UNIVERSITY OF JOS

Faculty of Pharmaceutical Sciences

RESEARCH PROPOSAL

**Topic:** Effect of cytochrome P450 2B6 (CYP2B6) single nucleotide polymorphism on the safety and effectiveness of efavirenz in HIV-1 infected Nigerians

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**Abstract**

The extremely high prevalence of HIV/AIDS in sub-Saharan Africa and limitations of current antiretroviral medicines such as high rates of adverse drug reactions and development of resistance in a significant proportion of patients, demand new tools to optimize therapy such as pharmacogenomics for person-to person variations. Efavirenz, a first line antiretroviral medicine in Nigeria, exhibits a pharmacogenetic dependent large plasma concentration variability which can impact its efficacy (safety and effectiveness).

**Aim:** This study aim to identify sources of variability in efavirenz adverse effects and effectiveness with a focus on genetic variation in CYP2B6 a the major enzyme in efavirenz metabolism

**Study design:** Using a prospective cohort study design, the functional single nucleotide polymorphisms (SNPs), CYP2B6:516G>T (rs3745274) defining the allele CYP2B6\*6 would be characterized in 124 HIV-infected adult Nigerian patients on efavirenz-containing highly active antiretroviral therapy (HAART) would be characterized. Study subjects will be followed for one year assessed for neuropsychiatrics toxicity, treatment adherence and immunologic and virologic failure.

**Data collection**: At baseline socio-demographic and clinical information shall be documented. Also venous blood shall be obtained for the following: genetic analysis, CD4 cell count determination, blood chemistry, hepatitis B serology. Neuropsychiatric screening shall be performed at baseline, 2, 4, 24, and 48 weeks, while drug refill adherence will be measured monthly for 12 months.

**Statistical analysis:** Hardy–Weinberg equilibrium will be calculated to evaluate the genotype frequency distribution. The association of the CYP2B6 G516T genotype with variations in serum levels of laboratory variables would be determined using the student T-test and Two way analysis of variance or non-parametric Mann–Whitney test and Kruskal–Wallis test and depending on the distribution of the variables and the number of sub-groups. Epidemiological measures of association between the main exposure CYP2B6 G516T genotype and the outcome measures: neuropsychiatric ADR, poor adherence, immunologic failure, and virologic failure) such as relative risk (risk ratio), risk difference (RD) or attributable risk (AR), and attributable proportion (attributable risk %) will be calculated.

# Background

In recent years, support from international agencies and donors have resulted in a rapid scale up in access to antiretroviral therapy (ART). As of December 2015, 17 million people living with HIV were accessing antiretroviral therapy, including 10.7 million people in sub-Saharan Africa (SSA) (UNAIDS, 2015). The introduction of combination antiretroviral therapy (cART) represents one of the most significant discoveries is changing the landscape of HIV related mortality (Montaner *et al*., 1998). ART substantially modified the natural history of HIV infection and reversed the curve of the epidemic. The number of AIDS-related deaths worldwide averted by access to ART between 1995 and 2013 is estimated to be 7.6 million, including 4.8 million deaths in sub-Saharan Africa (UNAIDS, 2014). Other benefits of ART documented in previous studies which has enormous public health benefits include prevention of disease progression, opportunistic infections, tuberculosis (TB) and HIV transmission, (Dieffenbach & Fauci, 2011; Palella  Jr *et al*., 1998). It is projected that expanded access to treatment in some settings could eventually lead to the elimination of HIV(Granich *et al*., 2015).

Nigeria is not left out in the scale up of access to ART. The country, with an estimated 3.5 million people living with HIV (about 10% of all persons living with HIV globally in 2013), has witnessed an upsurge in the number of HIV-infected persons accessing life-saving ART (UNAIDS | AIDinfo, 2014). A 2015 report by the National Agency for the Control of AIDS (NACA) shows that coverage of ART services in Nigeria increased from 25 health facilities in the year 2002 to 820 in 2013(National Agency for the Control of AIDS (NACA), 2015). According to the report, between 2009 and 2014, the number of persons receiving ART more than doubled, from 302073 to 747 382, respectively. Most persons on ART in Nigeria are on (efavirenz) based ART in accordance to the Nigerian National ART treatment Guideline (Federal Ministry of Health (FMoH) Nigeria, 2010).

Efavirenz is a non-nucleoside reverse transcriptase inhibitor that has demonstrated high antiretroviral efficacy in several clinical trials and is currently a first line drug for the treatment of human immunodeficiency virus (HIV) infection in several countries (Maggiolo, 2009b; Staszewski et al., 1999). The World Health Organization (WHO) in its 2013 consolidated guideline for the management of HIV infection in adults and adolescents recommended EFV-containing antiretroviral (ARV) regimen as the preferred first line regimen (World Health Organization (WHO), 2013). The implementation of this public health strategy for the management of HIV infection has resulted in rapid scale-up in the use of EFV-based regimens, especially in resource-limited settings including Nigeria. In Nigeria, it is the recommended first-line regimen for the management of HIV in adults and adolescents (Federal Ministry of Health (FMoH) Nigeria, 2010).

