PhD Proposal

Pharmacogenetics and Model-Informed Optimisation of Hydroxyurea Therapy in Sickle Cell Disease Patients of Nigerian Descent

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Abstract

The wide interindividual variability in clinical response to hydroxyurea therapy in the management of sickle cell disease has limited its use. These variabilities have been linked to differences in pharmacodynamics, pharmacokinetics, and pharmacogenetics. This study, therefore, aims to enhance understanding of these factors as they relate to hydroxyurea therapy, with the overall goal of developing a precision medicine algorithm.

The study will be a prospective cohort pharmacokinetic study of 100 Nigerian patients with sickle cell disease, including current hydroxyurea users and naive patients. Pharmacodynamic markers will be collected to evaluate response. PopPK and PK-PD models will be developed in Monolix, exposure-response relationships will be analysed in R, and pregnancy, lactation, and paediatrics PBPK models will be developed in Simcyp or PK-SIM to inform dose optimisation.

Overall, this study will provide scientific knowledge that can enhance clinical decision-making in sickle cell management within the Nigerian population, and the models could serve as a template to optimize hydroxyurea use in this population.

Keywords: Modelling, Genetic Polymorphism, Pharmacokinetics, Clinical Outcomes, Hydroxyurea, Sickle Cell Disease, Precision Medicine, Population Pharmacokinetics

Brief Background

Sickle Cell Disease (SCD) remains a major public health burden in Nigeria, which carries the largest global share of affected individuals. Worldwide, approximately eight (8) million people are living with the disease, with nearly 80% residing in Sub-Saharan Africa.(Adigwe et al., 2023, p.3503; Thomson et al., 2023, p.e589-e590) Earlier modelling by Piel et al. (2013) projected 404,190 new births with SCD globally by 2050; however, recent analyses indicate that this estimate has already been exceeded by 27% as of 2021.(Piel, Frederic B, Hay et al., 2013, p.e1001484; Thomson et al., 2023, p.594) Nigeria contributes disproportionately, with about 150,000 affected births annually and more than 2 – 3% of the population living with the disease (Okocha et al., 2022, p.2). This high prevalence is directly linked to persistently elevated morbidity and mortality rates despite therapeutic advances.(Adigwe et al., 2023, p.3503; Darshana et al., 2021, p.1) Addressing this burden requires more effective clinical interventions, among which hydroxyurea has emerged globally as an approved disease-modifying therapy.

Pharmacometric modelling provides a powerful tool to unify inter-individual differences in drug response and translate them into evidence-based dosing strategies.(Mould & Upton, 2012, p.2-3) Population pharmacokinetic-pharmacodynamic (PK/PD) models have been successfully used in other regions to optimise hydroxyurea therapy, demonstrating that model-informed approaches can reduce the time required to reach the maximum tolerated dose.(Dong et al., 2016, p.750; Wiczling et al., 2014, p.1021-1022) This is particularly relevant in low- and middle-income countries, where prolonged monitoring and high costs pose barriers to achieving optimal dosing.(Okocha et al., 2022, p.6) Furthermore, pharmacometric simulations offer an ethical means to explore hydroxyurea use in vulnerable groups such as pregnant and lactating women, who are typically excluded from clinical trials, but form a significant proportion of the affected population.(Sewaralthahab et al., 2024, p.10-11) By integrating pharmacogenetic, pharmacokinetic, and clinical outcome data, a Nigerian-specific pharmacometric model could provide a locally validated decision-support tool, reduce clinical hesitancy, and improve outcomes.(Mould & Upton, 2012, p.1)

Research Gap

Hydroxyurea is an approved, disease-modifying therapy for sickle cell disease. However, its adoption in Nigeria remains low because prescribers lack confidence in how to tailor treatment to their patients, (Akindele et al., 2022, p.6; Okocha et al., 2022, p.5-6; Sanyaolu et al., 2020, p.115) and are uncertain about the optimal dose for Nigerian patients. This uncertainty stems out of the wide inter-individual variability observed in individual responses to the drug reported in the literature, (Aisha L. Walker, Ryan M. Franke, Alex Sparreboom, 2011, p.2,3,6; Estepp et al., 2018, p. 1482-1483; Ware et al., 2011, p.4985, 4989)and the absence of national guidelines for its prescription.

The reported wide variability is believed to reflect differences in the drug's pharmacodynamic, pharmacokinetic, and pharmacogenetic properties. (McGann & Ware, 2015, p.6; Pressiat et al.,

2021, p.2283). Patients have reported varying degrees of dose tolerance, ranging from 15 mg/kg/day to 35 mg/kg/day before myelosuppression occurs, indicating the minimum toxic dose. However, most of these associations have been explored in non-African or in vitro studies.(Gambari et al., 2024, p.4263; Huang et al., 2004, p.3500) The absence of locally generated data for Africans, including Nigerian patients, hampers dose optimisation and perpetuates clinical hesitancy. This makes exploring pharmacogenetic and pharmacokinetic variability in Nigeria a pressing research priority.

Of particular concern is the varying reports of proteins such as drug metabolising enzymes and transporters suspected to be influencing the inter-individual variabilities. Studies in non-African cohorts have identified genetic polymorphisms in drug-metabolising enzymes and transporters – such as CYP2D6, catalase, and SLC14A1- that influence hydroxyurea metabolism and the eventual clinical response and toxicity. (Juul et al., 2010, p.21413-21414; Yahouédéhou et al., 2020, p.5-6) Other enzymes that have been implicated include CYP2E1, UGT1A1, and CYP2C9, based on the available enzymes that the authors could explore. (Nkya et al., 2025a, p.5-6; Yahouédéhou et al., 2018, p.736-737). Given the genetic heterogeneity of the African population,(Masimirembwa & Matimba, 2012, p.175; Pereira et al., 2021, p.292,303) and the rich diversity in the Nigerian population,(Bolaji et al., 2019, p.921) locally generated pharmacogenetic data are essential. Linking these genetic factors to hydroxyurea pharmacokinetics will be critical for understanding systemic exposure and clinical outcomes.

