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LABORATORY FOR PUBLIC HEALTH RESEARCH BIOTECHNOLOGIES

PHARMACOGENETIC STUDY OF EFAVIRENZ IN HIV PATIENTS: CYP 2B6 516G>T AND 983T>C POLYMORPHISMS IMPLICATION IN THE NEUROLOGICAL SIDE EFFECTS IN CAMEROON

'' THESIS ''

Submitted to the Department of Biochemistry in partial fulfillment of the requirements for the Award of a PhD Degree in Public Health Biotechnology

BY:

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ATTESTATION DE CORRECTION DE LA THESE DE DOCTORAT/PhD

Nous soussignons : **Prof. PENLAP NINTCHOM Véronique épouse BENG**, Présidente du jury et **Prof. ACHIDI Eric**, Membre du Jury, attestons que **NGUEFEU NKENFOU Carine épouse TCHINDA** a effectué les corrections conformément aux exigences du jury de soutenance de sa thèse de Doctorat / PhD en Biochimie option Biotechnologie de la Santé Publique avec pour thème « Pharmacogenetic study of Efavirenz in HIV patiernts : CYP 2B6 516G>T and CYP 2B6 983T>C polymorphisms implication in the neurological side effects in Cameroon » Cette thèse a été soutenue le 4 Fevrier 2022.

En foi de quoi, la présente attestation lui est établi et délivré pour lui servir et valoir ce que de droit.

15 SEPT 2022

Examinateur **Président du jury** **Chef de Département**

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I. Introduction

Acquired Immune Deficiency Syndrome (AIDS) remains one of the world's most serious health challenges. The World Health Organization (WHO) recently reported an estimated 37.9 million People Living with HIV (PLWHA) worldwide at the end of 2018. Africa remains the continent most heavily affected by this pandemic and accounting for approximately 70% of all the people infected around the world (UNAIDS/WHO, 2019). Cameroon is not excluded from this picture with an estimated average prevalence of 3.8% among the young adult population aged between 15–49 years (UNAIDS/WHO/UNICEF, Global AIDS Monitoring, 2017). Up to date, no effective cure or vaccine against HIV/AIDS exists; nevertheless, it is controlled by the administration of antiretroviral therapy (ART). Regarding the effective care, antiretroviral treatment was introduced in 2003 and has been available free-of-charge since 2007 at all levels of the health system. The ART coverage has been extended in a gradual process reaching 52 % in 2018 and the number of facilities dispensing ARV increased from 168 in 2013 to 481 in 2019. Cameroon meets national and international therapeutic standards. The first line recommended by WHO in resource limited countries, including Cameroon, consists of two Nucleoside Reverse Transcriptase Inhibitors (NRTI) plus one Non-Nucleoside Reverse Transcriptase Inhibitors (NNTIs) where Efavirenz (EFV) is the most commonly used (Kouanfack et al., 2011).

Since its introduction, ART has dramatically improved the pattern of morbidity and mortality among HIV infected patients, changing it to a chronic manageable infection (Anlay et al., 2016). ART, which continues to be the mainstay of HIV management, prevents viral multiplication and reduces the viral load in the blood, resulting in improved immune function in an HIV-infected person and a decrease in the risk of transmitting the virus to sexual partners and children(Cohen et al., 2016). Unfortunately, alongside these gains, anti-retrovirals, like many other administered drugs, are reported to be associated with adverse drug reactions (ADR). According to the WHO definition, an ADR is any noxious, unintended, and undesired effect of a drug, which occurs at doses used in humans for prophylaxis, diagnosis or therapy (Ejigu et al., 2018). Anti-HIV-related ADRs have been particularly found to occur in higher proportions at the beginning of ART (Tadesse et al., 2014). The incidence of ADRs among patients on ART vary across the world and ranged between 4.3%-90% (Roshni et al., 2016); (Kindie et al., 2017); (Mehari et al., 2017). Previous studies carried out around the world in developing and developed countries have described associations between ART used and a large spectrum of ADRs. These included lipodystrophy, fatigue, nausea and vomiting, abdominal pains, diarrhea, increase in liver function and liver failure, hypersensitivity syndrome reactions (rashes), central

nervous system adverse events, pancreas and kidney toxicities (Neuman et al., 2012); (Shubber et al., 2013). Central nervous system (CNS) side effects are associated with Efavirenz (To et al., 2009). There are documented incidences of 60–90% of short term neuropsychiatric symptoms such as dizziness, hang-over sensation, difficulty concentrating, headache, vertigo, euphoria, sleep disturbance (including somnolence, insomnia and vivid or abnormal dreams), irritability or mood changes, suicidal ideation and nervousness (Cavalcante et al., 2010); (Abers et al., 2014). More severe neurological effects have also been registered (in fewer than 2% of the patients), including severe depression, delirium, paranoia, depersonalization, hallucinations, anxiety, aggressive behaviour, abnormal thinking and mania (Scourfield et al., 2012). Drug toxicities can add to the complexity of HIV management by impairing patient compliance to treatment. The plausible resulting consequences could be the inferior clinical outcomes associated with higher costs to the public health system (Mehari et al., 2017). Some evidences showed that up to 25% of patients discontinued their initial ART regimen because of toxic effects (Kindie et al., 2017).

Drug treatment in HIV disease is characterized by a great variability in response, in terms of both efficacy and toxicity, that is why among individuals taking the same drug at the same doses, some will develop ADRs and others would not (Burger et al., 2006). Several factors may affect this variability and may include ethnicity, gender, age, body weight, drug–drug and drug–food interactions, binding to plasma proteins, hepatic impairment, disease status, pregnancy, and host genetic factors which are by far the most important (Michaud et al., 2012).The benefit of pharmacogenetic testing is to guide the choice of the initial drug regimen, thus increasing efficacy, and simultaneously avoiding ADRs (Bushyakanist et al., 2015). Genetic variations can impact the pathways of drug absorption, disposition, metabolism and excretion (ADME). A mutation in a gene coding for a drug-metabolizing enzyme can result in an enzyme with normal, low, or no activity (Banjoko, 2012). EFV is principally metabolized by cytochrome P450 2B6 (Zanger and Klein, 2013). The gene that encodes for CYP2B6 is highly polymorphic. CYP2B6 516G>T and 983T>C Single Nucleotide Polymorphisms (SNPs) have being reported to be of clinical relevance. Several studies performed in Africa have reported that the CYP2B6 516G>T allele can occur in 20 to over 49% of the individuals (Nyakutira et al., 2008) (Sarfo et al., 2014) (Gross et al., 2017).The second polymorphism is more frequent among African subjects with allele frequencies of 4–11%. These two polymorphisms have been associated with increased EFV plasma levels in several studies. A number of associations between these human genetic variants, high drug level and predisposition to EFV related drug toxicity have been described in recent years (Gounden et al., 2010) (Gallien et al., 2017)

Research question: What is the implication of CYP 2B6 516 G>T and 983 T>C polymorphisms on the response to EFV-based ART regimen in HIV patients in Cameroon?

OBJECTIVE

The general objective of this study was to obtain pharmacovigilance and pharmacogenetic data in a Cameroonian population regarding the CYP2B6 gene coding for an enzyme involved in the metabolism of Efavirenz and propose a procedure manual and an algorithm for the better management of HIV patients in Cameroon.

Specifically, we sought to:

1- Determine the general profile of anti-retrovirals associated adverse drug reactions and the prevalence of those associated to Efavirenz;

2- Investigate the prevalence of CYP2B6 516G>T polymorphism and its association with Efavirenz related central nervous system toxicities;

3- Investigate the prevalence of CYP2B6 983T>C polymorphism and its association with Efavirenz related central nervous system toxicities;

4- Develop a manual of procedures to detect CYP2B6 516G>T and CYP 2B6 983T>C polymorphisms.

II. MATERIALS AND METHODS

II.1. Determination of the general profile of ADRs and the prevalence of those associated to EFV

An ethical clearance was obtained from the National Ethics Committee for Research in Human Health of Cameroon (Nº 2014/12/670/CE/CNERSH/SP). A retrospective study was conducted in the Outpatient ART Center (Day Care Hospital) of the Central Hospital of Yaoundé (YCH). This center is one of the largest in the country, with approximately 200 HIV-positive individuals getting enrolled in the program each month. The data for this study were obtained by reviewing the medical records of patients enrolled in the Center between 1st January, 2013 and 31st December, 2013. The study population included all HIV-positive patients enrolled in the program at the ART Center during that period of time and who had had at least one follow-up clinical visit after commencing treatment. ADRs were identified based on patient complaints and/or observations made and reported in the patient's record by physicians during routine

clinical examination. Physicians considered that an adverse effect was associated to ART if it was absent prior its initiation, and to which other causes could not be attributed. Data were retrieved from available patient medical records found in standardized data collection forms. Baseline demographic characteristics (age, marital status, sex, employment status, geographic site of residence and educational level) and clinical/immunological factors. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 25 to relate variables and/or compare groups in terms of variables. Descriptive and univariate analyses were performed on quantitative data. The association between different factors and ADRs was estimated with a 95% Confidence Interval, using the Chi Square Test. A *P* value < 0.05 was considered to be statistically significant.