Despite the reported efficacy, EFV use has been associated with an array of adverse effects (Fumaz *et al*., 2002; Hawkins *et al*., 2005; Maggiolo, 2009). Neuropsychiatric disorders are the most common and significant adverse effects associated with EFV therapy. Others include rash, lipodystrophy, and gynecomastia (Agbaji *et al*., 2011; Fumaz *et al*., 2002; Hawkins *et al*., 2005). A recent study reported a neuropsychiatric disorder incidence 40.3 per 1000 among adult Nigerians exposed to EFV (Abah *et al*., 2015).

Adverse drug reactions associated with the use of antiretroviral medicines (ARVs) can rapidly reverse the gains of ART resulting in poor health outcomes (Syed, Sulaiman, Hassali, & Lee, 2015) and increased mortality (Keiser *et al*., 2007). Adverse drug reactions to ART is recognized as one of the leading causes of reduced quality of life in patients living with HIV/Acquired immunodeficiency syndrome (AIDS) and results in an increase in the direct and indirect cost of HIV management with economic burden to the HIV-infected patients as well as to the society (Kingston-Riechers, 2011). In addition, ADRs is one of the leading causes of non-adherence and treatment discontinuation among patients on HAART. Furthermore, Non-adherence to therapy resulting from ADRs can rapidly reverse the gains of antiretroviral therapy, resulting in poorer health outcomes, increased HIV transmission, and an emergence of drug resistance (Parienti *et al*., 2004; Renaud-Théry *et al*., 2007).

EFV exhibits significant inter-individual pharmacokinetic and pharmacodynamic variability as well as a narrow therapeutic window. Among the factors affecting EFV pharmacokinetics are ethnicity, host genetic factors, gender, body weight, drug interactions, binding to plasma proteins, hepatic impairment, disease status and (Burger et al., 2006). Studies have reported ethnic variability in the incidence of neuropsychiatric disorders associated with EFV therapy. In U.S and European cohorts, one-half of patients have neuropsychiatric symptoms after initiating EFV therapy, but these symptoms usually resolve within one month (Molina et al., 2000). People of African ancestry with a variant of hepatic enzyme CYP2B6 may experience slower clearance of EFV from plasma and increased neurotoxicity (Barrett et al., 2002; Pfister et al., 2003). Ngaimisi and his colleagues reported significant differences in efavirenz pharmacokinetics, the extent of auto-induction and immunologic recovery between Ethiopian and Tanzanian HIV patients, partly but not solely, due to pharmacogenetic variations (Ngaimisi et al., 2013). In the study, Patient country, CYP2B6\*6 and ABCB1 c.4036A.G (rs3842A.G) genotype were identified as significant predictors of plasma and intracellular efavirenz concentration.

CYP2B6 is primarily responsible for EFV metabolism, to the major metabolite 8-hydroxy-EFV (V Michaud et al., 2012). The human CYP2B6 gene is highly polymorphic. Variant alleles associated with the lower expression include CYP2B6\*6, CYP2B6\*16, and CYP2B6\*18 (V Michaud et al., 2012). Among the identified polymorphic alleles, CYP2B6\*6, predominantly the 516G>T polymorphism is the most significant allelic variant of the CYP2B6 gene, in which the TT genotype is related with a slower metabolism of EFV and a natural increase in the plasma concentration of the drug (V Michaud et al., 2012). This polymorphism occurs due to a switch from nucleotide G to T at position 516 of exon 4 of the CYP2B6 gene, which causes the replacement of the amino acid glycine (Gly) with histidine (His) at position 172 (Gounden, van Niekerk, Snyman, & George, 2010). The presence of the CYP2B6 G516T polymorphism promotes a reduction of up to 75% in EFV clearance and has been associated with increased EFV plasma concentrations. High plasma concentrations of these drugs may lead to adverse reactions, such as CNS and liver toxicity. These reactions may compromise the treatment adherence in these individuals and cause an increase in the viral load and the selection of viruses with resistance mutations, thus limiting ART options.

Of these, CYP2B6\*6 is more common in Africans than in Caucasians (Thorn CF, Lamba JK, Lamba V, Klein TE, 2010). The high frequency of the 2B6\*6 allelic expression in a variety of ethnic populations, suggest the conduct additional post-authorization safety or efficacy (PASS/PAES) studies to minimize risk associated with interethnic differences in EFV efficacy and safety due to variations in prevalence of pharmacogenetic polymorphisms. As part of pharmacovigilance plan, many drug regulatory authorities now require data regarding relevant genomic BMs relating to efficacy or safety of a new medicinal product, including patient selection or dose specification for genomic subpopulations, available at time of marketing authorisation. However, it is not always feasible to gather information about these subpopulations during clinical trials. Hence, pharmacogenomic data collection in the post-authorisation phase has a potential to elucidate any association with genomic BMs to improve the benefit-risk balance of the medicinal product in ethnic subpopulations. The pharmacogenetics and pharmacokinetics of EFV have been widely studied in western countries, but data in the Nigeria population are not available.

