCES

1. Rees DC, Williams TN, Gladwin MT: Sickle-cell disease. Lancet 376:2018-31, 2010
2. Platt OS, Brambilla DJ, Rosse WF, et al: Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 330:1639-44, 1994
3. Ware RE, de Montalembert M, Tshilolo L, et al: Sickle cell disease. Lancet 390:311-323, 2017
4. Kauf TL, Coates TD, Huazhi L, et al: The cost of health care for children and adults with sickle cell disease. Am J Hematol 84:323-7, 2009
5. Smith WR, Penberthy LT, Bovbjerg VE, et al: Daily assessment of pain in adults with sickle cell disease. Ann Intern Med 148:94-101, 2008
6. Sogutlu A, Levenson JL, McClish DK, et al: Somatic symptom burden in adults with sickle cell disease predicts pain, depression, anxiety, health care utilization, and quality of life: the PiSCES project. Psychosomatics 52:272-9, 2011
7. McClish DK, Penberthy LT, Bovbjerg VE, et al: Health related quality of life in sickle cell patients: the PiSCES project. Health Qual Life Outcomes 3:50, 2005
8. Howard J, Thomas VJ, Rawle HM: Pain management and quality of life in sickle cell disease. Expert Rev Pharmacoecon Outcomes Res 9:347-52, 2009
9. Edlund MJ, Sullivan M, Steffick D, et al: Do users of regularly prescribed opioids have higher rates of substance use problems than nonusers? Pain Med 8:647-56, 2007
10. Platt OS, Thorington BD, Brambilla DJ, et al: Pain in sickle cell disease. Rates and risk factors. N Engl J Med 325:11-6, 1991
11. Steinberg MH: Genetic etiologies for phenotypic diversity in sickle cell anemia. ScientificWorldJournal 9:46-67, 2009
12. Zhang D, Xu C, Manwani D, et al: Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. Blood 127:801-9, 2016
13. Kato GJ, Gladwin MT, Steinberg MH: Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. Blood Rev 21:37-47, 2007
14. Platt OS: Sickle cell anemia as an inflammatory disease. J Clin Invest 106:337-8, 2000
15. Belcher JD, Mahaseth H, Welch TE, et al: Critical role of endothelial cell activation in hypoxia-induced vasoocclusion in transgenic sickle mice. Am J Physiol Heart Circ Physiol 288:H2715-25, 2005
16. Shet AS, Aras O, Gupta K, et al: Sickle blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes. Blood 102:2678-83, 2003
17. Westerman M, Pizzey A, Hirschman J, et al: Microvesicles in haemoglobinopathies offer insights into mechanisms of hypercoagulability, haemolysis and the effects of therapy. Br J Haematol 142:126-35, 2008
18. Kasar M, Boga C, Yeral M, et al: Clinical significance of circulating blood and endothelial cell microparticles in sickle-cell disease. J Thromb Thrombolysis 38:167-75, 2014
19. Nebor D, Bowers A, Connes P, et al: Plasma concentration of platelet-derived microparticles is related to painful vaso-occlusive phenotype severity in sickle cell anemia. PLoS One 9:e87243, 2014
20. van Tits LJ, van Heerde WL, Landburg PP, et al: Plasma annexin A5 and microparticle phosphatidylserine levels are elevated in sickle cell disease and increase further during painful crisis. Biochem Biophys Res Commun 390:161-4, 2009
21. van Beers EJ, Schaap MC, Berckmans RJ, et al: Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease. Haematologica 94:1513-9, 2009
22. Gerotziafas GT, Van Dreden P, Chaari M, et al: The acceleration of the propagation phase of thrombin generation in patients with steady-state sickle cell disease is associated with circulating erythrocyte-derived microparticles. Thromb Haemost 107:1044-52, 2012
23. Werre JM, Willekens FL, Bosch FH, et al: The red cell revisited--matters of life and death. Cell Mol Biol (Noisy-le-grand) 50:139-45, 2004
24. Willekens FL, Roerdinkholder-Stoelwinder B, Groenen-Dopp YA, et al: Hemoglobin loss from erythrocytes in vivo results from spleen-facilitated vesiculation. Blood 101:747-51, 2003