Additionally, no pharmacometric model currently exists to guide individualised dosing in the Nigerian population.(Dong et al., 2016, p.750; Mould & Upton, 2012, p.1; Wiczling et al., 2014, p.1021) This lack of population-specific data and tools contributes to clinical hesitancy, suboptimal prescribing, and missed opportunities for better SCD health outcomes.

Aims and Objectives

This study aims to build a pharmacometric model by integrating differences in pharmacokinetics, pharmacodynamics, and pharmacogenetics of hydroxyurea, which could aid the optimisation of hydroxyurea for sickle cell patients in Nigeria.

Objectives

The objectives of the study are

i. To determine the prevalence of genetic polymorphisms in metabolic enzymes and transporters relevant to the disposition of hydroxyurea in the Nigerian sickle cell disease population,
ii. To develop and validate an analytical method for the quantification of hydroxyurea using high-performance liquid chromatography.
iii. To evaluate the influence of genetic and other covariates on hydroxyurea disposition in the Nigerian sickle cell disease population using population pharmacokinetic modelling,
iv. To investigate the relationship between hydroxyurea exposure and clinical outcomes (foetal haemoglobin, mean corpuscular volume, reduction in vaso-

occlusive crises (VOC), and improved blood count) using pharmacokinetic-pharmacodynamic modelling,

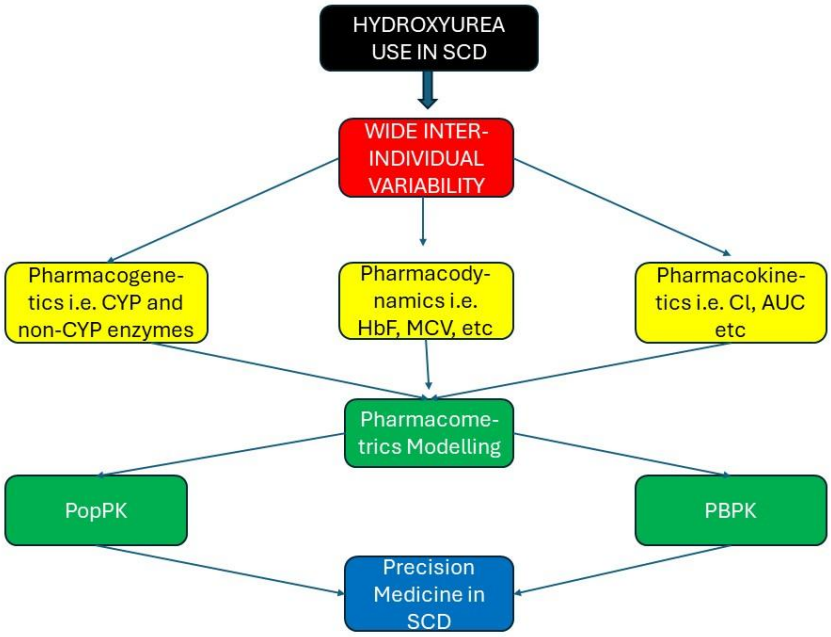
v. To develop physiologically-based pharmacokinetic (PBPK) models that could predict hydroxyurea concentrations in special populations of sickle cell disease patients in Nigeria i.e. pregnant women, lactating mothers, breastfed infants, and paediatrics.

vi. To develop a dosing guideline for hydroxyurea therapy in Nigerian sickle cell patients.

Research Questions

1. What is the prevalence of genetic polymorphisms in drug-metabolizing enzymes and transporters that affect hydroxyurea disposition among Nigerian patients with sickle cell disease?
2. What analytical method can be best suited for the quantification of hydroxyurea in human plasma in the light of available resources in Nigeria?
3. How do genetic variants and other patient covariates influence the pharmacokinetics of hydroxyurea in Nigerian sickle cell disease patients?
4. What is the relationship between hydroxyurea exposure and key clinical outcomes (foetal haemoglobin, mean corpuscular volume, frequency of vaso-occlusive crises, and blood count) in Nigerian sickle cell disease patients?
5. Can physiologically based pharmacokinetic (PBPK) models accurately predict hydroxyurea concentrations in special sickle cell disease populations in Nigeria, including pregnant women, lactating mothers, breastfed infants, geriatric, and paediatric patients?
6. What doses of hydroxyurea are best suited for different phenotypes of the sickle cell patients of Nigerian descent?

Conceptual Framework



This concept map begins with the obvious barrier to the use of hydroxyurea, namely, clinical hesitancy, due to the underlying reason of inter-individual variability that has hampered prescribers' and patients' confidence in the drug. This research aims to develop a population-specific pharmacometric model that integrates three interrelated concepts: pharmacogenetics, pharmacokinetics (PK), and pharmacodynamics (PD), as elucidated below.

Pharmacokinetics

Pharmacokinetics studies on hydroxyurea are non-existent in Nigeria and scarce in Africa. Differences in hydroxyurea pharmacokinetics have been reported across populations, emphasizing the need for locally relevant data. The NOHARM ancillary trial in Uganda demonstrated that African children had lower clearance and higher systemic exposure to hydroxyurea compared to their American counterparts, suggesting that both genetic and non-genetic factors may influence hydroxyurea disposition.(Marahatta et al., 2017, p.1-2) These findings suggest that dosing strategies developed in non-African populations may not be directly applicable to African populations. However, in Nigeria, no population-specific pharmacokinetic studies have been conducted, leaving clinicians without evidence-based guidance to tailor doses for safety and effectiveness.