# Literature Review

**Efavirenz in treatment of HIV**

The efficacy of EFV in the treatment of HIV infection has been documented in several studies. In comparison to other non nucleoside reverse transcriptase inhibitors, Bock, Fatti, & Grimwood, 2013, compared the efficacy of EFV and nevirapine in ART naïve African adults in a prospective multicentre cohort study. At initiation, 19 441 (71.1%) patients started EFV and 7909 (28.9%) started NVP treatment.  After a median follow-up period 9.5 months (IQR 4.6–17.7), viral load suppression at 6 months was higher in the EFV cohort overall (adjusted odds ratio [AOR] = 1.29, 95% CI 1.05–1.59), while mortality was similar in the two cohorts, (adjusted HR = 1.07, 95% CI 0.89–1.28). Patients starting on EFV were 47% less likely to change regimen (AOR = 0.53, 95% CI 0.48–0.59). The duration of follow up in this study was however too short to permit thorough safety evaluation (Bock, Fatti, & Grimwood, 2013).

When compared to protease inhibitors, the effectiveness of EFV has been shown to be non-inferior to protease inhibitors (PI)-based ART. Imaz *et al*., 2014, in a recent multicentre, observational cohort study, including 596 consecutive treatment-naive adults with plasma HIV-1 RNA>100,000 copies/ml initiating efavirenz or PI/ritonavir (r)-based ART between 2000 and 2010 in Spain showed HIV-1 RNA suppression to <50 copies/ml at week 48 was higher in the efavirenz compared to PI/r (84% versus 74% [difference 10%, 95% CI 3.4%, 16.7%; P=0.002]). The percentage of virological failures was similar (efavirenz 4% versus PI/r 4%; P=0.686), while voluntary discontinuations and toxicity-related treatment changes were higher with PI/r (4% versus 1%; P=0.006 and 11% versus 6%; P=0.069, respectively). However, resistance selection at failure was higher in patients receiving efavirenz (89% versus 50%; P=0.203). Efavirenz was significantly more effective than lopinavir/r or fosamprenavir/r, whereas no significant differences were observed between efavirenz and darunavir/r or atazanavir/r , (Imaz *et al*., 2014).

**Efavirenz metabolism and pharmacogenomics**

An overview of EFV metabolism illustrated in Michaud et al(2012) show that it is mainly metabolised by the cytochrome P450 2B6 (CYP2B6) isoenzyme. This enzyme belongs to the super-family cytochrome P450 enzyme system (CP450s). The main families of the CP450 enzyme are CYP1, CYP2, and CYP3. CYP2B is a sub-family of CYP2 and *CYP2B6* is the only identified gene belonging to the CYP2B sub-family in humans. Efavirenz is mainly metabolized by CYP2B6 into 8-hydroxyefavirenz and less so via accessory pathways involving CYP2A6, CYP3A4/5, and UGT2B7(Desta et al., 2007; Mutlib et al., 1999; Ward, 2003). The CYP2B6 protein is expressed mainly in the liver(Ortiz de Montellano, 2005). CYP2B6 is also found in various extrahepatic tissues such as the brain, kidneys, endometrium, peripheral circulating lymphocytes, and skin (Ding & Kaminsky, 2003; Gervot et al., 1999). In addition to being a substrate of CYP2B6, efavirenz can induce its own metabolism (self-inducer of CYP2B6) (Robertson et al., 2008; Zhu, Kaul, Nandy, Grasela, & Pfister, 2009). As such, the partial metabolic clearance of efavirenz would be responsible for around 90% of its systemic clearance (Ward, 2003).

**Frequency of CYP2B6 polymorphism in Africans**

The *CYP2B6* gene is highly polymorphic. To date, more than 28 alleles have been characterized and more than 100 mutations (SNPs) have been described for the *CYP2B6* gene. Among different variants, the *CYP2B6\*6* haplotype (*516 G*\_*T*, *785* *A*\_*G*) leads to reduced catalytic activity and a significant decrease in protein expression (Veronique Michaud et al., 2012). Variability has been reported in the frequency of *CYP2B6\*6* haplotype in different populations. The first report of the CYP2B6 allelic frequencies in African populations revealed that the CYP2B6\*6 allele was present at a considerably higher frequency in African–Americans (32.8%) and a Ghanaian population (46.9%), when compared to Asians (15.9–18.0%) and Caucasians (25.6%) (Klein *et al*., 2005). A review of different genomic studies in Africans by Alessandrini *et al* (2013)confirmed the increased frequency of CYP2B6\*6 in various African populations, ranging from 17% to as high as 60%. Other, African-focused studies have reported the 516G4T mutation, which is generally regarded as being specific for CYP2B6\*6, at frequencies of 30–35.3% in various South African populations(Cohen et al., 2012; Haas et al., 2005; Parathyras et al., 2009), 30.4% in Ugandans (Penzak *et al*., 2007), and 48.6% in Zimbabweans (Nyakutira *et al*., 2008). Matimba *et al.,* (Matimba *et al*., 2008) reported CYP2B6\*6 allelic frequency of 38%, 42% and 42% among Ibo, Hausa’s and Yoruba’s in Nigeria