25. Belcher JD, Chen C, Nguyen J, et al: Heme triggers TLR4 signaling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease. Blood 123:377-90, 2014
26. Hebbel RP, Yamada O, Moldow CF, et al: Abnormal adherence of sickle erythrocytes to cultured vascular endothelium: possible mechanism for microvascular occlusion in sickle cell disease. J Clin Invest 65:154-60, 1980
27. Hebbel RP, Boogaerts MA, Eaton JW, et al: Erythrocyte adherence to endothelium in sickle-cell anemia. A possible determinant of disease severity. N Engl J Med 302:992-5, 1980
28. Mohandas N, Evans E: Adherence of sickle erythrocytes to vascular endothelial cells: requirement for both cell membrane changes and plasma factors. Blood 64:282-7, 1984
29. Turhan A, Weiss LA, Mohandas N, et al: Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. Proc Natl Acad Sci U S A 99:3047-51, 2002
30. Camus SM, De Moraes JA, Bonnin P, et al: Circulating cell membrane microparticles transfer heme to endothelial cells and trigger vasoocclusions in sickle cell disease. Blood 125:3805-14, 2015
31. Camus SM, Gausseres B, Bonnin P, et al: Erythrocyte microparticles can induce kidney vaso-occlusions in a murine model of sickle cell disease. Blood 120:5050-8, 2012
32. Merciris P, Hardy-Dessources MD, Giraud F: Deoxygenation of sickle cells stimulates Syk tyrosine kinase and inhibits a membrane tyrosine phosphatase. Blood 98:3121-7, 2001
33. Nur E, Biemond BJ, Otten HM, et al: Oxidative stress in sickle cell disease; pathophysiology and potential implications for disease management. Am J Hematol 86:484-9, 2011
34. Monteiro HP, Arai RJ, Travassos LR: Protein tyrosine phosphorylation and protein tyrosine nitration in redox signaling. Antioxid Redox Signal 10:843-89, 2008
35. Monteiro HP, Stern A: Redox modulation of tyrosine phosphorylation-dependent signal transduction pathways. Free Radic Biol Med 21:323-33, 1996
36. George A, Pushkaran S, Konstantinidis DG, et al: Erythrocyte NADPH oxidase activity modulated by Rac GTPases, PKC, and plasma cytokines contributes to oxidative stress in sickle cell disease. Blood 121:2099-107, 2013
37. George A, Pushkaran S, Li L, et al: Altered phosphorylation of cytoskeleton proteins in sickle red blood cells: the role of protein kinase C, Rac GTPases, and reactive oxygen species. Blood Cells Mol Dis 45:41-5, 2010
38. Puchulu-Campanella E, Turrini FM, Li YH, et al: Global transformation of erythrocyte properties via engagement of an SH2-like sequence in band 3. Proc Natl Acad Sci U S A 113:13732-13737, 2016
39. Ferru E, Giger K, Pantaleo A, et al: Regulation of membrane-cytoskeletal interactions by tyrosine phosphorylation of erythrocyte band 3. Blood 117:5998-6006, 2011
40. Murphy M, Close J, Lottenberg R, et al: Effectiveness of imatinib therapy for sickle cell anemia and chronic myeloid leukemia. Am J Med Sci 347:254-5, 2014
41. Stankovic Stojanovic K, Thioliere B, Garandeau E, et al: Chronic myeloid leukaemia and sickle cell disease: could imatinib prevent vaso-occlusive crisis? Br J Haematol 155:271-2, 2011
42. Walters MC: Update of hematopoietic cell transplantation for sickle cell disease. Curr Opin Hematol 22:227-33, 2015
43. Nickel RS, Kamani NR: Ethical Challenges in Hematopoietic Cell Transplantation for Sickle Cell Disease. Biol Blood Marrow Transplant 24:219-227, 2018
44. Goodman MA, Malik P: The potential of gene therapy approaches for the treatment of hemoglobinopathies: achievements and challenges. Ther Adv Hematol 7:302-315, 2016
45. Hoban MD, Orkin SH, Bauer DE: Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. Blood 127:839-48, 2016
46. Traxler EA, Yao Y, Wang YD, et al: A genome-editing strategy to treat beta-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition. Nat Med 22:987-90, 2016
47. Sankaran VG, Weiss MJ: Anemia: progress in molecular mechanisms and therapies. Nat Med 21:221-30, 2015
48. Telen MJ: Beyond hydroxyurea: new and old drugs in the pipeline for sickle cell disease. Blood 127:810-9, 2016
49. Allan D, Limbrick AR, Thomas P, et al: Release of spectrin-free spicules on reoxygenation of sickled erythrocytes. Nature 295:612-3, 1982
50. Wun T, Paglieroni T, Rangaswami A, et al: Platelet activation in patients with sickle cell disease. Br J Haematol 100:741-9, 1998