Pharmacogenetics

Variants in drug-metabolising enzymes and transporters can influence both the efficacy and toxicity of hydroxyurea. For example, polymorphisms in variants of CYP*2D6* (rs3892097), CAT (rs7943316, rs1001179), and SLC14A1 (rs2298720) have been correlated with improved

hematologic parameters in Brazilian patients with sickle cell disease who are on hydroxyurea. (Yahouédéhou et al., 2018, 2020) The role of genetic differences was further supported by a Tanzanian study that identified CYP2C9 and CYP2E1 as implicated in HU metabolism.(Nkya et al., 2025b). Earlier in-vitro studies also demonstrated catalase-mediated hydroxyurea metabolism and its potential toxicity mechanisms.(Huang et al., 2004; Juul et al., 2010) It is worth noting that these studies examined a limited number of genes and may not provide a comprehensive picture of the extent of the genetic polymorphism effect, and none have been conducted in Nigeria. This is particularly important because Nigeria and Africa, as a whole, are known for their rich genetic diversity, with unique allelic variations that cannot be extrapolated from non-African studies.(Adehin et al., 2017, p.3; Bolaji et al., 2019; Masimirembwa & Matimba, 2012; Pereira et al., 2021)

Pharmacometrics

Pharmacometrics provides a methodological framework to integrate diverse sources of variability – pharmacogenetics, pharmacokinetics, and clinical outcomes – into unified models that can inform individualised therapy. Population pharmacokinetic-pharmacodynamic (PK/PD) modelling uses non-linear mixed-effects (NLME) approaches to quantify drug disposition and response across a population, accounting for covariates such as age, sex, weight, genetic variants, and co-morbidities(Bonate, 2011, p.303; Darwich et al., 2021, p.228-229; US FDA, 2022, p.2-7) This approach has been successfully applied in Europe and North America to optimise hydroxyurea therapy, demonstrating reductions in the time to reach the maximum tolerated dose through model-informed dosing.(Dong et al., 2016; Wiczling et al., 2014) Such strategies are especially valuable in resource-limited settings, where prolonged monitoring is costly and often impractical.(Okocha et al., 2022) Ultimately, this model will provide a decision-support tool that enables safer, personalized treatment with hydroxyurea.

DATA MANAGEMENT AND ANALYSIS PLAN

STUDY DESCRIPTION

The study will be a three-phase prospective cohort study of sickle cell patients of Nigerian descent receiving hydroxyurea therapy and hydroxyurea-naive patients who are willing to be placed on hydroxyurea therapy for the duration of the study (6 months). The three phases are as follows:

Pre-Field Phase – Objective 1&2

The study will start with the analysis of available pharmacogenetic data from sickle cell patients to explore genetic polymorphisms in metabolic enzymes, including CYP2D6, CYP2C9, CYP2C19, and CYP3A5, in Nigerian populations. (Data is available from the iProtecta -Implementation of Pharmacogenetic Testing for Effective Care and Treatment in Africa: Sickle Cell Disease - study portfolio). A preliminary physiologically based

pharmacokinetic model will be developed using data available in the literature, which will guide the determination of a more precise sample size, sampling time, and probable minimum dose for the pharmacokinetic study phase.

The analytical method for hydroxyurea will be developed using adapted methods from the tables below.

Table 1: HPLC Methods - 1

Parameters/Variables	**Legrand et al., 2017 (Legrand et al., 2017)**	**Bachir et al., 2007 (Bachir et al., 2007)**	**Bruna et al., 2014 (Bruna et al., 2014)**
Instrumentation	HPLC-UV	HPLC	UPLC
Main Analyte (Source)	Hydroxyurea (Sigma-Aldrich)	Hydroxyurea (Sigma)	Hydroxyurea (>98%)
Internal Standard (Source)	Methylurea (Sigma-Aldrich)	Methylurea	Methylurea (97%)
Matrix			Plasma
Derivatization Compound	Xanhydrol (Sigma-Aldrich)		
Chemicals/Reageants (Source)	• Acetonitrile • Isopropanol – HPLC grade) • Methanol – HPLC grade • Ammonium Acetate • 37% Chlorhydric acid (All from VWR, Fontenay-sous-Bois, France) • HCl • Drug Free Human Plasma	• Urea (Sigma) • Diacetylmonoxime • Thiosemicarbazid • FeCl3 • Sufuric acid • Orthophosphoric acid • Perchloric acid • Acetonitrile (VWR, Darmstadt, Germany) • Plasma • Urine	• Diacetyl monoxime • Tiosemicarbazide • Ferric chloride (From Sigma) • Sodium chloride (Labsynth) • Sulfuric acid • Orthophosphoric acid • Trichloroacetic acid • Acetonitrile
Chromatographic Instrument	Ultimate* 3000 (Thermo-Fisher)		A pumping system with diode array detector (PDA) (Accela Thermo Scientific)
Column	Kinetex C18 analytical column (4.6 x 150 mm, 5um) + Security guard column C18	Nucleosil C18, 250-4.6 column (5um particle size; Macherrey-Nagel, Hoerdt, France) filled to a	Nucleosil C18, 250_4.6 column (5 um particle sie) from Phenomenex and pre-column C18 (4x30 mm)

	(4.6 mm) both from Phenomenex, France	pre-column Nuceosil 8-4.6.	
Flow rate	1.0 ml/min	1.2 ml/min	1 ml/min
Mobile phase	20mM ammonium acetate and acetonitrile	Water/Acetonitrile (13/87)	Acetonitrile/water (16.7/83.3)
Mode			
Quantification mode			
m/z for HU			
m/z for Internal standard			
Detection wavelength	240 nm	449 nm	440 nm
Retention time for Hydroxyurea	Xan-HU = 6.0 min		6.763 min
Retention time for I.S	Xan-MeU = 10.6 min		11.472 min
Run time			
PK Sampling times			

Table 2: HPLC Methods 2

Parameters/Variables	**Jong et al., 2003 (Jong et al., 2003)**	**Pujari et al., 1997**	Yan et al., 2005
Instrumentation	HPLC – Electrochemical detection	HPLC – Electrochemical detection (LC-4C amperometric detector, a CC-4 cell system consisting of a glassy-carbon dual electrode cell and an Ag/AgCl reference cell	HPLC HPLC-Electrochemical detection (ESA coulochem, guard cell 0.65 V
Main Analyte (Source)	Hydroxyurea (Sigma)	Hydroxyurea	Hydroxyurea
Internal Standard (Source)	2-thiouracil (Sigma)		
Matrix	Plasma		
Derivatization Compound			
Chemicals/Reagents (Source)	• Sodium acetate • Trihydrate • Acetic acid	• Perchloric acid • Methanol	