**Effect of Genetic polymorphisms of CYP2B6 on EFV pharmacokinetics and pharmacodynamics**

Oral administration of a daily dose of 600 mg of efavirenz is associated with wide inter-individual variability in plasma concentrations (Csajka et al., 2003; Marzolini et al., 2001; Wang et al., 2006). Many studies have reported an association between genetic polymorphisms of CYP2B6 and the pharmacokinetics of efavirenz (Haas *et al*., 2005; Carr *et al*., 2010; Chen *et al*., 2010). Tsuchiya *et al* (2004) reported an increase in efavirenz plasma concentrations among *CYP2B6\*6/\*6* individuals. Wang *et al*. in 2006 showed that the concentrations of steady-state efavirenz were higher in Africans who were carriers of the *CYP2B6\*16* allelic variant than in other patients (Wang et al., 2006).

Cabrera *et al* (2009) developed a population pharmacokinetics model to study the effects of various covariables (such as gender, age, weight, duration of antiretroviral treatment and genetic polymorphisms of CYP2B6, CYP3A4, and the ABCB1 transporter) on the pharmacokinetics of efavirenz. Their study reported that the genetic polymorphism of *CYP2B6* could explain around 27% of the variance in efavirenz clearance (Cabrera et al., 2009). Lubomirov et al (2011) evaluated the association of recognized and proposed genetic markers of toxicity or elevated plasma drug levels over time to antiretroviral discontinuation during the first year of a first-line regimen. They reported an association between various genetics variants with different rates of efavirenz discontinuation. Their analysis indicates that loss of CYP2B6 function (homozygous, loss or decrease of functional alleles; *CYP2B6\*6*, *\*11*, *\*15*, *\*18*) with a concomitant reduction of function in accessory metabolic pathways (CYP2A6 and/or CYP3A4) was associated with a higher risk of discontinuation (Lubomirov et al., 2011). Patients having the highest genetic risk score discontinued efavirenz more frequently than those with a lower genetic risk scores (cumulative rates of 72 versus 28%, respectively)

**Pharmacogenetics and Toxicity Associated with Efavirenz**

Generally, there appears to be a good relationship between CYP2B6 genotype and phenotype. Clinical studies to date have reported that alterations associated with decreased metabolism (primarily CYP2B6\*6, \*16 and \*18) tend to result in increased substrate plasma concentrations, decreased drug clearance and neuropsychiatric side effects (Haas et al., 2004; Wang et al., 2006).

**Summary of literature review**

Several studies have demonstrated that the appearance of neuropsychiatric symptoms might be associated with high plasma concentrations of efavirenz and genetic polymorphism of *CYP2B6* could explain about 27% to 50% of the variance in efavirenz clearance. Hence patients with high plasma concentrations of efavirenz were more likely to experience adverse effects in the central nervous system. However, what is not very clear is the interaction between the pharmacogenetically modified EFV pharmacokinetics and the host CD4 + response and viral suppression. The results of CYP 2B6 polymorphism on the HIV treatment response are mixed. An analysis of the role of CYP2B6 SNP on clinical response among Ghanians showed that immunological failure was significantly associated with the GG genotype of CYP2B6 516G>T compared with the TT genotype; hazards ratio of 1.70 (1.04–2.76; P=0.03) (Sarfo et al., 2014). In contrast to the findings of Sarto et al., 2014, a Brazilian study reported that Individuals of EFV based therapy with the TT polymorphic genotype presented significantly lower CD4+T-cell counts compared to patients with other genotypes (Queiroz et al., 2017).

**Statement of problem**

Despite the reported efficacy of efavirenz (Staszewski *et al*., 1999), and wide acceptance of EFV in the management of HIV infection, EFV use has been associated with an array of adverse effects(Fumaz et al., 2002; Hawkins et al., 2005; Maggiolo, 2009a). Neuropsychiatric disorders are the most common and significant adverse effects associated with EFV therapy. A recent study reported a neuropsychiatric disorder incidence 40.3 per 1000 of among adult Nigerians exposed to EFV (Abah et al., 2015). Others include rash, lipodystrophy, and gynecomastia (Agbaji et al., 2011; Fumaz et al., 2002; Hawkins et al., 2005). EFV-associated adverse events may compromise adherence to treatment and lead to treatment discontinuation. Some studies have reported treatment discontinuation rates ranging from 4% to 46% related to neuropsychiatric side effects of EFV (Bartlett, Chen, & Quinn, 2007; Spire, Carrieri, Garzot, L’henaff, & Obadia, 2004). Poor adherence to treatment and treatment discontinuation are risk factors for treatment failure and development and selection of drug-resistant virus. The development of drug resistance has a public health implication for transmission of drug-resistant HIV virus and the need to implement treatment with much expensive second-line and salvage therapies. This could be a major threat to ART program success and sustainability in sub-Saharan Africa where most of the programs are donor dependent.