51. Hoover R, Rubin R, Wise G, et al: Adhesion of normal and sickle erythrocytes to endothelial monolayer cultures. Blood 54:872-6, 1979
52. Tantawy AA, Adly AA, Ismail EA, et al: Circulating platelet and erythrocyte microparticles in young children and adolescents with sickle cell disease: Relation to cardiovascular complications. Platelets 24:605-14, 2013
53. Browne P, Shalev O, Hebbel RP: The molecular pathobiology of cell membrane iron: the sickle red cell as a model. Free Radic Biol Med 24:1040-8, 1998
54. Pantaleo A, Ferru E, Pau MC, et al: Band 3 Erythrocyte Membrane Protein Acts as Redox Stress Sensor Leading to Its Phosphorylation by p (72) Syk. Oxid Med Cell Longev 2016:6051093, 2016
55. Champagne MA, Capdeville R, Krailo M, et al: Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. Blood 104:2655-60, 2004
56. Millot F, Guilhot J, Nelken B, et al: Imatinib mesylate is effective in children with chronic myelogenous leukemia in late chronic and advanced phase and in relapse after stem cell transplantation. Leukemia 20:187-92, 2006
57. Kesely KR, Pantaleo A, Turrini FM, et al: Inhibition of an Erythrocyte Tyrosine Kinase with Imatinib Prevents Plasmodium falciparum Egress and Terminates Parasitemia. PLoS One 11:e0164895, 2016
58. Pantaleo A, Kesely KR, Pau MC, et al: Syk inhibitors interfere with erythrocyte membrane modification during P falciparum growth and suppress parasite egress. Blood 130:1031-1040, 2017
59. Tran H, Gupta M, Gupta K: Targeting novel mechanisms of pain in sickle cell disease. Blood 130:2377-2385, 2017
60. Vincent L, Vang D, Nguyen J, et al: Mast cell activation contributes to sickle cell pathobiology and pain in mice. Blood 122:1853-62, 2013
61. Ivanova A, Qaqish BF, Schell MJ: Continuous toxicity monitoring in phase II trials in oncology. Biometrics 61:540-5, 2005
62. Cayman Assay. https://www.caymanchem.com/pdfs/700540.pdf (Accessed 8/14/2017)
63. Thermo Scientific, http://www.phadia.com/en-GB/5/Products/ImmunoCAP-Assays/ImmunoCAP-Tryptase/Test-Principle-ImmunoCAP-Tryptase/ (Accessed 8/14/2017).
64. Hoban MD, Orkin SH, Bauer DE. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. Blood. 2016;127(7):839-48. doi: 10.1182/blood-2015-09-618587. PubMed PMID: 26758916; PMCID: PMC4760089.
65. Traxler EA, Yao Y, Wang YD, Woodard KJ, Kurita R, Nakamura Y, Hughes JR, Hardison RC, Blobel GA, Li C, Weiss MJ. A genome-editing strategy to treat beta-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition. Nature medicine. 2016;22(9):987-90. doi: 10.1038/nm.4170. PubMed PMID: 27525524.
66. Ward P, Equinet L, Packer J, Doerig C. Protein kinases of the human malaria parasite Plasmodium falciparum: the kinome of a divergent eukaryote. BMC Genomics. 2004;5:79. doi: 10.1186/1471-2164-5-79. PubMed PMID: 15479470; PMCID: PMC526369.
67. Solyakov L, Halbert J, Alam MM, Semblat JP, Dorin-Semblat D, Reininger L, Bottrill AR, Mistry S, Abdi A, Fennell C, Holland Z, Demarta C, Bouza Y, Sicard A, Nivez MP, Eschenlauer S, Lama T, Thomas DC, Sharma P, Agarwal S, Kern S, Pradel G, Graciotti M, Tobin AB, Doerig C. Global kinomic and phospho-proteomic analyses of the human malaria parasite Plasmodium falciparum. Nat Commun. 2011;2:565. doi: 10.1038/ncomms1558. PubMed PMID: 22127061.
68. Telen MJ. Beyond hydroxyurea: new and old drugs in the pipeline for sickle cell disease. Blood. 2016;127(7):810-9. doi: 10.1182/blood-2015-09-618553. PubMed PMID: 26758919; PMCID: PMC4760087.
69. Sankaran VG, Weiss MJ. Anemia: progress in molecular mechanisms and therapies. Nature medicine. 2015;21(3):221-30. doi: 10.1038/nm.3814. PubMed PMID: 25742458; PMCID: PMC4452951.
70. Li C, Ding L, Sun CW, Wu LC, Zhou D, Pawlik KM, Khodadadi-Jamayran A, Westin E, Goldman FD, Townes TM. Novel HDAd/EBV Reprogramming Vector and Highly Efficient Ad/CRISPR-Cas Sickle Cell Disease Gene Correction. Sci Rep. 2016;6:30422. doi: 10.1038/srep30422. PubMed PMID: 27460639; PMCID: PMC4961958.