	(Merck, Darmstatdt, Germany) • Milli-Q water • Acetonitrile (HPLC grade)	• Acetonitrile (HPLC grade)	
Chromatographic Instrument			
Column		Econosil C18, 250x4.6 mm, 10um particle size (from Altech)	Beckman Ultrasphere ODS, 5u, 250 x 4.6 mm; guard column: Brownlee RP-18, 7u, 15 x 3 mm
Flow rate			1.0 ml/min
Mobile phase		0.2M sodium perchlorate-methanol (95:5, v/v) adjusted to pH 5.0 using perchloric acid.	5% acetonitrile in 50 mP sodium acetate, 5mM tetrabutylammonium-hydrogen sulfate (TBAHS), pH 6.77
Run time			5 minutes

Table 3 - HPLC Methods 3

Parameters/Variables	**Marahatta & Ware, 2017**
Instrumentation	HPLC-UV
Main Analyte (Source)	Hydroxyurea
Internal Standard (Source)	Methylurea
Matrix	Plasma
Chemicals/Reagents (Source)	• Acetonitrile
Column	Zorbax Eclipse XDB C18 (250 mm x 4.6 mm, 5uM particle size)
Flow rate	1 ml/min
Mobile phase	870 ml MILLIQ water and 130 ml Acetonitrile (13% of Acetonitrile)
Detection wavelength	449 nm

Table 4: LC-MS/MS Methods

Parameters/Variables	**Hai et al, 2017 (Hai et al., 2017)**	**Marahatta et al., 2016 ((Anu Marahatta, Vandana Megaraj, Patrick T. McGann, Russell E. Ware, 2016)**
Instrumentation	LC-MS/MS	LC-MS/MS
Main Analyte (Source)	Hydroxyurea (Qilu Tianhe Pharmaceutica, China)	Hydroxyurea (Sigma)
Internal Standard (Source)	Hydroxyurea-13C1,15N2 – Isotope labelled (Toronto Research Chemicals)	Hydroxyurea-13C1,15N2 – Isotope labelled (Toronto Research Chemicals)
Matrix	Plasma	Whole blood - DBS
Chemicals/Reagents (Source)	• Acetonitrile -HPLC grade (Thermo Fisher) • Ammonium Formate – HPLC grade (Sigma-Aldrich) • Drug Free Human Plasma (Healthy donors)	• Ammonium formate (Sigma) • Water (HPLC-MS grade) • Formic acid (HPLC-MS grade) • Acetonitrile (HPLC-MS grade) From Fischer Scientific • DMPK-C cards • Whatman 903 specimen collection paper • FTA classic cards From GE health • VAMS devices (Neoteryx, Torrance, USA)

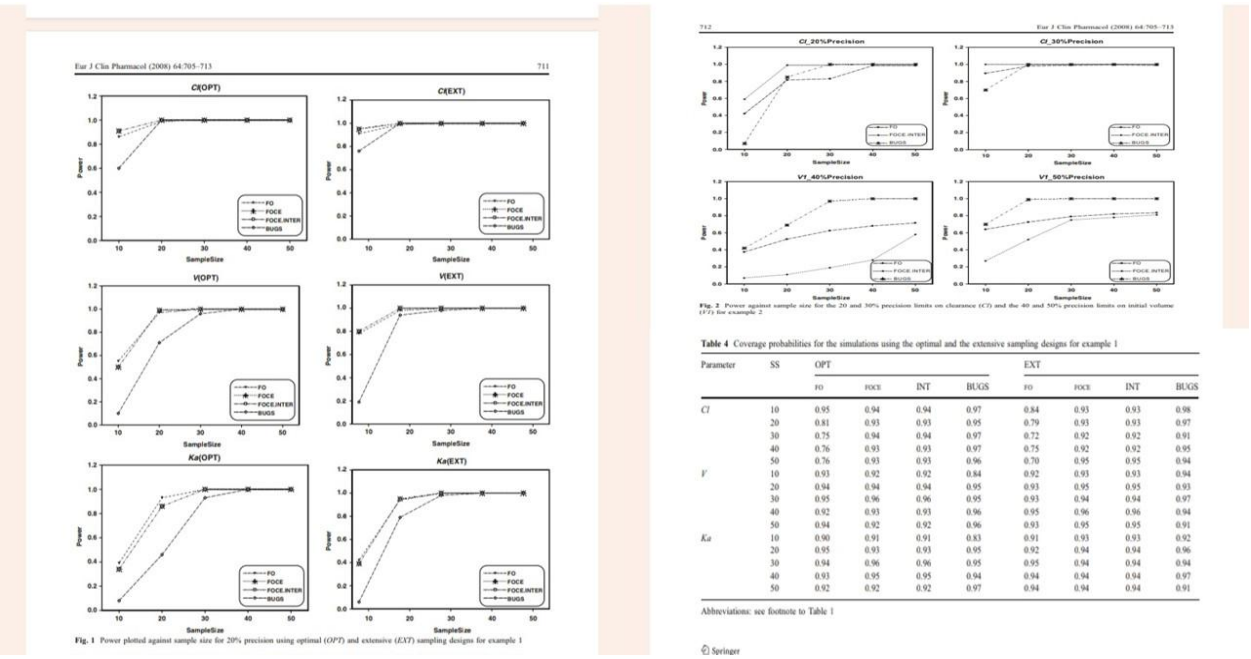
		• Nitrogen gas
Chromatographic Instrument	Shimadzu ultra fast liquid chromatography 20A system	Shimadzu LC-2-AD coupled to an AB Sciex 5500 Q-Trap MS
Column	Phenomenex Luna Silica Column (100 x 2 mm, 3um)	Atlantis HILIC Silica 3um column (2.1 x 100 mm)
Flow rate	0.35 ml/min	0.4 ml/min
Mobile phase	Acetonitrile-water containing 1.5mM ammonium formate (90:10, V:V)	10 mmol/l ammonium formate and acetonitrile with 0.1% formic acid
Mode	Electrospray ionization (ESI) in positive ion mode	
Quantification mode	Multiple reaction monitoring (MRM) mode	
m/z for HU	77.2 – 44.1	44
m/z for Internal standard	80.2 – 46.1	46
Run time		5 min
PK Sampling times	0, 0.25h, 0.5 h, 1h, 2h, 4h, 6h, 8h	

Pharmacokinetic/Pharmacodynamic Field Phase

The pharmacokinetic/pharmacodynamic study phase will involve adult sickle cell patients of Nigerian descent including both current hydroxyurea users and naive patients, stratified by metabolizing enzyme phenotypes, with CYP2D6 serving as the reference enzyme (Yahoudehou et al., 2020, p. 5-6).