II.2.To evaluate the prevalence of CYP 2B6 516G>T SNP and investigate its association with EFV related CNS toxicities

The study was conducted in the Outpatients ART Centre of the Yaoundé Central Hospital (YCH) and the Bertoua Regional Hospital (BRH). HIV-infected individuals already under ART, with or without ADRs, were selected retrospectively, based on information reported in their medical records by clinicians after consultation. A list of patients with their telephone numbers was thus constituted. ADRs were diagnosed, based on patient complaints and recorded by physicians during routine clinical visit. Controls were recruited from the same Health Centers and were selected on the basis of the absence of ADR development during at least 2 years of treatment regimen containing EFV. Participants were prospectively recruited after contacting them through phone calls. Demographic, clinical and therapeutic data were obtained from clinical records of HIV/AIDS patients under ART who gave their informed consent. Such data included: sex, age, weight, CD4 cell counts, hemoglobin level, ART regimen, treatment initiation date, complaints after treatment initiation, ADR onset time, treatment modification and information on treatment observance or adherence.

Five (5) mL of venous blood were collected from each participant who gave consent and used to prepare dried blood spots on filter paper that were stored until genomic analysis. DNA was extracted using the Chelex method. The final supernatant (DNA) was transferred into a fresh tube and stored in TE buffer at −20°C for further pharmacogenetic analyses. The CYP2B6 516G>T (rs3745274) polymorphism was investigated using PCR-RFLP. The primer sequences used to amplify the gene were CYP 516 F: 5′-GGT CTG CCC ATC TATAAA C-3′ and CYP 516 R: 5′-CTG ATT CTT CAC ATG TCT GCG-3′. The amplification was done in a T3 thermal cycler (Biometra, UK). The reaction mixture was prepared to a total volume of 25 µL,

containing 17.75 µL of nuclease free water (NFW), 2.5µL of 10X thermopol buffer from New England Biolabs (NEB), 0.5µL of 10mM dNTPs (200µM of each deoxyribonucleotide), 0.5 µL of each primer and 0.25 µL of 5U/ µL *Thermophillus acquaticus* (Taq) polymerase (NEB) and 3 µL of DNA extract. For 516 G>T, after initial denaturation at 95°C for 10 min, 35 cycles of amplification were carried out with denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 60 s, followed by a final extension at 72°C for 10 min and then conservation at 4–8°C for 48 h. To confirm the presence of CYP 2B6 gene, the expected amplicon sizes of the PCR products 526bp were verified on 2% agarose gel electrophoresis. The RFLP reaction conditions for digestion with *BSrI* (New England Biolabs, USA) was set at 65°C for 16 h. The products of the digestion reaction were separated on a 2% agarose gel stained with ethidium bromide. Polymorphisms were determined according to specific fragment patterns as follows:

(516 G/G): 268 bp, 236 bp, 22 bp

(516 G/T): 504 bp, 268 bp, 236 bp, 22 bp

(516 T/T): 504 bp, 22 bp

All data were entered into Excel files and analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., USA) statistical software. Frequencies of the CYP2B6 516G>T genotypes in the study population were obtained by descriptive statistics. The Chi-square test was used to assess the association between the genotype/phenotype of the CYP2B6 516G>T and adverse effects of EFV in the study population. The odds ratios (ORs) at 95% confidence intervals (CIs) were also calculated and the cut-off for statistical significance was set at a p value of <0.05.

II.3.To determine the prevalence of CYP 2B6 983 T>C SNPs and evaluate its association with EFV related CNS toxicities

This polymorphism was evaluated on all DNA samples as for the CYP 2B6 516 G>T in II.2. For 983T>C, the PCR conditions were almost the same as in II.1. The only differences were at the level of annealing (59°C for 25 s) and the total number of cycles was 45. The RFLP reaction conditions for digestion were done with *BSmAI* (New England Biolabs, USA) and set at 55°C. Polymorphisms were determined according to specific fragment patterns as follows:

(983 T/T): 759 bp

(983 C/T): 759 bp, 637 bp, 122 bp

(983 C/C): 637 bp, 122 bp

Statistical analyses were the same as in II.2

II.4. Writing up of the manual of procedures

The development of the manual was inspired from experience on the field and in the laboratory, and so took into account all the challenges faced. The manual will be structured in chapters and standard operating procedures (SOPs), which provide amendments to these challenges. A standard operating procedure, is a step-by-step set of instructions to guide researcher to perform tasks in a consistent manner. SOPs are particularly important for complex tasks that must conform to regulatory standards and are also critical to ensuring efficient effort with little variation and high quality in output. In the laboraratory each protocol was tested and standardized.

III. Results

III.1.The medical files of 1254 HIV/AIDS patients who initiated ART were included in this study and among them 306 (24.40%) have reported to develop at least one ADR. The most common systems affected by adverse reactions were hematological, systemic, gastro-intestinal dermatological and the central nervous system with 37.58%, 12.75%, 12.75%, 12.60 and 10.78% respectively.

III.2. A total number of 425 samples were collected from participants, among which 174 from Yaoundé Central Hospital and 251from the Bertoua regional Hospital. The genotyping of the CYP2B6 516G>T SNP was done successfully on all the participants. The number of individuals presenting the CYP2B6 516GG wild-type genotype (extensive metabolizers) in Yaoundé and Bertoua was 13(7.47%) and 22(8.76%) respectively. One hundred and twenty one (69.54%) participants had the heterozygous GT genotype (intermediate metabolizers) in YCH and 206(82.07%) in BRH. The number of participants expressing the homozygous TT genotype considered as poor metabolizers was 40 (22.98%) and 23(9.16%) in YCH and BRH respectively. Allele frequencies for the CYP2B6 516G>T SNP in YCH were 42.24% for the G allele and 57.76% for the T allele. In BRH we obtained 49.80% for the G allele and 50.20% for the T allele. Association analysis of the CYP2B6 516G>T SNP showed that individuals homozygous for the wild-type allele (GG) were likely to have some degree of protection against CNS adverse events with a statistically significant difference observed in the participants from Yaoundé (p=0.000, OR=0.865). Heterozygous individuals (GT) showed about a two times risk for developing ADRs although with no significant difference in YCH (p=0.079, OR=1.703).

Additionally, the results showed that individuals homozygous mutants (TT) had a seven times higher risk of developing EFV associated adverse reactions in BRH with a statistically significant difference (P=0.002, OR=6.723).

III.3. The detection of the 983T>C polymorphism was successful on all the 174 samples from Yaoundé and on 235 samples from Bertoua (detection unsuccessful for 16 samples). Three types of metabolizers were identified: extensive (TT), intermediate (TC) and slow (CC). Prevalences of 88.50% TT, 5.75% TC and 5.75% CC were obtained in Yaoundé and 85.10% TT, 14.46% TC and 0.85% CC in Bertoua. Allele frequencies for the CYP2B6 983T>C SNP in YCH were 91.38% for the T allele and 8.62% for the C allele. In BRH we obtained 92% for the T allele and 8% for the C allele. Association study showed that, individuals homozygous for the wild-type allele (983TT) were less likely to develop EFV associated ADRs compared to other genotypes although with no significant difference observed in Yaoundé (OR=0.630, P=0.231). Slow metabolizers (CC) were shown to be about two times more at risk to develop CNS adverse events compared to extensive and intermediate metabolizers in Yaoundé (OR=1.92, p=0.252).

III.4. The manual of standard operating procedures

At the end of this research work, a manual of procedures was produced. This manual is structured in chapters and standard operating procedures (SOPs), which provide amendments to the challenges faced on the field. In the manual of procedures, there are 5 parts and each consists of a set of SOPs which entail a series of tasks and procedures to follow and there by facilitating the reader to carry out his own pharmacogenetics study successfully. All the SOPs were prepared by Carine Nguefeu Nkenfou Tchinda, proofread by Prof. Atogho Barbara Tiedeu and Dr. Jean Paul Chedjou and validated by Pr. Wilfred Fon Mbacham.

CONCLUSION

In this study, we assessed the pharmacovigilance and pharmacogenetics profiles of HIV patients under ART and the following conclusions could be drawn:

1-The spectrum of adverse events in this study was wide and varied. The overall prevalence of adverse drug reactions was 24.40%. The prevalence of EFV associated ADRs was 10.78%.

2- The CYP 2B6 516G>T SNP was strongly associated with susceptibility to adverse drug reactions induced by Efavirenz in HIV/AIDS-infected Cameroonians. The GG genotype was protective while the risk of developing ADRs with TT genotype was about seven times higher.

3-Individuals homozygous for the wild-type allele (983TT) were less likely to develop EFV associated ADRs compared to other genotypes although with no significant difference. Slow metabolizers (CC) were shown to be about two times more at risk to develop CNS adverse events.

Additionally, we showed that haplotypes was also associated with the susceptibility of developing CNS adverse effects.

Pharmacogenetics and pharmacokinetics combined with therapeutic drug monitoring should be used to guide EFV dosages. Thus, CYP2B6 genotyping should be introduced in routine clinical practice because it would help the patients to avoid or reduce the adverse neuropsychiatric events after the initiation of EFV-based antiretroviral regimen.

4- The manual developed an presented take into account all the challenges in the context of the standards and means available in our country, to guide healthcare professionals, program managers and others researchers in systematically conducting further pharmacogenetic studies.

PERPECTIVES

- To include participants from others regions, for purposes of capturing the different genetic representation of the country.
- To study the relationship between EFV plasma drug levels and the presence of SNPs.
- To study the implication of others CYP 2B6 polymorphisms on the treatment outcomes.