APPENDIX A: IMATINIB TABLETS DOSING GUIDELINES

Imatinib 340mg/m2/day		
Body Surface Area (m2)	Total Daily Dose (Rounded to Nearest 50 mg)	Recommended Administration
≤ 0.36	100 mg	1 x 100 mg tablet once daily
0.37 – 0.51	150 mg	1 ½ x 100 mg tablets once daily
0.52 – 0.66	200 mg	2 x 100 mg tablets once daily
0.67 – 0.81	250 mg	2 ½ x 100 mg tablets once daily
0.82 – 0.95	300 mg	3 x 100 mg tablets once daily
0.96 – 1.10	350 mg	3 ½ x 100 mg tablets once daily
1.11 – 1.25	400 mg	4 x 100 mg tablets once daily
1.26 – 1.39	450 mg	4 ½ x 100 mg tablets once daily
1.4 – 1.54	500 mg	5 x 100 mg tablets once daily
1.55 – 1.69	550 mg	5 ½ x 100 mg tablets once daily
≥1.70	600 mg	6 x 100 mg tablets once daily

Imatinib 260mg/m2/day		
Body Surface Area (m2)	Total Daily Dose (Rounded to Nearest 50mg)	Recommended Administration
≤ 0.29	50 mg	½ x 100mg tablet once daily
0.3 – 0.48	100 mg	1 x 100mg tablet once daily
0.49 – 0.67	150 mg	1 ½ x 100mg tablet once daily
0.68 – 0.86	200 mg	2 x 100mg tablet once daily
0.87 – 1.05	250 mg	2 ½ x 100mg tablet once daily
1.06 – 1.24	300 mg	3 x 100mg tablet once daily
1.25 – 1.44	350 mg	3 ½ x 100mg tablet once daily
1.45 – 1.63	400 mg	4 x 100mg tablet once daily
1.64 – 1.82	450 mg	4 ½ x 100mg tablet once daily
1.83 – 2.01	500 mg	5 x 100mg tablet once daily
≥ 2.02	550 mg	5 ½ x 100mg tablet once daily