Sample Size

100 patients will be recruited to participate in this phase of the study, as this sample size can estimate the 95% confidence interval with a power of 0.8 to 0.9 (Ogungbenro & Aarons, 2008; Sukarnjanaset & Wattanavijitkul, 2018), as depicted in the figure below.



Power plots and tables for optimal sample size calculations sourced from Ogungbenro & Aarons, 2008.

The U.S. Department of Health and Human Services, Food and Drug Administration (FDA) Population Pharmacokinetics Guidance for Industry recommends a sample size that can estimate a covariate effect with high precision and statistical power (US FDA, 2022).The power plots as simulated by Ogungbenro & Aarons, 2008 (and referenced by the FDA guideline) shows that a sample size of 30 for each covariate population and rich sampling can give a statistical power of 0.8 to 0.95 (Ogungbenro & Aarons, 2008).

Sample size per covariate group = 30
The major covariate groups is the metabolizing enzyme phenotypic group i.e. Fast, Normal, and Slow metabolizer
Hence, Cohort 1 = Patients that are fast metabolizers = 30
Cohort 2 = Patients that are normal metabolizers = 30
Cohort 3 = Patients that are slow metabolizers = 30
An additional 10 study participants will be recruited to make up for voluntary withdrawal from the study.
Therefore, Sample size = 100

Blood samples will be collected from all recruited patients at a minimum of 6 time points from the pool of the stated time points; 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours (Ogungbenro & Aarons, 2008; Paule et al., 2011; Wiczling et al., 2014). Corresponding pharmacodynamic data including HbF level and blood counts which will be used as the response biomarkers, will also be collected. The blood will be centrifuged, the plasma collected and stored at -80 ºC until analysis. The validated HPLC method in plasma will be used to quantify HU and used for the analysis. The patients will be followed up for at least 6 months , as follows (Hebert et al., 2020).

Pharmacodynamic Data to be collected

Parameters	Baseline	3 months	6 months
Foetal Haemoglobin	✓	X	✓
Total Haemoglobin	✓	✓	✓
Red Blood Cell Count	✓	✓	✓
Mean Corposcular Volume	✓	✓	✓
Mean Corposcular Haemoglobin	✓	✓	✓
Neutrophils Count	✓	✓	✓
Platelet Count	✓	✓	✓
Reticulocyte Count	✓	✓	✓
Serum Creatinine	✓	✓	✓
Lactate Dehydrogenase	✓	✓	✓
Urea	✓	✓	✓
Direct Bilirubin	✓	✓	✓
Total Bilirubin	✓	✓	✓
Aspartate aminotransferase	✓	✓	✓
Alanine aminotransferase	✓	✓	✓
Number of blood transfusions (last 6 months)	✓	✓	✓
Number of vaso-occlusive crises (last 6 months)	✓	✓	✓
Number of hospitalisations (last 6 months)	✓	✓	✓

The determination of foetal haemoglobin will be performed using a validated cation-exchange HPLC method with customised Bio-Rad HPLC equipment (Chumchuen et al., 2023; Leonova et al., 1996a). The quantitation of blood counts, i.e., erythrocytes, leucocytes, and platelets, will be performed using the Mindray BC-5000 haematology analyser, which has proven to be useful for high precision and accuracy and is comparable with conventional methods for determining blood counts (Xiang et al., 2015a). An alternative blood counter is the haematological autoanalyzer (SFRI blood count counter, H18 light, Saint Jean d'Ilac, France) (Asafa, Ahmed, Umar, et al., 2024). The severity of vaso-occlusive pain will be reported using the visual analogue scale (VAS), in which patients will be asked to rate their pain on a scale of 0 to 100, with 0 indicating 'no pain' and 100 indicating 'worst possible pain' (Chibueze & Chima, 2021a; Lopez et al., 2007a). The number of transfusions and hospitalisations over the last six months will be obtained from the participant's case files.

Other variables that will be collected include Weight, Height, Gender, Tribe, and Age; other disease conditions; date of birth; blood pressure readings; pulse; history of surgical procedures; other concomitant medications; smoking history; alcohol history; adherence; and other related covariates. Overall, the exposure variable for this study is hydroxyurea, and the outcome variable is sickle cell patient health outcomes i.e. pharmacodynamic data and clinical outcomes e.g. foetal haemoglobin and the frequency and severity of vaso-occlusive crises.

Post-Field Modelling Phase

Population pharmacokinetic and pharmacokinetic/pharmacodynamic models will be developed using the latest version of the Monolix Suite at the time of analysis. Exposure-response relationships, model diagnostics, and statistical summaries will be conducted in R. The previously developed PBPK model will subsequently be validated using clinical data, and subsequently, the model will be adapted for use in pregnant women and lactating mothers (Dong et al., 2023, p.426). The pregnancy and lactation PBPK models will be developed in Simcyp or PK-SIM to inform dose optimisation in special populations.

Setting and Target Population

The population will consist of sickle cell disease patients whose genetic profiles of the considered enzymes are available in the iProtecta (Implementation of Pharmacogenetic Testing for Effective Care and Treatment in Africa: Sickle Cell Disease) study portfolio. The study sites will be the Obafemi Awolowo University Teaching Hospital Complex in Ile-Ife and the Sickle Cell Foundation in Ibadan, Nigeria.

Data Sources

Objective 1: Data will be obtained from the iProtecta (Implementation of Pharmacogenetic Testing for Effective Care and Treatment in Africa: Sickle Cell Disease) study portfolio.

Objective 2: Data for method development and validation will be obtained from literature.

Objective 3 & 4: Data from Pharmacokinetic/Pharmacodynamic Field Phase will be used.

Objective 5: Data from the Pharmacokinetic/Pharmacodynamic field phase and literature (physiological data, and physicochemical properties of hydroxyurea).

Objective 6: Data from Objectives 2, 4, & 5.