PUBLICATIONS FROM THIS WORK

1. Carine Nguefeu Nkenfou, Barbara Atogho Tiedeu, Celine Nguefeu Nkenfou, Akindeh M. Nji, Jean Paul Chedjou, Calvino Tah Fomboh, Charles Kouanfack, Wilfred Fon Mbacham. Adverse drug reactions associated to CYP 2B6 polymorphisms in HIV/AIDS-treated patients in Yaoundé, Cameroon. *The Application of Clinical Genetics 12; 261-268 (2019)*

2. **Carine Nguefeu Nkenfou-Tchinda**, Barbara Atogho Tiedeu, Celine Nguefeu Nkenfou, Akindeh M. Nji, Aristid Ekollo Mbange, Jean Paul Chedjou, Calvino Tah Fomboh, Charles Kouanfack, Wilfred Fon Mbacham. ''Adverse Drug Reactions and associated factors among adult HIV-positive patients taking art at the Yaoundé Central Hospital, Cameroon''. *Journal of Applied Pharmaceutical Science 10(11); 67-73(2020)*

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UNIVERSITÉ DE YAOUNDÉ I **Faculté des Sciences** Division de la Programmation et du Suivi des Activités Académiques		THE UNIVERSITY OF YAOUNDE I **Faculty of Science** Division of Programming and Follow-up of Academic Affaires
LISTE DES ENSEIGNANTS PERMANENTS		**LIST OF PERMANENT TEACHING STAFF**

ANNÉE ACADEMIQUE 2021/2022
(Par Département et par Grade)
DATE D'ACTUALISATION 22 septembre 2021

ADMINISTRATION

DOYEN : TCHOUANKEU Jean- Claude, *Maître de Conférences*
VICE-DOYEN / DPSAA : ATCHADE Alex de Théodore, *Maître de Conférences*
VICE-DOYEN / DSSE : NYEGUE Maximilienne Ascension, *Professeur*
VICE-DOYEN / DRC : ABOSSOLO Monique, *Maître de Conférences*
Chef Division Administrative et Financière : NDOYE FOE Marie C. F., *Maître de Conférences*
Chef Division des Affaires Académiques, de la Scolarité et de la Recherche DAASR : AJEAGAH Gideon AGHAINDUM, *Professeur*

1- DÉPARTEMENT DE BIOCHIMIE (BC) (38)

N°	NOMS ET PRÉNOMS	GRADE	OBSERVATIONS
1	BIGOGA DAIGA Jude	Professeur	En poste
2	FEKAM BOYOM Fabrice	Professeur	En poste
3	FOKOU Elie	Professeur	En poste
4	KANSCI Germain	Professeur	En poste
5	MBACHAM FON Wilfried	Professeur	En poste
6	MOUNDIPA FEWOU Paul	Professeur	Chef de Département
7	NINTCHOM PENLAP V. épse BENG	Professeur	En poste
8	OBEN Julius ENYONG	Professeur	En poste
9	ACHU Merci BIH	Maître de Conférences	En poste
10	ATOGHO Barbara Mma	Maître de Conférences	En poste
11	AZANTSA KINGUE GABIN BORIS	Maître de Conférences	En poste
12	BELINGA née NDOYE FOE M. C. F.	Maître de Conférences	Chef DAF / FS
13	BOUDJEKO Thaddée	Maître de Conférences	En poste
14	DJUIDJE NGOUNOUE Marcelline	Maître de Conférences	En poste
15	EFFA NNOMO Pierre	Maître de	En poste

		Conférences	
16	EWANE Cécile Anne	Maître de Conférences	En poste
17	MOFOR née TEUGWA Clotilde	Maître de Conférences	Inspecteur de Service MINESUP
18	NANA Louise épouse WAKAM	Maître de Conférences	En poste
19	NGONDI Judith Laure	Maître de Conférences	En poste
20	NGUEFACK Julienne	Maître de Conférences	En poste
21	NJAYOU Frédéric Nico	Maître de Conférences	En poste
22	TCHANA KOUATCHOUA Angèle	Maître de Conférences	En poste

23	AKINDEH MBUH NJI	Chargé de Cours	En poste
24	BEBEE Fadimatou	Chargée de Cours	En poste
25	BEBOY EDJENGUELE Sara Nathalie	Chargé de Cours	En poste
25	DAKOLE DABOY Charles	Chargé de Cours	En poste
26	DJUIKWO NKONGA Ruth Viviane	Chargée de Cours	En poste
27	DONGMO LEKAGNE Joseph Blaise	Chargé de Cours	En poste
28	FONKOUA Martin	Chargé de Cours	En poste
29	KOTUE KAPTUE Charles	Chargé de Cours	En poste
30	LUNGA Paul KEILAH	Chargé de Cours	En poste
31	MANANGA Marlyse Joséphine	Chargée de Cours	En poste
32	MBONG ANGIE M. Mary Anne	Chargée de Cours	En poste
33	Palmer MASUMBE NETONGO	Chargé de Cours	En poste
34	PECHANGOU NSANGOU Sylvain	Chargé de Cours	En poste

35	MBOUCHE FANMOE Marceline Joëlle	Assistante	En poste
36	OWONA AYISSI Vincent Brice	Assistant	En poste
37	WILFRIED ANGIE Abia	Assistante	En poste

2- DÉPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES (BPA) (46)

1	AJEAGAH Gideon AGHAINDUM	Professeur	*DAARS/FS*
2	BILONG BILONG Charles-Félix	Professeur	Chef de Département
3	DIMO Théophile	Professeur	En Poste
4	DJIETO LORDON Champlain	Professeur	En Poste
5	DZEUFIET DJOMENI Paul Désiré	Professeur	En Poste
6	ESSOMBA née NTSAMA MBALA	Professeur	*Vice Doyen/FMSB/UYI*

7	FOMENA Abraham	Professeur	En Poste
8	KAMTCHOUING Pierre	Professeur	En poste
9	KEKEUNOU Sévilor	Professeur	En poste
10	NJAMEN Dieudonné	Professeur	En poste
11	NJIOKOU Flobert	Professeur	En Poste
12	NOLA Moïse	Professeur	En poste
13	TAN Paul VERNYUY	Professeur	En poste
14	TCHUEM TCHUENTE Louis Albert	Professeur	*Inspecteur de service* *Coord.Progr./MINSANTE*
15	ZEBAZE TOGOUET Serge Hubert	Professeur	*En poste*

16	BILANDA Danielle Claude	Maître de Conférences	En poste
17	DJIOGUE Séfirin	Maître de Conférences	En poste
18	JATSA BOUKENG Hermine épse MEGAPTCHE	Maître de Conférences	En Poste
19	LEKEUFACK FOLEFACK Guy B.	Maître de Conférences	En poste
20	MEGNEKOU Rosette	Maître de Conférences	En poste
21	MONY Ruth épse NTONE	Maître de Conférences	En Poste
22	NGUEGUIM TSOFACK Florence	Maître de Conférences	En poste
23	TOMBI Jeannette	Maître de Conférences	En poste

24	ALENE Désirée Chantal	Chargée de Cours	En poste
25	ATSAMO Albert Donatien	Chargé de Cours	En poste
26	BELLET EDIMO Oscar Roger	Chargé de Cours	En poste
27	DONFACK Mireille	Chargée de Cours	En poste
28	ETEME ENAMA Serge	Chargé de Cours	En poste
29	GOUNOUE KAMKUMO Raceline	Chargée de Cours	En poste
30	KANDEDA KAVAYE Antoine	Chargé de Cours	En poste
31	MAHOB Raymond Joseph	Chargé de Cours	En poste
32	MBENOUN MASSE Paul Serge	Chargé de Cours	En poste
33	MOUNGANG Luciane Marlyse	Chargée de Cours	En poste
34	MVEYO NDANKEU Yves Patrick	Chargé de Cours	En poste
35	NGOUATEU KENFACK Omer Bébé	Chargé de Cours	En poste
36	NGUEMBOK	Chargé de Cours	En poste
37	NJUA Clarisse Yafi	Chargée de Cours	Chef Div. UBA
38	NOAH EWOTI Olive Vivien	Chargée de Cours	En poste
39	TADU Zephyrin	Chargé de Cours	En poste
40	TAMSA ARFAO Antoine	Chargé de Cours	En poste
41	YEDE	Chargé de Cours	En poste

42	BASSOCK BAYIHA Etienne Didier	Assistant	En poste
43	ESSAMA MBIDA Désirée Sandrine	Assistante	En poste
44	KOGA MANG DOBARA	Assistant	En poste
45	LEME BANOCK Lucie	Assistante	En poste
46	YOUNOUSSA LAME	Assistant	En poste

3- DÉPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE VÉGÉTALES (BPV) (33)			
1	AMBANG Zachée	Professeur	Chef Division/UYII
2	BELL Joseph Martin	Professeur	En poste
3	DJOCGOUE Pierre François	Professeur	En poste
4	MBOLO Marie	Professeur	En poste
5	MOSSEBO Dominique Claude	Professeur	En poste
6	YOUMBI Emmanuel	Professeur	Chef de Département
7	ZAPFACK Louis	Professeur	En poste
8	ANGONI Hyacinthe	Maître de Conférences	En poste
9	BIYE Elvire Hortense	Maître de Conférences	En poste
10	KENGNE NOUMSI Ives Magloire	Maître de Conférences	En poste
11	MALA Armand William	Maître de Conférences	En poste
12	MBARGA BINDZI Marie Alain	Maître de Conférences	CT/ MINESUP
13	NDONGO BEKOLO	Maître de Conférences	*CE / MINRESI*
14	NGODO MELINGUI Jean Baptiste	Maître de Conférences	En poste
15	NGONKEU MAGAPTCHE Eddy L.	Maître de Conférences	En poste
16	TONFACK Libert Brice	Maître de Conférences	En poste
17	TSOATA Esaïe	Maître de Conférences	En poste
18	DJEUANI Astride Carole	Chargé de Cours	En poste
19	GOMANDJE Christelle	Chargée de Cours	En poste
20	MAFFO MAFFO Nicole Liliane	Chargé de Cours	En poste
21	MAHBOU SOMO TOUKAM. Gabriel	Chargé de Cours	En poste
22	NGALLE Hermine BILLE	Chargée de Cours	En poste
23	NGOUO Lucas Vincent	Chargé de Cours	En poste
24	NNANGA MEBENGA Ruth Laure	Chargé de Cours	En poste
25	NOUKEU KOUAKAM Armelle	Chargé de Cours	En poste
26	ONANA JEAN MICHEL	Chargé de Cours	En poste
27	GODSWILL NTSOMBAH NTSEFONG	Assistant	En poste
28	KABELONG BANAHO Louis-Paul-	Assistant	En poste

	Roger		
29	KONO Léon Dieudonné	Assistant	En poste
30	LIBALAH Moses BAKONCK	Assistant	En poste
31	LIKENG-LI-NGUE Benoit C	Assistant	En poste
32	TAEDOUNG Evariste Hermann	Assistant	En poste
33	TEMEGNE NONO Carine	Assistant	En poste

4- DÉPARTEMENT DE CHIMIE INORGANIQUE (CI) (33)

1	AGWARA ONDOH Moïse	Professeur	*Chef de Département*
2	DJOUFAC WOUMFO Emmanuel	Professeur	En poste
3	Florence UFI CHINJE épouse MELO	Professeur	*Recteur Univ.Ngaoundere*
4	GHOGOMU Paul MINGO	Professeur	*Ministre Chargé deMiss.PR*
5	NANSEU Njiki Charles Péguy	Professeur	En poste
6	NDIFON Peter TEKE	Professeur	*CT MINRESI*
7	NDIKONTAR Maurice KOR	Professeur	*Vice-Doyen Univ. Bamenda*
8	NENWA Justin	Professeur	En poste
9	NGAMENI Emmanuel	Professeur	*DOYEN FS UDs*
10	NGOMO Horace MANGA	Professeur	*Vice Chancelor/UB*

11	ACAYANKA Elie	Maître de Conférences	En poste
12	BABALE née DJAM DOUDOU	Maître de Conférences	*Chargée Mission P.R.*
13	EMADACK Alphonse	Maître de Conférences	En poste
14	KAMGANG YOUBI Georges	Maître de Conférences	En poste
15	KEMMEGNE MBOUGUEM Jean C.	Maître de Conférences	En poste
16	KONG SAKEO	Maître de Conférences	En poste
17	NDI NSAMI Julius	Maître de Conférences	En poste
18	NJIOMOU C. épse DJANGANG	Maître de Conférences	En poste
19	NJOYA Dayirou	Maître de Conférences	En poste
20	TCHAKOUTE KOUAMO Hervé	Maître de Conférences	En poste

21	BELIBI BELIBI Placide Désiré	Chargé de Cours	CS/ ENS Bertoua
22	CHEUMANI YONA Arnaud M.	Chargé de Cours	En poste

23	KENNE DEDZO GUSTAVE	Chargé de Cours	En poste
24	KOUOTOU DAOUDA	Chargé de Cours	En poste
25	MAKON Thomas Beauregard	Chargé de Cours	En poste
26	MBEY Jean Aime	Chargé de Cours	En poste
27	NCHIMI NONO KATIA	Chargé de Cours	En poste
28	NEBA nee NDOSIRI Bridget NDOYE	Chargée de Cours	CT/ MINFEM
29	NYAMEN Linda Dyorisse	Chargée de Cours	En poste
30	PABOUDAM GBAMBIE A.	Chargée de Cours	En poste

31	NJANKWA NJABONG N. Eric	Assistant	En poste
32	PATOUOSSA ISSOFA	Assistant	En poste
33	SIEWE Jean Mermoz	Assistant	En Poste

5- DÉPARTEMENT DE CHIMIE ORGANIQUE (CO) (34)			
1	DONGO Etienne	Professeur	Vice-Doyen/FSE/UYI
2	GHOGOMU TIH Robert Ralph	Professeur	Dir. IBAF/UDA
3	NGOUELA Silvère Augustin	Professeur	Chef de Département UDS
4	NYASSE Barthélemy	Professeur	En poste
5	PEGNYEMB Dieudonné Emmanuel	Professeur	*Directeur/ MINESUP/* Chef de Département
6	WANDJI Jean	Professeur	En poste

7	Alex de Théodore ATCHADE	Maître de Conférences	Vice-Doyen / DPSAA
8	AMBASSA Pantaléon	Maître de Conférences	En poste
9	EYONG Kenneth OBEN	Maître de Conférences	En poste
10	FOLEFOC Gabriel NGOSONG	Maître de Conférences	En poste
11	FOTSO WABO Ghislain	Maître de Conférences	En poste
12	KEUMEDJIO Félix	Maître de Conférences	En poste
13	KEUMOGNE Marguerite	Maître de Conférences	En poste
14	KOUAM Jacques	Maître de Conférences	En poste
15	MBAZOA née DJAMA Céline	Maître de Conférences	En poste
16	MKOUNGA Pierre	Maître de Conférences	En poste
17	MVOT AKAK CARINE	Maître de	En poste

		Conférences	
18	NGO MBING Joséphine	Maître de Conférences	Sous/Direct. MINERESI
19	NGONO BIKOBO Dominique Serge	Maître de Conférences	C.E/ MINESUP
20	NOTE LOUGBOT Olivier Placide	Maître de Conférences	C.S/ MINESUP
21	NOUNGOUE TCHAMO Diderot	Maître de Conférences	En poste
22	TABOPDA KUATE Turibio	Maître de Conférences	En poste
23	TAGATSING FOTSING Maurice	Maître de Conférences	En poste
24	TCHOUANKEU Jean-Claude	Maître de Conférences	*Doyen /FS/ UYI*
25	TIH née NGO BILONG E. Anastasie	Maître de Conférences	En poste
26	YANKEP Emmanuel	Maître de Conférences	En poste
27	ZONDEGOUMBA Ernestine	Maître de Conférences	En poste

28	KAMTO Eutrophe Le Doux	Chargé de Cours	En poste
29	NGNINTEDO Dominique	Chargé de Cours	En poste
30	NGOMO Orléans	Chargée de Cours	En poste
31	OUAHOUO WACHE Blandine M.	Chargée de Cours	En poste
32	SIELINOU TEDJON Valérie	Chargé de Cours	En poste

33	MESSI Angélique Nicolas	Assistant	En poste
34	TSEMEUGNE Joseph	Assistant	En poste

6- DÉPARTEMENT D'INFORMATIQUE (IN) (25)

1	ATSA ETOUNDI Roger	Professeur	*Chef Div.MINESUP*
2	FOUDA NDJODO Marcel Laurent	Professeur	*Chef Dpt ENS/Chef IGA.MINESUP*

3	NDOUNDAM Réné	Maître de Conférences	En poste

4	ABESSOLO ALO'O Gislain	Chargé de Cours	En poste
5	AMINOU Halidou	Chargé de Cours	*Chef de Département*
6	DJAM Xaviera YOUH - KIMBI	Chargé de Cours	En Poste
7	DOMGA KOMGUEM Rodrigue	Chargé de Cours	En poste
8	EBELE Serge Alain	Chargé de Cours	En poste
9	KOUOKAM KOUOKAM E. A.	Chargé de Cours	En poste

10	MELATAGIA YONTA Paulin	Chargé de Cours	En poste
11	MONTHE DJIADEU Valery M.	Chargé de Cours	En poste
12	MOTO MPONG Serge Alain	Chargé de Cours	En poste
13	OLLE OLLE Daniel Claude Delort	Chargé de Cours	Directeur adjoint Enset. Ebolowa
14	TAPAMO Hyppolite	Chargé de Cours	En poste
15	TINDO Gilbert	Chargé de Cours	En poste
16	TSOPZE Norbert	Chargé de Cours	En poste
17	WAKU KOUAMOU Jules	Chargé de Cours	En poste

18	BAYEM Jacques Narcisse	Assistant	En poste
19	EKODECK Stéphane Gaël Raymond	Assistant	En poste
20	HAMZA Adamou	Assistant	En poste
21	JIOMEKONG AZANZI Fidel	Assistant	En poste
22	MAKEMBE. S . Oswald	Assistant	En poste
23	MESSI NGUELE Thomas	Assistant	En poste
24	MEYEMDOU Nadège Sylvianne	Assistante	En poste
25	NKONDOCK. MI. BAHANACK.N.	Assistant	En poste

7- DÉPARTEMENT DE MATHÉMATIQUES (MA) (30)