Imatinib 200mg/m2/day		
Body Surface Area (m2)	Total Daily Dose (Rounded to Nearest 50mg)	Recommended Administration
≤ 0.37	50 mg	½ x 100mg tablet once daily
0.38 – 0.62	100 mg	1 x 100mg tablet once daily
0.63 – 0.87	150 mg	1 ½ x 100mg tablet once daily
0.88 – 1.12	200 mg	2 x 100mg tablet once daily
1.13 – 1.37	250 mg	2 ½ x 100mg tablet once daily
1.38 – 1.62	300 mg	3 x 100mg tablet once daily
1.63 – 1.87	350 mg	3 ½ x 100mg tablet once daily
1.88 – 2.12	400 mg	4 x 100mg tablet once daily
2.13 – 2.37	450 mg	4 ½ x 100mg tablet once daily
≥ 2.38	500 mg	5 x 100mg tablet once daily

APPENDIX B: Drugs known to be metabolized by CYP450 isoenzymes 2D6 and 3A4

CYP2D6	
Substrates	
Amitriptyline (hydroxylation) Amphetamine Betaxolol Bisoprolol Brofaromine Buturolol Bupropion Captopril Carvedilol Cevimeline Chlorpheniramine Chlorpromazine Cinnarizine Clomipramine (hydroxylation) Clozapine (minor pathway) Codeine (hydroxylation, o-demelhylation) Cyclobenzaprine (hydroxylation) Cyclophosphamide Debrisoquin Delavirdine Desipramine Dexfenfluramine Dextromethorphan (o-demethylation) Dihydrocodeine Diphenhydramine Dolasetron Donepezil Doxepin Encainide Fenlluramine Flecainide Fluosetine (minor pathway) Fluphenazine Halofantrine Haloperidol (minor pathway) Hydrocodone Hydrocortisone Hydroxyamphetamine Imipramine (hydroxylation) Labetalol Loratadine Maprotiline m-Chlorophenylpiperazine (m-CPP) Meperidine Methadone	Methamphetamine Metoclopramide Metoprolol Mexitetine Mianserin Mirtazapine (hydroxylation) Molindone Nortriptyline (hydroxylation) Olanzapine (minor, hydroxymethylation) Ondansetron Orphenadrine Oxycodone Papaverine Paroxetine (minor pathway) Penbutolol Pentazocine Perhexiline Perphenazine Phenformin Pindolol Promethazine Propafenone Propranolol Quetiapine Remoxipride Risperidone Ritonavir (minor) Ropivacaine Selegiline Sertindole Sertratine (minor pathway) Sparteine Tamoxifen Thioridazine Tiagabine Timolol Tolterodine Tramadol Trazodone Trimipramine Tropisetron Venlafaxine (o-desmethylation) Yohimbine
Inhibitors	
Amiodarone	Methadone

Celecoxib	Mibefradil
Chloroquine	Moclobemide
Chlorpromazine	Nortluoxeline
Cimelidine	Paroxetine
Citalopram	Perphenazine
Clomipramine	Propafenone
Codeine	Quinacrine
Delavirdine	Quinidine
Desipramine	Ranitidine
Dextropropoxyphene	Risperidone (weak)
Diltiazem	Ritonavir
Doxorubicin	Sertindole
Entacapone (high dose)	Sertraline (weak)
Fluoxetine	Thioridazine
Fluphenazine	Vaiprolc acid
Fluvoxamine	Venlafaxine (weak)
Haloperidol	Vinblastine
Labetalol	Vincristine
Lobeline	Vinorelbine
Lomustine	Yohimbine

CYP3A3/4	
Substrates	
Acetaminophen	Chlorpromazine
Alfentanil	Cimetidine
Alosetron	Cisapride
Alprazolam	Citalopram
Amiodarone	Clarithromycin
Amitriptyline (minor)	Clindamycin
Amlodipine	Clomipramine
Anastrozole	Clonazepam
Androsterone	Clozapine
Antipyrine	Cocaine
Astemizole	Codeine (demethylation)
Atorvastatin	Cortisol
Benzphetamine	Cortisone
Bepridil	Cyclobenzaprine (demethylation)
Bexarotene	Cyclophosphamide
Bromazepam	Cyclosporine
Bromocriptine	Dapsone
Budesonide	Dehydroepiandrostendione
Bupropion (minor)	Delavirdine
Buspirone	Desmethyldiazepam
Busutfan	Dexamethasone
Caffeine	Dextromethorphan (minor, N-demethylation)
Cannabinoids	Diazepam (minor; hydroxylation, N-demethylation)
Carbamazepine	Nefazodone
Cevimeline	Nelfinavir
Cerivastatin	Nevirapine
Digitoxin	Nicardipine