DATA COLLECTION METHODS

RedCap was used to capture the clinical data for the iProtecta study, and in the process to synchronize the data collection, it will be used to collect all clinical data in this study. Two research assistants will collect data in English; hence, there will be no need to translate the tools. Data, i.e., Clinical data, will be collected at baseline (start of field data collection), at 3 months, and after 6 months, using the REDCap interface, over an 8-month period. The quality of the data collected will be ensured through daily quality checks by me, the Principal Investigator. Where discrepancies are observed, a review of the data will be conducted with the research assistant, and, if necessary, a re-collection of the data will be conducted within 7 days of the first data collection. Prior to the onset of clinical data collection, the two research assistants will be trained by me on obtaining consent, extracting data from participants' case files, and documentation. A trained phlebotomist will be hired to collect blood samples and perform haematological analyses.

DATA TYPES AND FORMATS

All data collected during this study will be quantitative. As detailed in Table 2 above. Data will be saved as CSV files for importation into the analysis software.

DATA STORAGE AND BACKUP

Data will be extracted weekly from the REDCap account, and each version will be saved on my personal laptop, OneDrive account, Google Drive account, and an external hard drive as backups. These transfers will be done every Friday on or before 12 midnight.

DATA SECURITY AND CONFIDENTIALITY

Data entered will be transformed after collection to remove the primary personal identifiers, which will only be available to me. At any point where the data will be relayed to my supervisors or analysed with any software, identifiers will have been extracted to ensure confidentiality and anonymity. The folders containing the results on my personal laptop, cloud drives, and the external hard drive will be protected by passwords available only to me.

All hard-copy entries (in case of internet failure or power outage during data collection) will be stored in an enclosed file in my physical office at Obafemi Awolowo University, Ile-Ife, Nigeria, after being entered into the REDCap account.

DATA CLEANING AND PROCESSING

The data collected per day will be assessed for missing data and outliers. If any data is missing, efforts will be made the following day to capture it. If an outlier, attempts will be made to recapture the data. If data recapturing is impossible, the analysis will be attempted with and without the outlier, and the decision to keep or remove the outlier will be made based on the effects of the change. General cleaning of the dataset will be done each week to remove duplicates and resolve any other inconsistencies.

DATA ANALYSIS PLAN

Inclusion Criteria

For a patient's data to be deemed fit for inclusion in the pop PK analysis, the patient must have received at least one dose of HU and have at least six measurable HU concentrations using the validated quantitative analytical method. The criteria for including patient data in the exposure-response/PK-PD data are similar to the Pop PK criteria. However, additional criteria include a measurable Cmax concentration and sufficient time points for AUC calculation.

Handling of Missing, Outlier, and Erroneous Data

The different methods of handling missing data and concentrations below the limit of quantification (BLQs), as described by Beal et al, would be explored, i.e., the M1 to M6 method (Beal, 2001a).

Exploratory Data Analysis will be done before model building, and in the process, outliers will be identified. These outliers will be flagged and may be excluded from the analysis if they significantly affect model and covariate model building. In accordance with accepted population PK reporting guidelines, the final population PK model will be rerun using the entire dataset (i.e., including the outliers). A list of the excluded individuals and/or data points, along with the reasons for exclusion, will be provided in the report.

Non-Compartmental Analysis

Individual PK parameters will be estimated using non-compartmental analysis methods. Parameters to be estimated with the non-compartmental analysis include the AUC and the C_{max}, which would be used for the exposure-response modelling.

Software

The Monolix software, a software package for nonlinear mixed-effect analysis (Lixoft, France) will be used for population PK modelling. Simulx (also a part of the Monolix Suite) and/or R will be used for simulations to derive exposure metrics for the subsequent exposure-response analysis. R will be used for graphical analysis, model diagnostics, and statistical summaries. Exposure-response (E-R) analyses will be performed using R, and software version numbers will be documented in the report.

Model Development

The dataset will be run with model files for one-, two-, and three-compartment models written in Mlxtran to determine the structural model. Different absorption models, such as those that include lag time and transit compartments, will also be explored. Improvements in objective function values, likelihood ratio tests, and inspection of diagnostic plots will guide the choice of the structural model. An exploration of the different error models (constant, exponential, proportional, and combined) will also be conducted to determine which model best fits the structural model. Basic goodness-of-fit plots (arithmetic/log scale) of individual fits, observations versus predictions, scatter plots of residuals, distribution of residuals, visual predictive check, numerical predictive check, and prediction distribution will be used to diagnose the structural and statistical models. The Student t-test, Pearson correlation test, and/or ANOVA will be used to analyse the statistical models.

Covariate Selection for the Population PK Model

The covariates to be tested in the model are ethnicity, weight, age, BMI, and genetic polymorphism, as they are clinically relevant in pharmacokinetics. A covariate will be included in the final model if it is available for at least 80% of the study population and if it can be explained biologically and clinically. Significant covariates in the developed model with an α (significance level) of 0.10, adjudged from a combination of relevant statistics (Wald's test, Likelihood ratio test, etc.), will be selected using a forward selection/backward deletion approach.

Correlations between Random Effects

Correlation coefficients between random effects will be explored in a stepwise process. A significant reduction in the objective function value will be used as the criterion for adding the correlation to the model. The correlations will be biologically and statistically justified.

Final Population PK Model

The final pop PK model, after optimising the base model and developing the covariate model, should meet the following criteria.

- No estimates close to a boundary.
- Relative errors of the estimates should preferably be < 30% for fixed effect parameters and <50% for random effect parameters.
- No acceptable trends in goodness of fit (GOF) plots.

Evaluation of the Final Population PK Model

A nonparametric bootstrap analysis will be conducted to evaluate the stability of the final model and to estimate confidence intervals (CIs) for the model parameters. The bootstrap analysis will be performed with 1000 replicates of the dataset, generated by random resampling of subjects from the original dataset with replacement. Visual predictive checks (VPCs) will be

used to evaluate the predictive ability of the final model. If required, VPCs will be performed with prediction correction. Plots of observed data distributions will be compared to simulated distributions to demonstrate the model's ability to predict the data on which the model is based adequately. VPCs may be based on 500 simulations and may be stratified by covariates of potential interest. Other goodness-of-fit plots that will be used include observations versus prediction plots, scatter plots of residuals, distribution of the residuals, visual predictive checks, numerical predictive checks, and prediction distribution.