1	AYISSI Raoult Domingo	Professeur	Chef de Département
2	EMVUDU WONO Yves S.	Professeur	*Inspecteur MINESUP*

3	KIANPI Maurice	Maître de Conférences	En poste
4	MBANG Joseph	Maître de Conférences	En poste
5	MBEHOU Mohamed	Maître de Conférences	En poste
6	MBELE BIDIMA Martin Ledoux	Maître de Conférences	En poste
7	NKUIMI JUGNIA Célestin	Maître de Conférences	En poste
8	NOUNDJEU Pierre	Maître de Conférences	*Chef service des programmes & Diplômes/FS/UYI*
9	TCHAPNDA NJABO Sophonie B.	Maître de Conférences	Directeur/AIMS Rwanda
10	TCHOUNDJA Edgar Landry	Maître de Conférences	En poste

11	AGHOUKENG JIOFACK Jean Gérard	Chargé de Cours	Chef Cellule MINPLAMAT
12	CHENDJOU Gilbert	Chargé de Cours	En poste
13	DJIADEU NGAHA Michel	Chargé de Cours	En poste
14	DOUANLA YONTA Herman	Chargé de Cours	En poste
15	FOMEKONG Christophe	Chargé de Cours	En poste
16	KIKI Maxime Armand	Chargé de Cours	En poste
17	MBAKOP Guy Merlin	Chargé de Cours	En poste
18	MENGUE MENGUE David Joe	Chargé de Cours	En poste
19	NGUEFACK Bernard	Chargé de Cours	En poste
20	NIMPA PEFOUKEU Romain	Chargée de Cours	En poste
21	POLA DOUNDOU Emmanuel	Chargé de Cours	En poste
22	TAKAM SOH Patrice	Chargé de Cours	En poste
23	TCHANGANG Roger Duclos	Chargé de Cours	En poste
24	TETSADJIO TCHILEPECK M. E.	Chargé de Cours	En poste
25	TIAYA TSAGUE N. Anne-Marie	Chargée de Cours	En poste

26	BITYE MVONDO Esther Claudine	Assistante	En poste
27	MBATAKOU Salomon Joseph	Assistant	En poste
28	MBIAKOP Hilaire George	Assistant	En poste
29	MEFENZA NOUNTU Thiery	Assistant	En poste
30	TCHEUTIA Daniel Duviol	Assistant	En poste

8- DÉPARTEMENT DE MICROBIOLOGIE (MIB) (18)

1	ESSIA NGANG Jean Justin	Professeur	*Chef de Département*
2	NYEGUE Maximilienne Ascension	Professeur	*VICE-DOYEN / DSSE*
3	NWAGA Dieudonné M.	Professeur	En poste

4	ASSAM ASSAM Jean Paul	Maître de Conférences	En poste
5	BOYOMO ONANA	Maître de Conférences	En poste
6	RIWOM Sara Honorine	Maître de Conférences	En poste
7	SADO KAMDEM Sylvain Leroy	Maître de Conférences	En poste

8	BODA Maurice	Chargé de Cours	En poste
9	BOUGNOM Blaise Pascal	Chargé de Cours	En poste
10	ESSONO OBOUGOU Germain G.	Chargé de Cours	En poste
11	NJIKI BIKOÏ Jacky	Chargée de Cours	En poste
12	TCHIKOUA Roger	Chargé de Cours	En poste

13	ESSONO Damien Marie	Assistant	En poste
14	LAMYE Glory MOH	Assistant	En poste
15	MEYIN A EBONG Solange	Assistante	En poste
16	NKOUDOU ZE Nardis	Assistant	En poste
17	SAKE NGANE Carole Stéphanie	Assistante	En poste
18	TOBOLBAÏ Richard	Assistant	En poste

9. DEPARTEMENT DE PYSIQUE(PHY) (40)

1	BEN- BOLIE Germain Hubert	Professeur	En poste
2	DJUIDJE KENMOE épouse ALOYEM	Professeur	En poste
3	EKOBENA FOUDA Henri Paul	Professeur	*Vice-Recteur. UN*
4	ESSIMBI ZOBO Bernard	Professeur	En poste
5	KOFANE Timoléon Crépin	Professeur	En poste
6	NANA ENGO Serge Guy	Professeur	En poste
7	NANA NBENDJO Blaise	Professeur	En poste
8	NDJAKA Jean Marie Bienvenu	Professeur	Chef de Département
9	NJANDJOCK NOUCK Philippe	Professeur	En poste
10	NOUAYOU Robert	Professeur	En poste
11	PEMHA Elkana	Professeur	En poste
12	TABOD Charles TABOD	Professeur	Doyen FS Univ/Bda
13	TCHAWOUA Clément	Professeur	En poste
14	WOAFO Paul	Professeur	En poste
15	ZEKENG Serge Sylvain	Professeur	En poste

16	BIYA MOTTO Frédéric	Maître de Conférences	DG/HYDRO Mekin
17	BODO Bertrand	Maître de Conférences	En poste
18	ENYEGUE A NYAM épse BELINGA	Maître de Conférences	En poste
19	EYEBE FOUDA Jean sire	Maître de Conférences	En poste
20	FEWO Serge Ibraïd	Maître de Conférences	En poste
21	HONA Jacques	Maître de Conférences	En poste
22	MBANE BIOUELE César	Maître de Conférences	En poste
23	MBINACK Clément	Maître de Conférences	En poste
24	NDOP Joseph	Maître de	En poste

		Conférences	
25	SAIDOU	Maître de Conférences	MINRESI
26	SIEWE SIEWE Martin	Maître de Conférences	En poste
27	SIMO Elie	Maître de Conférences	En poste
28	VONDOU Derbetini Appolinaire	Maître de Conférences	En poste
29	WAKATA née BEYA Annie	Maître de Conférences	*Directeur/ENS/UYI*

30	ABDOURAHIMI	Chargé de Cours	En poste
31	CHAMANI Roméo	Chargé de Cours	En poste
32	EDONGUE HERVAIS	Chargé de Cours	En poste
33	FOUEDJIO David	Chargé de Cours	Chef Cell. MINADER
34	MBONO SAMBA Yves Christian U.	Chargé de Cours	En poste
35	MELI'I Joelle Larissa	Chargée de Cours	En poste
36	MVOGO ALAIN	Chargé de Cours	En poste
37	OBOUNOU Marcel	Chargé de Cours	DA/Univ Inter Etat/Sangmalima
38	WOULACHE Rosalie Laure	Chargée de Cours	En poste

39	AYISSI EYEBE Guy François Valérie	Assistant	En poste
40	TEYOU NGOUPOU Ariel	Assistant	En poste

10- DÉPARTEMENT DE SCIENCES DE LA TERRE (ST) (43)

1	BITOM Dieudonné	Professeur	*Doyen / FASA / UDs*
2	FOUATEU Rose épse YONGUE	Professeur	En poste
3	NDAM NGOUPAYOU Jules-Remy	Professeur	En poste
4	NDJIGUI Paul Désiré	Professeur	Chef de Département
5	NGOS III Simon	Professeur	En poste
6	NKOUMBOU Charles	Professeur	En poste
7	NZENTI Jean-Paul	Professeur	En poste

8	ABOSSOLO née ANGUE Monique	Maître de Conférences	*Vice-Doyen / DRC*
9	BISSO Dieudonné	Maître de Conférences	*Directeur/Projet Barrage Memve'ele*
10	EKOMANE Emile	Maître de Conférences	En poste
11	GANNO Sylvestre	Maître de Conférences	En poste
12	GHOGOMU Richard TANWI	Maître de Conférences	CD/Uma
13	MOUNDI Amidou	Maître de	*CT/ MINIMDT*

		Conférences	
14	NGUEUTCHOUA Gabriel	Maître de Conférences	CEA/MINRESI
15	NJILAH Isaac KONFOR	Maître de Conférences	En poste
16	NYECK Bruno	Maître de Conférences	En poste
17	ONANA Vincent Laurent	Maître de Conférences	*Chef service Maintenance & du Matériel/UYII*
18	TCHAKOUNTE J. épse NOUMBEM	Maître de Conférences	*Chef.cell / MINRESI*
19	TCHOUANKOUE Jean-Pierre	Maître de Conférences	En poste
20	TEMDJIM Robert	Maître de Conférences	En poste
21	YENE ATANGANA Joseph Q.	Maître de Conférences	*Chef Div. /MINTP*
22	ZO'O ZAME Philémon	Maître de Conférences	*DG/ART*

23	ANABA ONANA Achille Basile	Chargé de Cours	En poste
24	BEKOA Etienne	Chargé de Cours	En poste
25	ELISE SABABA	Chargé de Cours	En poste
26	ESSONO Jean	Chargé de Cours	En poste
27	EYONG JOHN TAKEM	Chargé de Cours	En poste
28	FUH Calistus Gentry	Chargé de Cours	*Sec. D'Etat/MINMIDT*
29	LAMILEN BILLA Daniel	Chargé de Cours	En poste
30	MBESSE CECILE OLIVE	Chargée de Cours	En poste
31	MBIDA YEM	Chargé de Cours	En poste
32	METANG Victor	Chargé de Cours	En poste
33	MINYEM Dieudonné-Lucien	Chargé de Cours	*CD/Uma*
34	NGO BELNOUN Rose Noël	Chargée de Cours	En poste
35	NGO BIDJECK Louise Marie	Chargée de Cours	En poste
36	NOMO NEGUE Emmanuel	Chargé de Cours	En poste
37	NTSAMA ATANGANA Jacqueline	Chargé de Cours	En poste
38	TCHAPTCHET TCHATO De P.	Chargé de Cours	En poste
39	TEHNA Nathanaël	Chargé de Cours	En poste
40	TEMGA Jean Pierre	Chargé de Cours	En poste