Diltiazem	Nifedipine
Disopyramide	Niludipine
Docetaxel	Nimodipine
Dolasetron	Nisoldipine
Donepezil	Nitrendipine
Doxorubicin	Omeprazole (sulfonation)
Doxycycline	Ondansetron
Dronabinol	Oral contraceptives
Enalapril	Orphenadrine
Erythromycin	Paclitaxel
Estradiol	Pantoprazole
Ethinyl estradiol	Pimozide
Ethosuximide	Pioglitazone
Etoposide	Pravastatin
Exemestene	Prednisone
Dofetilide (minor)	Progesterone
Felodipine	Proguanil
Fentanyl	Propafenone
Fexotenadine	Quercetin
Finaxteride	Quetiapine
Fluoxetine	Quinidine
Flutamide	Quinine
Glyburide	Repaglinide
Granisetron	Retinoic acid
Halofantrine	Rifampin
Hydrocortixone	Risperidone
Hydroxyarginine	Ritonavir
Ifosfamide	Salmeterol
Imipramine	Saquinavir
Indinavir	Sertindole
Isradipine	Sertraline
Itraconazole	Sibutramine
Ketoconazole	Sildenafil citrate
Lansoprazole (minor)	Simvastatin
Letrozole	Sirolimus
Levobupivicaine	Sufentanil
Lidocaine	Tacrolimus
Loratadine	Tamoxifen
Losartan	Temazepam
Lovastatin	Teniposide
Methadone	Terfenadine
Mibefradil	Testosterone
Miconazole	Tetrahydrocannabinol
Midazolam	Theophylline
Mifepristone	Tiagabine
Mirtazapine (N-demethylation)	Tolterodine
Montelukast	Vincristine
Navelbine	Warfarin (R-warfarin)
Toremifene	Yohimbine
Trazodone	Zaleplon (minor pathway)
Tretinoin	Zatoestron

Triazolam Troglitazone Troleandomycin Venlafaxine (N-demethylation) Verapamil Vinblastine	Zileuton Ziprasidone Zolpidem Zonisamide
Inducers	
Carbamazepine Dexamethasone Ethosuximide Glucocorticoids Griseofulvin Nafcillin Nelfinavir Nevirapine Oxcarbazepine Phenobarbital Phenylbutazone	Phenytoin Primidone Progesterone Rifabutin Rifampin Rofecoxib (mild) St John's wort Sulfadimidine Sulfinpyrazone Troglitazone
Inhibitors	
Amiodarone Anastrozole Azithromycin Cannabinoids Cimetidine Clarithromycin Clotrimazole Cyclosporine Danazol Delavirdine Dexamethasone Diethyldithiocarbamate Diltiazem Dirithromycin Disulfiram Entacapone (high dose) Erythromycin Ethinyl estradiol Fluconazole (weak) Fluoxetine Fluvoxamine Gestodene Grapefruit juice Indinavir Isoniazid Itraconazole	Ketoconazole Metronidazole Mibefradil Miconazole (moderate) Nefazodone Nelfinavir Nevirapine Norfloxacin Norfluoxetine Omeprazole (weak) Oxiconazole Paroxetine (weak) Propoxyphene Quinidine Quinine Quinupristin and dalfopristin Ranitidine Ritonavir Saquinavir Sertindole Sertraline Troglitazone Troleandomycin Valproic acid (weak) Verapamil Zafirlukast Zileuton

(Adapted from Cytochrome P-450 Enzymes and Drug metabolism. In : Lacy CF, Armstrong LL, Goldman MP, Lance LL eds. Drug Information Handbook 8th ed. Hudson, OH; LexiComp Inc. 2000: 1364-1371