EFV exhibits significant inter-individual pharmacokinetic and pharmacodynamic variability as well as a narrow therapeutic window. The enzyme CYP2B6, which is primarily responsible for EFV metabolism to the major metabolite 8-hydroxy-EFV (Desta et al., 2007) is highly polymorphic. There is a high frequency of the 2B6\*6 allelic expression in a variety of ethnic populations: CYP2B6\*6 is more common in Africans than in Caucasians (Thorn CF, Lamba JK, Lamba V, Klein TE, 2010), in South Africans, the frequency of CYP2B6\*6 allele ranged from 30-36% (Warnich, Drögemöller, Pepper, Dandara, & Wright, 2011), 37% in Botswana (Caudle, Yang, Mittendorf, & Kuerer, 2008) and 49% in Ghanaians and Zimbabweans (Klein et al., 2005; Nyakutira et al., 2008).

Africans show exhibit the greatest genetic diversity in CYP2B6, thus making it difficult to extrapolate findings from one population to another. Furthermore, despite the rapid scale up in the use of EFV in many African countries since 2013, following the adoption of the WHO recommendation on the use of EFV as part of first line antiretroviral regime for the treatment and management of HIV in adults and adolescents(World Health Organization (WHO), 2013), Africans have been largely left out in pharmacogenomic studies. There are little or no data on the impact of EFV pharmacogenomics on its toxicity profile and treatment outcomes among Nigerian HIV infected patients on EFV-based regimen. The frequency of 2B6\*6 allele in HIV patient population is known. Nigeria is home to10% of all persons living with HIV globally. An estimated 639,397 persons were on ART in Nigeria in 2013 (National Agency for the Control of AIDS (NACA), 2014), with over 60% of these being on EFV-based regimen in accordance with the national treatment guideline.  There is, therefore, an overarching need to conduct locally relevant pharmaco-epidemiological studies to inform programmatic decisions given the shift towards EFV-containing regimen for the management of HIV infection.  Additionally, there is paucity of prospective data on the effect of CYP2B6 SNP on treatment outcomes such as CD4 cell improvement and HIV viral suppression

**Justification for the study**

The purpose of this study is to assess the role of selected SNP in the CYP 2B6 gene on EFV pharmacodynamics including ADRs in a clinic cohort of HIV-infected Nigerian adults on ART. The study will also evaluate the effect CYP 2B6\*6 allele on the incidence of neuropsychiatric disorders and related this to key HIV treatment outcomes of immunological responses and viral suppression. Understanding the risk factors for poor treatment response in a given population can facilitate the identification of patients at risk of ADRs which can result in appropriate steps such as the use of alternative regimens or closer monitoring of patients for early detection and management of ADRs when they occur. This will contribute to the improved durability of first-line regimens (Perović Mihanović, Haque, Rutherford, Zekan, & Begovac, 2013). In addition, understanding the association between ADRs and viral suppression can be exploited in the rational selection of ARVs regimens to achieve maximal and durable viral suppression as well as foster retention of patients on treatment (Perović Mihanović et al., 2013). Furthermore, data derived from within the country or region may have greater relevance and educational value and may encourage national regulatory decision-making (World Health Organization (WHO), 2002).

**Research questions**

1. How common is CPY2B6 SNP polymormphism in HIV-1 infected patients in our study setting
2. Does CYP2B6 SNP increase the risk of neuropsychiatric effect in patients on EFV-based ART during the first year of ART?
3. Does CYP2B6 SNP increase the risk of heptatic toxicities during the first year of EFV-based ART?
4. Can CYP2B6 SNP independently predict the risk of neuropsychiatric disorders during the first year of EFV-based ART?
5. What is the predictive value of CPY2B6 SNP on poor treatment outcomes of poor adherence, immunologic failure, and virologic failure during first year of EFV-based ART

**Research hypothesis**

1. HO1: CYP2B6:516G > T SNP does not significantly increase the risk of neuropsychiatric disorder in the first year of EFV-based ART
2. HO2 CYP2B6:516G > T SNP does not significantly increase the risk of hepatic impairment in the first year of EFV-based ART
3. H03: CYP2B6: 516G > T SNP does not significantly impact on adherenc to EFV-based ART in the first year of treatment
4. H04: CYP2B6: 516G > T SNP does not significantly increase the risk of immunologic failure in the first year of EFV-based ART
5. H05: CYP2B6:516G > T SNP is not an independent predictors of virologic failure in the first year of EFV-based ART

# General Aims and Specific Objectives

**Aim**

This study aims to identify the frequency of CYP2B6:516G > T SNP in HIV-1 infected persons on ART at the study site and to determined whether the presence of the polymorphism was related to neuropsychiatric and liver toxicity and changes in CD4+ count and the plasma HIV-1 viral load. Furthermore, the study will evaluate the relationship of neuropsychiatric toxicities to treatment adherence during the first year of ART.