41	FEUMBA Roger	Assistant	En poste
42	MBANGA NYOBE Jules	Assistant	En poste

Répartition chiffrée des Enseignants de la Faculté des Sciences de l'Université de Yaoundé I

NOMBRE D'ENSEIGNANTS

DÉPARTEMENT	Professeurs	Maîtres de Conférences	Chargés de Cours	Assistants	Total
BCH	8 (01)	14 (10)	13 (05)	3 (02)	**38 (18)**
BPA	15 (01)	8 (06)	18 (05)	05 (02)	**46 (14)**
BPV	07 (01)	10 (01)	9 (06)	07 (01)	**33 (9)**
CI	10 (01)	10 (02)	10 (02)	03 (0)	**33 (5)**
CO	6 (0)	21 (05)	05 (02)	02 (0)	**34(7)**
IN	2 (0)	1 (0)	14 (01)	08 (01)	**25 (2)**
MAT	2 (0)	8 (0)	15 (01)	05 (02)	**30 (3)**
MIB	3 (0)	4 (02)	05 (01)	06 (02)	**18 (5)**
PHY	15 (0)	14 (02)	09 (03)	02 (0)	**40 (5)**
ST	7 (1)	15 (01)	18 (05)	02 (0)	**42(7)**
Total	**75 (5)**	**105 (29)**	**116 (31)**	**43 (10)**	**339 (75)**

Soit un total de **339 (**75**)** dont :

- Professeurs **75 (5)**
- Maîtres de Conférences **105 (**29**)**
- Chargés de Cours **116 (31)**
- Assistants **43** (10**)**

( ) = Nombre de Femmes **75**

DEDICATION

To my wonderful husband Tchinda Jules Ygor and my sweet children Daniella, Maelle, Arielle and Gabriela

ACKNOWLEDGEMENTS

The accomplishment of this project would not have been possible without the tireless and sacrificial inputs of the following persons:

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For all those I could not list, please find here the expression of my sincere gratitude.

Above all I thank the Almighty God who gave me the strength to persevere and without whom nothing is possible.

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3TC	2'3'-didéoxy-3'-thiacytidine -Lamivudine
ADRs	Adverse Drug Reactions
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
AZT	Zidovudine
BMI	Body Mass Index (BMI)
CCR5	Chemokine Co-Receptor 5
CD4	Cluster of Differentiation Class 4
CRFs	Circulating Recombinant Forms
CNS	Central Nervous System
CYP	Cytochrome P450
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
EFV	Efavirenz
EIA	Enzyme Immunoassay
EIs	Entry Inhibitors
ELISA	Enzyme-Linked Immunosorbent Assay
EM	Extensive Metabolizer
FIs	Fusion Inhibitors
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IFA	Immunofluorescence Assay
IM	Intermediate Metabolizer
INIs	Integrase Inhibitors
LAV	Lymphadenopathy-associated Virus
NEB	New England Biolabs
NFW	Nuclease Free Water
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine

PCR	Polymerase Chain Reaction
PI	Protease Inhibitor
PM	Poor Metabolizer
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
SIV	Simian Immunodeficiency Virus
SM	Slow Metabolizer
Taq	*Thermophillus aquaticus*
TDF	Tenofovir
TDM	Therapeutic Drug Monitoring
UM	Ultra-rapid Metabolizer
URFs	Unique Recombinant Forms
WB	Western blot
WHO	World Health Organization

ABSTRACT

The introduction of antiretroviral therapy (ART) as standard of care has considerably enhanced the life span and quality of life among people living with HIV/AIDS. Unfortunately, these drugs are associated with some adverse drug reactions (ADRs) that have been identified as a serious barrier to adherence which is the most important factor for success of therapy. As a consequence of this, there is an increased risk of viral resistance and treatment failure. The first line treatment recommended by WHO in resource limited countries, including Cameroon, consists of two Nucleoside Reverse Transcriptase Inhibitors (NRTI) plus one Non-Nucleoside Reverse Transcriptase Inhibitor (NNTIs) where Efavirenz (EFV) is the most commonly used. EFV was shown to be associated with many Central nervous system (CNS) side effects. Drug treatment in HIV disease is characterized by a great variability in response, in terms of both efficacy and toxicity that is why amongst individuals taking the same drug at the same doses, some will develop ADRs and others will not. Several factors may affect this variability among which, host genetic factors are by far the most important. EFV is principally metabolized by cytochrome P450 2B6 enzyme (CYP2B6). The gene that encodes for CYP2B6 is highly polymorphic. Up to date, about 60 allelic variants have been reported. Of these, CYP2B6 516G>T and 983T>C single nucleotide polymorphisms (SNPs) have been reported to be of clinical relevance. These polymorphisms were shown to be associated with increased plasma levels of EFV and the increased likelihood of developing central adverse effects. The general aim of this study was to obtain pharmacovigilance and pharmacogenetic data in a Cameroonian population on CYP2B6 gene coding for an enzyme involved in the metabolism of EFV and propose a procedure manual for the better management of HIV patients in Cameroon.

To assess the general profile of ADRs and the prevalence of those associated with EFV, a retrospective study was carried out at the day care unit in the Central Hospital of Yaoundé in Cameroon. The patients recruited into this study were enrolled in the ART programme between January 2013 and December 2013. Data were obtained from clinical records of HIV/AIDS patients and gathered using a structured questionnaire. The collected data were entered in an excel sheet and statistical analyses were done using the Statistical Package for the Social Sciences software (SPSS) version 25. The pharmacogenetic study was conducted on the Outpatients enrolled at the day care unit of the Yaoundé Central Hospital (YCH) and the Bertoua Regional Hospital (BRH). HIV-infected individuals already under ART, with or without ADRs, were selected retrospectively, participants were prospectively recruited after

contacting them through phone calls. Five (5) mL of venous blood were collected from participants who gave their consent. Blood was spotted on filter paper for further analysis by PCR. DNA was extracted using the Chelex method. The two polymorphisms under study were detected by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). For CYP2B6 516 G>T and CYP2B6 983 T>C, the restriction enzymes used were *Bsr*I and *Bsm*AI respectively. The respective genotypes were determined according to a specific fragment pattern on agarose gel electrophoresis. Results were compared between patients with and without EFV-related side effects.

A total of 1254 individuals were included in the retrospective study. The spectrum of adverse events in the study was wide and varied. The overall prevalence of adverse drug reactions was 24.40% (306/1254). The most common systems affected by adverse reactions were hematological, systemic, gastro-intestinal, dermatological and the central nervous system with 37.58%, 12.75%, 12.75%, 12.60 and 10.78% respectively. A total number of 425 participants among which 174 from Yaoundé Central Hospital and 251 from the Bertoua regional Hospital were included in the pharmacogenetic study. This study demonstrated that the CYP2B6 516G>T SNP was strongly associated with susceptibility to develop Central Nervous System (CNS) adverse events induced by Efavirenz in HIV/AIDS-infected Cameroonians. The 516GG genotype (extensive or normal metabolizers) was protective (OR=0.865, p=0.000) while the TT (slow metabolizers) was about seven times at risk of developing ADRs (OR=6.723, p=0.002). Individuals homozygous for the wild-type allele (983TT) appeared to be less likely to develop EFV associated ADRs compared to other genotypes although with no significant difference. Slow metabolizers (983CC) were shown to be more susceptible to develop hallucinations (OR=7.3, p=0.057), vision reduction (OR=22.700, p=0.011) and memory loss (OR=27.522, p=0.004). Additionally we showed that haplotypes were also associated with the susceptibility of developing CNS adverse effects. Individuals harboring the wild type genotype for the two polymorphisms (516GG/983TT) were shown to be protected against neurotoxicity (OR=0.356, p=0.011). CYP2B6 genotyping should be introduced in clinical practice because it would help to determine patients at high risk to develop adverse drug reactions and guide to choose a better therapeutic option thereby preventing or decreasing the adverse neuropsychiatric events after the initiation of EFV-based antiretroviral regimen.

Keywords: Pharmacogenetics, CYP2B6, Efavirenz, adverse drug reactions, central nervous system, HIV/AIDS.

RESUME

L'introduction de la thérapie antirétrovirale comme standard de prise en charge a significativement amélioré l'espérance et la qualité de vie des personnes vivant avec le VIH/SIDA. Malheureusement ces médicaments sont associés à des effets indésirables qui ont un sérieux impact négatif sur l'adhérence au traitement, ce dernier étant un facteur très important pour le succès du traitement. Comme conséquences de ces effets indésirables, nous avons une augmentation du risque de développement de la résistance virale et l'échec thérapeutique. La première ligne de traitement proposée par l'organisation mondiale de la santé (OMS) pour les pays en voie de développement, incluant le Cameroun est constituée de deux inhibiteurs nucléotidiques de la transcriptase inverse plus un inhibiteur non nucléotisique de la transcriptase inverse dont l'Efavirenz (EFV) est le plus utilisé. L'Efavirenz est associé à de nombreux effets indésirables au niveau du système nerveux central. On observe une grande variabilité dans la réponse au traitement du VIH, en termes d'efficacité et de toxicité. C'est pour cette raison que parmi des individus prenant les mêmes médicaments, aux mêmes doses, certains auront des effets indésirables et d'autres pas. Plusieurs facteurs contribuent à cette variabilité, au rang desquels les facteurs génétiques sont parmi les plus importants. EFV est principalement métabolisé par l'enzyme Cytochrome P450 2B6 (CYP2B6). Le gène codant pour cette enzyme est très polymorphique. A ce jour, environ 60 formes alléliques ont déjà été identifiées. Les polymorphismes mononucléotidiques CYP2B6 516G>T et 983T>C sont d'une grande importance clinique. Ces deux polymorphismes génétiques ont été associés à une augmentation des concentrations plasmatiques d'EFV et une plus grande susceptibilité au développement des effets indésirables au niveau du système nerveux central. L'objectif général de cette étude était d'obtenir des données de pharmacovigilance et de pharmacogénétique dans une population Camerounaise sur le gène CYP2B6 impliqué dans le métabolisme de l'EFV et proposer un manuel de procédure pour ameliorer les lignes guides de prise en charge des personnes infectées par le VIH.