Objective 3: **EXPOSURE-RESPONSE ANALYSIS**

The final population PK model of HU will be used to predict rich concentration-time profiles based on Posterior Bayesian estimates. Exposure metrics, such as the area under the curve over 24 hours ($AUC_{0\text{-}24}$), the AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), and the maximum concentration (C_{max}), will be derived from the non-compartmental analysis. The relationship between response data (Crisis versus No Crisis in 6 months) and exposure metrics (AUC0-∞, Cmax) will be established using nonlinear regression. Various structural models, such as linear and saturable structures, will be applied to the data and assessed for their capacity to characterise the exposure-response relationship sufficiently. Simulations will be performed to evaluate the efficacy of different treatment dose options.

An assumption that there is a direct non-linear relationship between the exposure ($AUC_{0\text{-}\infty}$) and the response (crisis or no crisis):

$$Probability\ of\ no\ crisis = \frac{E_{max} \times AUC_{0-\infty}^{\gamma}}{E_{50}^{\gamma} + AUC_{0-\infty}^{\gamma}}$$

Where,

E_{max} = maximum effect

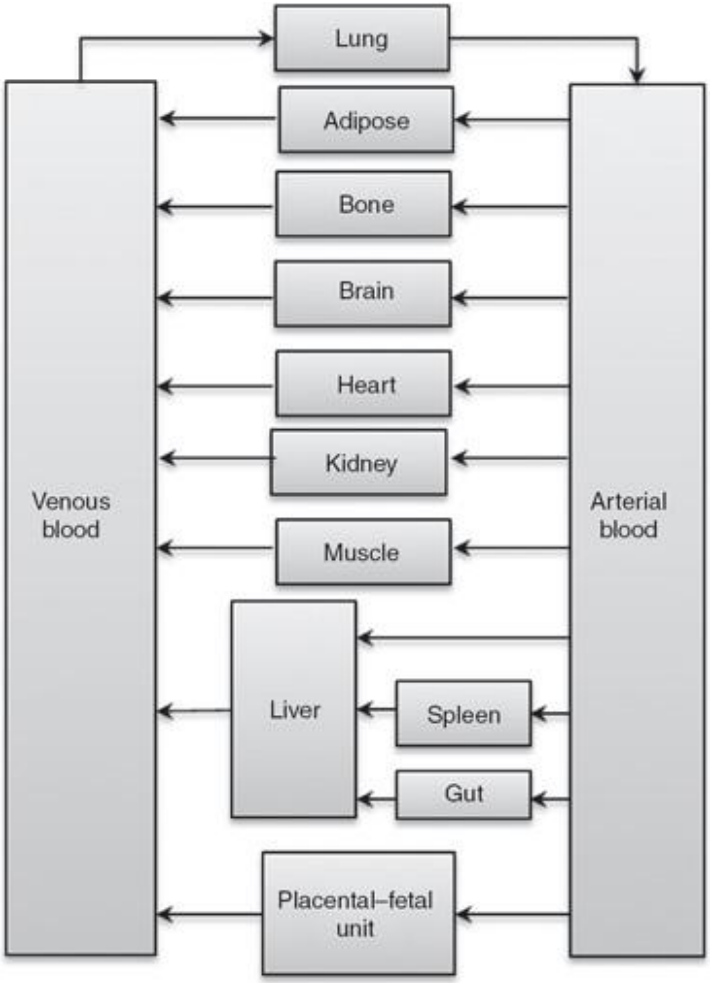
$AUC_{0\text{-}\infty}$= Exposure

E_{50} = Exposure that will produce 50% of the maximum effect

γ = slope factor

Objective 4: PBPK Analysis

The method of developing the pregnancy physiologically-based pharmacokinetic model (PBPK) model will be used as detailed by Atoyebi et al. (2024) (Atoyebi et al., 2024a). Summarily, the PBPK model will be developed by incorporating mathematical equations that describe the human biological system and HU-specific parameters. The anatomical, physiological, and demographic data available in the literature and obtained from the pharmacokinetic analysis performed in 'objective 1' above would be used to define the biological system parameters. The drug-specific parameters will comprise data on the physicochemical properties of the drug (e.g. pKa and lipophilicity) and in vitro data available (e.g. fraction of the unbound drug in plasma and intrinsic enzymatic clearance of the drug).



Schematic description of pregnancy physiologically based pharmacokinetic (p-PBPK) model Source: Gaohua et al., 2012 (Gaohua et al., 2012a).

The full-body adult PBPK and paediatric model will be developed in Simcyp®, a product of Certara, U.S.A. The modelling process will start with the simulation of a virtual cohort of 100 healthy adult individuals and a healthy paediatric population, which will be used to validate the non-pregnant adult PBPK model and paediatric population. The ratio of males to females in this virtual cohort will be set at 50:50 to reflect the mixed-gender composition of clinical studies in non-pregnant and paediatric populations. Another cohort of 200 healthy volunteers, comprising 100 females and 100 paediatric patients, will also be simulated using the pregnancy PBPK model and the paediatric PBK model, respectively, to represent the pregnant and paediatric populations.

Organ weights will be determined using an anthropometric equation (Bosgra et al., 2012a). Organ volumes will be calculated from the organ weights and the respective organ densities, and regional blood flows to organs and tissues will also be calculated as fractions of the cardiac output (Brown et al., 1997a). A foetal component will be included in the pregnancy PBPK model to accommodate the estimation of the drug concentration in the foetus. Similarly, a lactation PBPK will be built to estimate the disposition of HU in lactating mothers and their infants.

Ethical Considerations

Ethical Approval: Ethical Approval will be obtained from the Research Ethics Committee of the Obafemi Awolowo University Teaching Hospital Committee.

Informed Consent: The study protocol will be explained to the study participants. Upon acceptance to participate in the study, participants will be given an informed consent form to review and sign. Questions related to the study protocol will be addressed, and participants will then provide written informed consent. Participants will not be subject to any form of coercion to participate in the study and may withdraw at any time.

Benefits to Patients: The study will guide tailoring hydroxyurea use for each patient, and its results will be published for the benefit of society.