**Specific objectives**

1. To describe the prevalence of CYP2B6:516G > T SNP in HIV-1 infected patients on EFV-based ART in our study setting
2. To compare the one-year incidence of neuropsychiatric disorder in patients on EFV-based ART with or without CYP2B6:516G > T SNP
3. To compare the one year risk of hepatic toxicities of patients on EFV-based ART with or without CYP2B6:516G > T SNP
4. To evaluate the effect of CYP2B6:516G > T SNP on poor treatment outcomes, of poor adherence, immunologic failure, and virologic failure during the first year of EFV-based ART

# Research Methodology

**Study setting**

Jos University Teaching (JUTH) is located in North-Central Nigeria and provides comprehensive HIV care services for the city of Jos and serves as a referral centre for health facilities in Plateau State and other states in the region. The comprehensive HIV treatment and support program started in 2002 as part of the Government of Nigeria (GoN) ART initiative. Rapid scale-up of ART services started in 2004 through the collaboration between AIDS Prevention Initiative in Nigeria (APIN) Lte./Gte., Harvard School of Public Health (HSPH), the University of Jos and JUTH, with support from a United States President’s Emergency Plan for AIDS Relief (PEPFAR) grant. By 2012, over 16,000 patients had been cumulatively enrolled into care at the treatment centre.

**Study design**

A prospective cohort study design shall be utilized. A cohort of HIV-1 infected patients (>15 years), treatment naive, newly initiated on EFV-containing regimen will be followed for a period of one year. Previous study in the study setting suggest that the period of greatest risk of EFV associated neuropsychiatric disorder is within the first year of treatment (Abah, et al., 2015) Additionally, early virologic failure can be detected in the first year of ART. The prospective cohort study is a quantitative analytical study design best suited for measuring the incidence of an event (Bonita & Beaglehole, 2006), hence making suitable for measuring the incidence of neuropsychiatric disorder. In addition, the method supports the design and collection of quality data to address the research question. Hence, will be useful in assessing predictors of incidence of EFV-induced neuropsychiatric disorder, hepatotoxicity, immunologic and virologic response (Gordis, 2008). Misclassification and measurement bias is less with the prospective cohort study. A major disadvantage of using prospective cohort study design to measure the incidence of a rare event is that a long follow period will be required to detect a rare event. This makes it too expensive and unrealistic to conduct in a limited time frame.   Alternatively, retrospective cohort study could be employed to explore the incidence of EFV-related neuropsychiatric disorder in a cohort of patients.  A retrospective cohort study design is relatively quick and easy to conduct as there are no long periods of follow-up, and data on all variables are collected at once (Bonita & Beaglehole, 2006). However, in settings with poor data collection and storage, unavailability of essential data is a major limitation.

**Study population**

**Inclusion criteria:** HIV-1 infected patients ≥15 years of age, treatment with EFV-based ART, treatment naïve at ART initiation

**Exclusion criteria:** Patients with background neuropsychiatric disorder, those taking drugs that potentially may interact with EFV metabolism (i.e Rifampicin, Ritonavir, Carbamazepine, Phenytoin, phenobarbitone, St John’s Wort), pregnant women and patients with hepatic dysfunction as indicated by: a) Transaminases > 5-10× the upper limit of normal, b) ALP> 5-10× the upper limit of normal

**Sampling procedure**

Eligible HIV-1 infected patients, newly initiated on EFV-based ART will be consecutively recruited for the study until the sample size is reached. Eligible patients include HIV-1 positive, treatment naive adult (aged >15 years) commencing ART containing EFV at the treatment during the study period. Patient with background neuropsychiatric disorder at enrolment shall be excluded. Eligibility for ART at the clinic is based on the Nigerian National Adult ART Guidelines (Nigeria, 2010).   
**Sample size determination**

To determine the prevalence of CYP2B6:516G > T SNP in the study population a sample size of 124 will be utilized based on the formula for sample proportions with finite correction factor : ***n* = [DEFF\*Np(1-p)]/ [(d2/Z21-α/2\*(N-1)+p\*(1-p)]**(Kasiulevičius, Šapoka, & Filipavičiūtė, 2006); population size(for finite population correction factor or fpc)(N)= 200 (about 200 patients are expected to be initiated on ART yearly at the study site), hypothesized % frequency of outcome factor in the population (p)=30%+/-5 (Matimba et al., 2008), confidence limits as % of 100(absolute +/- %)(d)= 5%, design effect (for cluster surveys-DEFF)= 1. The sample size of 124 is adequate to detect a difference in incidence of neuropsychiatric disorder in patients with or without CYP2B6:516G > T SNP. The minimum sample size required to detect a difference between the exposed and unexposed is 46 in both arms, giving a total of 96:using a Two-sided significance level(1-alpha) of 95; power(1-beta, % chance of detecting) of 80; ratio of sample size, Unexposed/Exposed of 1; percent of Unexposed with Outcome of 50 (Haas et al., 2004); Prevalence Ratio: 1.6 based (Sarfo et al., 2014). The sample size was determined using OpenEpi epidemiological calculator and the method by Kelsey et al., (1996): Table 12-15.