Une étude rétrospective a été menée à l'hôpital du jour de l'hôpital Central de Yaoundé pour déterminer le profil général des effets indésirables des ARVs et la prévalence de ceux associés à l'Efavirenz. Les patients inclus dans cette étude ont été enregistrés dans le programme de prise en charge entre Janvier et Décembre 2013. Les données ont été collectées à partir des dossiers médicaux des patients et reportées dans des questionnaires structurés. Les analyses statistiques ont été faites avec le logiciel SPSS 25 (Statistical Package for the Social Sciences software) L'étude pharmacogénétique a été menée à l'hôpital Central de Yaoundé et

à l'hôpital Régional de Bertoua. Des individus infectés par le VIH et déjà sous traitement ayant développé ou non des effets indésirables liées à l'Efavirenz ont été sélectionnés de façon rétrospective sur la base des informations contenues dans les dossiers médicaux. Ces patients ont été ensuite contactés par appels téléphoniques. Après obtention du consentement éclairé, 5 mL de sang veineux ont été collectés pour chaque participant et utiliséé pour réaliser des spots sur papier filtre. Les échantillons de sang sur papier filtre ont été ensuite utilisés pour l'extraction d'ADN génomique par la méthode au chelex. La technique de PCR-RFLP a été utilisée pour l'étude des polymorphismes affectant un seul nucléotide (SNP) sur le gène CYP2B6 au moyen des enzymes de restriction *Bsr*I and *Bsm*AI pour CYP2B6 516 G>T et CYP2B6 983T>C respectivement. Les génotypes étaient déterminés en se référant au poids moléculaires des différentes bandes attendues. Les résultats ont été comparés entre les participants avec ou sans effets indésirables.

Un total de 1254 individus a été inclus dans l'étude rétrospective. Le spectre des effets indésirables était large et varié avec une prévalence de 24,4 % (306/1254). Les systèmes les plus affectés étaient : hématologique, systémique, gastro-intestinal, dermatologique et le système nerveux central avec des prévalences de 37,58%, 12,75%, 12,75%, 12,60% et 10,78% respectivement. 425 participants ont été recrutés pour l'étude pharmacogénétique dont 174 à l'hôpital Central de Yaoundé et 251 à l'hôpital Régional de Bertoua. Cette étude a permis de montrer qu'il y avait une association significative entre le polymorphisme CYP2B6 516G>T et la susceptibilité à développer les effets indésirables liées au système nerveux central et induits par l'Efavirenz chez les patients vivant avec le VIH au Cameroun. Les individus ayant le génotype 516GG (métaboliseurs normaux) présentaient un dégrée de protection contre les effets indésirables (OR=0,865, p=0,000) tant dis que les métaboliseurs lents (516TT) étaient plus susceptibles à développer ces effets (OR=6,723, p=0,002). Les participants homozygotes pour l'allèle sauvage (983TT) étaient moins susceptibles à développer les effets indésirables, cependant il n'y avait pas de différence significative. Les métaboliseurs lents (983CC) étaient plus susceptibles au développement des effets tels que : hallucinations (OR=7,3, p=0,057), baisse de la vision (OR=22,700, p=0,011) et perte de mémoire (OR=27,522, p=0,004). Il a été également observé qu'il existait une relation entre les haplotypes et le risque de développer les effets indésirables liés au système nerveux central. Les participants qui étaient homozygotes sauvages pour les deux polymorphismes (516GG/983TT) ont présenté un dégrée de protection contre le développement des effets indésirables. Ces résultats fournissent des informations utiles et importantes pour l'utilisation du génotypage CYP2B6 en pratique clinique. Ceci aiderait à identifier les patients plus à

risque de développer les effets indésirables et guiderait sur le choix d'une meilleure option thérapeutique. On aboutira ainsi à la prévention ou à la réduction des troubles neuropsychiatriques liés à l'initiation d'un traitement antirétroviral contenant l'Efavirenz.

Mots clés: Pharmacogénétique, CYP2B6, Efavirenz, effets indésirables, Système nerveux central, VIH/SIDA.

INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) remains one of the world's most serious health challenges. Since its first discovery in 1981, the HIV and AIDS pandemic has escalated over the years. The first AIDS cases were reported in Cameroon in 1985 (Mbanya *et al*., 2008). The World Health Organization (WHO) recently reported an estimated 37.7 million People Living with HIV worldwide at the end of 2020. Africa remains the continent most heavily affected by this pandemic accounting for approximately 70% of all the people infected around the world (UNAIDS/WHO, 2021). Cameroon is not excluded from this picture with an estimated average prevalence of 3 % amongst the young adult population aged between 15–49 years (UNAIDS/WHO/UNICEF, 2020).

Up to date, no effective cure or vaccine against HIV/AIDS exists; nevertheless, it is controlled by the administration of antiretroviral therapy (ART). Regarding the effective care, antiretroviral treatment was introduced in 2003 and has been available free-of-charge since 2007 at all levels of the health system. The ART coverage has been extended gradually reaching 52 % in 2018 and the number of facilities dispensing ARV increased from 168 in 2013 to 481 in 2019. Cameroon meets national and international therapeutic standards. The first line recommended by WHO in resource limited countries, including Cameroon, consists of two Nucleoside Reverse Transcriptase Inhibitors (NRTI) plus one Non-Nucleoside Reverse Transcriptase Inhibitors (NNTIs) where Efavirenz (EFV) is the most commonly used (Kouanfack *et al.,* 2011).

Since its introduction, ART has dramatically improved the pattern of morbidity and mortality among HIV infected patients, changing it to a chronic manageable infection (Anlay *et al*., 2016). ART, which continues to be the mainstay of HIV management, prevents viral multiplication and reduces the viral load in the blood, resulting in improved immune function in an HIV-infected person and a decrease in the risk of transmitting the virus to sexual partners and children (Cohen *et al.,* 2016). Unfortunately, alongside these gains, anti-retrovirals, like many other administered drugs, are reported to be associated with adverse drug reactions. ADRs are also one of the most common causes of withdrawal of a certain drug from the market, with consequent enormous financial implications for the pharmaceutical industry (Alomar *et al*., 2019).

According to the WHO definition, an ADR is any noxious, unintended, and undesired effect of a drug, which occurs at doses used in humans for prophylaxis, diagnosis or therapy (Ejigu *et al*., 2018). Anti-HIV-related ADRs have been particularly found to occur in higher proportions at the beginning of ART (Tadesse *et al*., 2014). The incidence of ADRs among patients on ART vary across the world and ranged between 4.3%-90% (Roshni *et al*., 2016;

Kindie *et al*., 2017; Mehari *et al*., 2017). Previous studies carried out around the world in developing and developed countries have described associations between ART used and a large spectrum of ADRs. These included lipodystrophy, fatigue, nausea and vomiting, abdominal pains, diarrhea, increase in liver function and liver failure, hypersensitivity syndrome reactions (rashes), central nervous system adverse events, pancreas and kidney toxicities (Neuman *et al*., 2012; Shubber *et al*., 2013). Central nervous system (CNS) side effects are associated with Efavirenz (To *et al*., 2009). There are documented incidences of 60–90% of short term neuropsychiatric symptoms such as dizziness, hang-over sensation, difficulty concentrating, headache, vertigo, euphoria, sleep disturbance (including somnolence, insomnia and vivid or abnormal dreams), irritability or mood changes, suicidal ideation and nervousness (Cavalcante *et al*., 2010; Abers *et al*., 2014). More severe neurological effects have also been registered (in fewer than 2% of the patients), including severe depression, delirium, paranoia, depersonalization, hallucinations, anxiety, aggressive behavior, abnormal thinking and mania (Scourfield *et al*., 2012). Drug toxicities can add to the complexity of HIV management by impairing patient compliance to treatment. The plausible resulting consequences could be the inferior clinical outcomes associated with higher costs to the public health system (Mehari *et al*., 2017). Some evidences showed that up to 25% of patients discontinued their initial ART regimen because of toxic effects (Kindie *et al*., 2017).