Harm to Study Participants: Study Participants will be exposed to minimal risks, including slight pain and discomfort, during blood sample collection. There is also a minimal risk of infection, as all protocols will be followed to protect all parties involved from cross-infection.

Confidentiality and Data Security: Study participants will be assigned personal identifiers, with the code linking the data to the participant's personal information only available to me, the principal investigator.

Conclusions

Expected Outcomes

This study will therefore provide scientific knowledge on the varying responses of sickle cell disease patients to hydroxyurea, as well as the effects of genetic polymorphism and other covariates on the pharmacokinetics and pharmacodynamics of hydroxyurea in the Nigerian population. Using a physiologically based pharmacokinetic model developed for pregnant women, lactating mothers, and neonates, the safety and efficacy of hydroxyurea could be assessed, and possible dose adjustments recommended for these special populations. Using pharmacometrics tools, this study will demonstrate how technology-driven analysis can be instrumental in clinical decision-making, particularly in low-resource settings such as Africa, where conducting full-scale clinical trials is challenging due to resource constraints and cultural factors.

Proposed Research Outputs

Objective 1:

Paper 1 – Genetic Polymorphisms of Metabolic Enzymes and Transporters Associated with Medications in Sickle Cell Disease Management in Nigeria

Paper 2: Pharmacogenetics in the Disposition of Hydroxyurea in Sickle Cell Disease Patients: A Scoping Review

Objective 2:
Paper 3: Development and Validation of an Analytical Method for the Quantification of Hydroxyurea in Human Matrices

Objective 3:
Paper 4: Population Pharmacokinetics Modelling of Hydroxyurea in Sickle Cell Disease Patients of Nigerian Descent

Objective 4:
Paper 5: Pharmacokinetic-Pharmacodynamic Modelling of Hydroxyurea in Sickle Cell Disease Patients of Nigerian Descent.

Objective 5
Paper 6: Development of a Physiologically Based Pharmacokinetic Modelling of Hydroxyurea in Pregnant Women of African Descent.

Scope and Limitations
This study is limited to the Southwestern part of Nigeria; however, the data collected will still be representative of the Nigerian population because the teaching hospital, the central data collection site, welcomes people from different parts of the country. Also, the study participants will be the HbSS patients only, as they represent the major haemoglobinopathy group. Development of a method for routine quantification of foetal haemoglobin might be the most challenging, as there are no clearly documented, reproducible methods in the literature for low and middle-income economies. Hence, the option of the mean corpuscular volume as an alternative to foetal haemoglobin.

Overall, this study will provide scientific knowledge that can enhance clinical decision-making in sickle cell management for the Nigerian population. The models could serve as a template to optimise hydroxyurea use in this population, and this approach can be extrapolated to the broader African population.

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Appendices

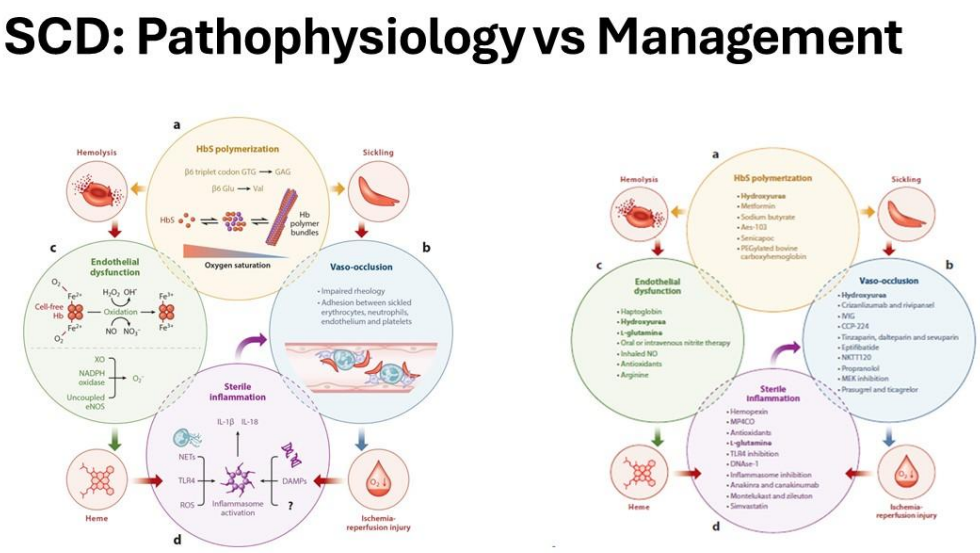
Appendix 1: Glossary of Acronyms/Abbreviations

1. SCD – Sickle Cell Disease
2. SCA – Sickle Cell Anaemia
3. VOC – Vaso-occlusive Crisis
4. HU – Hydroxyurea
5. PK – Pharmacokinetics
6. PD – Pharmacodynamics
7. PGx – Pharmacogenetics
8. PBPK – Physiologically Based Pharmacokinetic Modelling
9. HbF – Foetal Haemoglobin
10. MTD – Minimum Toxic Dose / Maximum Tolerable Dose
11. MCV – Mean Corpuscular Volume
12. IIV – Inter-individual Variability
13. Pop PK – Population Pharmacokinetics
14. CYP – Cytochrome
15. HPLC – High Power Liquid Chromatography
16. LC-MS/MS – Liquid Chromatography – Mass Spectrometry/Mass spectrometry
17. Hb – Haemoglobin
18. NLME – Non-Linear Mixed Effects

Appendix 2: Brief Explanation of Technical Terms

1. Pharmacokinetics is the study of how the body affects drugs i.e. it describes how a drug is absorbed, distributed, metabolised and eliminated. The concept of pharmacokinetics originated from the work of Paracelsus (medieval alchemist), whose philosophy was ‘only the dose makes the poison’, such that therapeutic effects are achieved when the drug fall within a particular window, when it is below the range, there is no therapeutic effect, and when it is above it, there are harmful effects.
2. Pharmacodynamics is the effect of the drug on the body.
3. Pharmacogenetics is the study of the influence of differing genetic variability on drug response, either on the pharmacodynamics or the pharmacokinetics.

Appendix 3: Figure showing the comparison of SCD Pathophysiologic and management paths



Adapted from Sundd et al., 2019

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