**Data collection**

At baseline socio-demographic and clinical information shall be documented. Also venous blood shall be obtained for the following: genetic analysis, CD4 cell count determination, blood chemistry, hepatitis B and C serology. Genotyping of patient samples

Genotyping of patient samples

Total genomic DNA would be isolated from leukocytes of whole blood samples using a modified salting-out method as described earlier by Miller et al., 1988. polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) would be used for accurate genotyping of the selected SNP. Sequencing would be performed using a Spectrumedix SCE 400 Genetica analysis system (Spectrumedix LCC, USA). PCR of relevant amplicons Sequences of the primer that would be used.

Assessment of drug toxicity

*Clinical toxicity assessment*

Neuropsychiatric assessment would be conducted at 2, 4, 8 and 12 weeks, and thereafter at 12-week intervals. A questionnaire developed by Gounden et al, 2010 based on the AIDS Clinical Trials Group Study A5095(Clifford, 2005) would adopted for the neuropsychiatric assessment

*Laboratory toxicity assessment*

At baseline and 12 months, quantification of urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) would be performed with Architect 8000 equipment (Abbott Diagnostics, Illinois, USA) and the enzymatic/automated method using DiaSys kits (Diagnostic Systems, Holzheim, Germany), according to the manufacturer’s instructions.

Adherence assessment

Pill count and drug refill adherence would be utilized. Drug refill adherence which had been previously described (Abah et al., 2014) would be calculated as the total number of days behind schedule for drug refill divided by the total number of days the patient was assumed to be exposed to ART given the dispensed number of pills multiplied by 100. Drug refill adherence shall be determined monthly from the date of ART commencement up to the date of last ARV refill or the end of the study period. Pill count will conducted every 2 months.

Assessment of ART efficacy

Six months after initiation of treatment, drug response in all patients would be measured by a CD4-cell count and viral load measurement. Subsequent measurements would taken every 6 months, except in cases in which the patients experienced an increase in viral load after 6 months despite ART; in such cases, a repeat viral load measurement would be performed after another 3 months.

T-lymphocyte count (CD4+) would be measured by Partec flow Cytometry, Munster Germany

HIV-1 RNA viral load would be measured using the Roche Cobas Amplicor HIV-1 Monitor, version 1.5 (Roche Diagnostics GmbH, Mannheim, Germany).

Summary of assessment schedule

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SN** | **Description** | **Month of assessment** | | | | | | | | | | | | |
|  |  | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | Adherence assessment |  | **X** | **X** | **X** | **X** | **X** | **X** |  |  |  |  |  | **X** |
| 2 | Neuropsychiatric assessment | **X** | **X** | **X** | **X** |  |  | **X** |  |  |  |  |  | **X** |
| 3 | Genotyping | **X** |  |  |  |  |  |  |  |  |  |  |  |  |
| 4 | Alanine aminotransferase (AST) | **X** |  |  |  |  |  | **X** |  |  |  |  |  | **X** |
| 5 | Aspartate aminotransferase (AST) | **X** |  |  |  |  |  | **X** |  |  |  |  |  | **X** |
| 6 | CD4+ cell count | **X** |  |  |  |  |  | **X** |  |  |  |  |  | **X** |
| 7 | HIV plasma RNA level |  |  |  |  |  |  |  |  |  |  |  |  | **X** |

**Statistical analysis**

Hardy–Weinberg equilibrium will be calculated to evaluate the genotype frequency distribution. The Kolmogorov-Smirnov test would be performed to look for normality of the laboratory variables. The association of the CYP2B6 G516T genotype with variations in serum levels of laboratory variables would be determined using the student T-test and Two way analysis of variance or non-parametric Mann–Whitney test and Kruskal–Wallis test and depending on the distribution of the variables and the number of sub-groups. Epidemiological measures of association between the main exposure CYP2B6 G516T genotype and the outcome measures: neuropsychiatric ADR, poor adherence, immunologic failure, and virologic failure) such as relative risk (risk ratio), risk difference (RD) or attributable risk (AR), and attributable proportion (attributable risk %) will be calculated.

A multivariable logistic regression analysis will be performed to adjust for the effect of confounders on the primary outcome. All statistical tests will be two-tailed and a p-value <0.05 will be considered statistically significant. Stata version 13 (College Station, TX) will be used for the statistical analyses

# Ethical consideration

Only patients who consent to the study will be enrolled.Patients who decide to take part in the study will give a written informed consent. Subjects will be allowed to withdraw from the study at any time without affecting further care at the clinic. An application for ethical clear will be submitted to the Jos University Teaching Hospital ethics. Subjects will be interviewed using a questionnaire adopted from the AIDS Clinical Trials Group Study A5095 to obtain biodata and information of drug toxicity. Ten (10)mls of venous blood will then be obtained from the anticubital fossa. The blood draw may cause minimal discomfort to the patients. Additional information will be retrieved from the clinic electronic database Filemaker Pro version 12 (FileMaker Pro, FileMaker, Inc. USA) developed for this purpose. All patient data will be maintained in an encrypted, password protected computer and will be accessed only by key personnel and the data entry clerk

# Project management

The timeline for the various project components in shown in the Gantt chart below

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| SN | Description | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
| 1 | Proposal defence |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | Submission ethic approval request |  |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 | Ethics approval |  |  |  |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 4 | Approval to use facility for study |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 5 | Patient recruitment/data collection |  |  |  |  |  |  |  |  | X | X | X | X | X | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 6 | Patient follow up |  |  |  |  |  |  |  |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |  |  |
| 7 | Laboratory analysis of samples |  |  |  |  |  |  |  |  | X | X | X | X | X | X | X |  |  |  |  |  |  |  |  |  | X | X |  |  |  |  |
| 8 | Data analysis |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X | X |  |  |  |  |  |  |  |  |  | X | X |  |  |  |
| 9 | Compilation of research report |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X | X |  |  |  |  |  |  |  |  |  |  |  | X | X | X |

**Research Budget**

The project total cost is estimated at about Fifteen million, one hundred and twenty thousand Naira only. The details budget is presented below.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SN | Description | Test month | | | Total number of test | Unit cost (Naira) | Cost per patient (Naira) | Total cost (Naira) for 150 patients | Cost in US dollars |
|  | **Direct cost** | 0 | 6 | 12 |  |  |  |  |  |
| 1 | Genotyping | 1 |  |  | 1 | 30800 | 30800 | 4620000 | 11,550.00 |
| 2 | Alanine aminotransferase (AST) | 1 | 1 | 1 | 3 | 500 | 1500 | 225000 | 562.50 |
| 3 | Aspartate aminotransferase (AST) | 1 | 1 | 1 | 3 | 500 | 1500 | 225000 | 562.50 |
| 4 | CD4+ cell count | 1 | 1 | 1 | 3 | 2000 | 6000 | 900000 | 2,250.00 |
| 5 | HIV plasma RNA level |  |  | 1 | 1 | 49000 | 49000 | 7350000 | 18,375.00 |
|  | **Total direct cost of lab test** |  |  |  |  |  |  | **13,320,000.00** | **33,300.00** |
|  | **Indirect cost** |  |  |  |  |  |  |  |  |
| 6 | Transport of 150 samples for genotyping to South Africa |  |  |  | 1 | 300000 |  | 300,000.00 | 750.00 |
| 7 | Air ticket (return) |  |  |  | 1 | 540000 |  | 540,000.00 | 1,350.00 |
| 8 | Accommodation |  |  |  | 30 | 20000 |  | 600,000.00 | 1,500.00 |
| 9 | DSA |  |  |  | 30 | 12000 |  | 360,000.00 | 900.00 |
|  | **Total indirect cost** |  |  |  |  |  |  | **1,800,000.00** | **4,500.00** |
|  | **Total direct + indirect cost** |  | | | | | | **15,120,000.00** | **37,800.00** |

# Risk analysis

Limitations:While this study goes some to way provide information on the role genetic polymorphism on treatment outcomes, the study will focus on only a selected SNP which account for about 27% to 50% of the variance in EFV clearance and hence limited in explaining all the efactors results are limited to the selected SNP.

Potential challenges and steps to mitigate same

Loss to follow up is a challenge in follow up studies. To accommodate attrition due to loss to follow, the final sample size will provide for 20% attrition.

Cost of genotyping and viral load

The cost of viral load assay and genotyping are major cost in this study. Research grant shall be sort to cover the cost of genotyping

**Potential outcomes**

The identification of subpopulations with either increased or decreased sensitivity to EFV due to genomic factors could provide important information that could be used to mitigate the risk of side effects and the risk of lack of efficacy in those subpopulations. Characterization and categorization of individuals based on genotype or phenotype to genomic subpopulations may lead to a significant increase in therapy benefit, decreased risks or both.

# Conclusion

The study will provide genomic data on CYP 2B6 SNP among HIV infected patients. Depending on the direction of the study findings, the information on the effect of CYP 2B6 SNP on treatment outcomes can be exploited in precision medicine for HIV infected patients. Further studies to determine the safe and effective dose of EFV in Nigerian HIV infected adults would be recommended.

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