Drug treatment in HIV disease is characterized by a great variability in response, in terms of both efficacy and toxicity, that is why among individuals taking the same drug at the same doses, some will develop ADRs and others would not (Burger *et al*., 2006). Several factors may affect this variability and may include ethnicity, gender, age, body weight, drug–drug and drug– food interactions, binding to plasma proteins, hepatic impairment, disease status, pregnancy, and host genetic factors which are by far the most important (Michaud *et al*., 2012). The factors for the growing importance of pharmacogenetic testing are the necessity to prevent adverse drug reaction, obtain maximum benefits from drug therapy and reduce therapeutic failure. In addition, individualized dosing has the potential of better therapeutic outcome (Bushyakanist *et al*., 2015). Genetic variations can impact the pathways of drug absorption, disposition, metabolism and excretion. A mutation in a gene coding for a drug-metabolizing enzyme can result in an enzyme with normal, low, or no activity (Banjoko, 2012). EFV is principally metabolized by cytochrome P450 2B6 (Zanger and Klein, 2013). The gene that encodes for CYP2B6 is highly polymorphic. CYP2B6 516G>T and 983T>C Single Nucleotide Polymorphisms (SNPs) have being reported to be of clinical relevance.

Several studies performed in Africa have reported that the CYP2B6 516G>T allele can occur in 20 to over 49% of the individuals (Nyakutira *et al*., 2008; Sarfo *et al*., 2014; Gross *et al*., 2017). The second polymorphism is more frequent among African subjects with allele frequencies of 4–11%. These two polymorphisms have been associated with increased EFV plasma levels in several studies. A number of associations between these human genetic variants, high drug level and predisposition to EFV related drug toxicity have been described in recent years (Gounden *et al*., 2010; Gallien *et al*., 2017).

Many clinical trials and modeling studies have reported successful dose reduction that still maintains therapeutic success (Mello *et al*., 2011; Mukonzo *et al*., 2014; Dickinson *et al*., 2015; Damronglerd *et al*., 2015; Schalkwijk *et al*., 2018) . This may reduce drug-related adverse events and medication costs. Even small reductions in the annual per-patient cost of treatment would lead to important reductions in the global cost of HIV treatment. These are the reasons why this study was carried out on a Cameroonian population to determine factors influencing response to treatment among HIV patients and improve on their management.

RATIONALE

ART has become a standard of care in HIV patients; these drugs once started have to be continued lifelong. Hence, toxicity to these drugs is a very important issue in the management of HIV-infected patients, as this determines the compliance and adherence of patients to the treatment and is the major cause of treatment changes. Information on the prevalence of antiretroviral ADRs and their associated factors give rise to important information for treatment guideline review, pharmaceutical planning and decision-making. Little is known about the risk of short- and long-term neuropsychiatric toxicity in Cameroon where EFV, one of the commonly used NNRTI is widely used in first-line combination ART. A well-structured, efficient pharmaco-vigilance system for early detection and prevention of ADRs, which assesses and monitors safety profiles and impact of antiretroviral medicines, is very much needed in Cameroon. More research is needed to develop algorithms for prediction of adverse effects of the existing regimens, along with generation of more efficacious and less toxic drugs. Treatment of the adverse reactions, including dose adjustment and the choice of an appropriate regimen, are key strategies for improving compliance among patients initiating ART and subsequently reduce patient suffering and improve quality of life. It is well known that people respond differently to medications. The same medication can be well tolerated and/or effective in some individuals, but lead to severe adverse reactions and/or be ineffective

in others. This heterogeneity in drug response poses immense clinical challenges and underscores the importance of individualized medicine efforts, i.e., tailor the medications to individuals in order to optimize treatment, prevent adverse reactions, and improve patient care. It was shown that genetic make-up of individuals was among the most important factors causing this large inter-individual variability to treatment response. Besides, reports of several studies indicate that subjects carrying certain alleles do not benefit from drug therapy due to ultra-rapid metabolism caused by multiple genes or by induction of gene expression or, alternatively, suffer from adverse effects of the drug treatment due to the presence of defective alleles (Ingelman-Sundberg and Rodriguez-Antona, 2005). This implies that the genetic profiles of humans could influence treatment or intervention outcomes. Pharmacogenetics is an important area of study because it permit gene profiling to answer questions about drug responses and promote the design of better and safer drugs for individualized HIV pharmacotherapy. Additionally, since the introduction of ARVs for HIV treatment in Cameroon, there has been very limited or no report on the pharmacovigilance and pharmacogenetic profiles of patients under ART.

HYPOTHESIS

CYP 2B6 516G>T and 983T>C polymorphisms are presnt in the Cameroonian population and could influence response to ART with regard to CNS ADRs associated to EFV.

RESEARCH QUESTIONS

1- What are the different types and frequencies of adverse drug reactions that are developed by HIV patients under ART in Cameroon?
2- What are the frequencies of CYP 2B6 516G>T and 983T>C polymorphisms in HIV patients on ART?
3- Do any association exist between these SNPs and the response to EFV-based ART regimen such as risk of Central nervous system toxicity among Cameroonian HIV-infected patients?

OBJECTIVES

The general objective of this study was to obtain pharmacovigilance and pharmacogenetic data in a Cameroonian population regarding the CYP2B6 gene coding for an enzyme involved in the metabolism of Efavirenz and propose a procedure manual for the detection of these two SNPs and therefore better management of HIV patients in Cameroon. Specifically, we sought to:

1- Study the general profile and proportions of anti-retrovirals associated adverse drug reactions and the prevalence of those associated to Efavirenz;
2- Estimate the prevalence of CYP2B6 516G>T polymorphism and investigate its association with Efavirenz related central nervous system toxicities;
3- Evaluate the prevalence of CYP2B6 983T>C polymorphism and investigate its association with Efavirenz related central nervous system toxicities.

CHAPTER I: LITERATURE REVIEW

I.1. Introduction on HIV/AIDS

I.1.1. Definition, Classification and Structure of HIV

HIV refers to human immunodeficiency virus. HIV infection progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumors (Sepkowitz, 2001). HIV initially referred to as either the Lymphadenopathy-associated Virus (LAV) or the Human T-cell Lymphotropic Virus type III (HTLV-III) and was first identified as the causative agent of the Acquired Immunodeficiency Syndrome (AIDS) in 1983 (Barre-Sinoussi *et al*., 1983; Clavel *et al*., 1986) . AIDS appeared as a long-lasting disease, with an extremely long lag time between exposure to the causative agent and the profound state of immune suppression (Gallo and Montagnier, 2003). The virus is a genetically related member of the sub-family of *Lentivirus* genus belonging to the *Retroviridae* family meaning its genetic information is stored in the form of ribonucleic acid (RNA) instead of deoxyribonucleic acid (DNA) and the virus therefore requires reverse transcription in order to multiply and invade the host system (Liu *et al*., 2010). These retroviruses are characterized by the lengthy time period between infection and clinical manifestation of symptoms and they primarily infect white blood cells (Benveniste *et al*., 1986). One of the major characteristics of lentiviruses is their extensive genetic variability which is the result of the high error rate, the recombinogenic properties of the reverse transcriptase enzyme and the fast turnover of virions in HIV-infected individuals (Sharp and Hahn, 2011).

As shown in figure 1 below, HIV viral particle is spherical in shape and 100nm in diameter. This virus is essentially a capsule composed of proteins, glycoproteins (proteins covalently attached to different sugar molecules), fat molecules and the ribonucleic acids (RNA) (Murgatroyd *et al*., 2016).

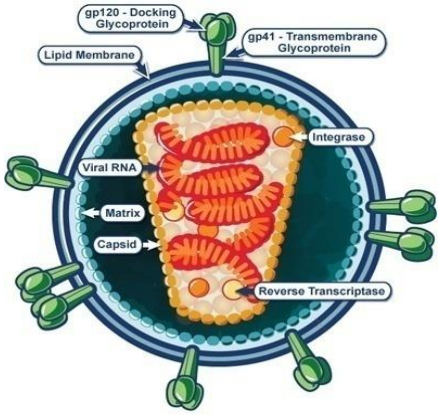

Figure 1: Structure of the HIV viral particle. (NIAID, 2018)

Its envelope is derived from the fatty cell membrane of the human host cell. Seventy-two projections composed of two different glycoproteins, gp120 and gp41, extend from the interior of the virus through its envelope to the exterior (Davenport *et al*., 2016). Within the viral envelope lies the nucleocapsid or the viral core. The outer layer of this core is composed of a protein called p17 while the inner core is made up of p24 proteins. The core of the viral particle holds the HIV genome made up of two single-stranded RNA molecule (Chan *et al*., 1997). These molecules are associated with several other proteins needed for its propagation and survival, namely reverse transcriptase which converts the RNA genome into DNA, and an integrase which integrates the DNA into the host cell's genome (Sundquist and Krausslich, 2012). The HIV-1 genome encodes a total of nine genes namely gag, pol, env, tat, rev, nef, vif, vpr and vpu that are flanked by long terminal repeat sequences required for genome integration and regulation (Kogan and Rappaport, 2011).

I.1.2. Transmission and epidemiology of HIV

There are three main routes for HIV transmission. Many reports have shown that unprotected sexual intercourses are responsible for nearly 70% of HIV-1 infections worldwide with the remainder largely attributable to the vertical transmission from mother to child (during pregnancy, at birth or through breast feeding) and the parenteral route (Shaw and Hunter, 2012). This last one which is the least common include: sharing skin-piercing or cutting instruments or injecting equipment, transfusion of contaminated blood or blood products and transplantation of tissues or organs obtained from HIV positive donors (MacArthur *et al*., 2014). It is also apparent that HIV-1 transmission risk for heterosexual exposures depends on others confounding risk factors: the presence of other sexually transmitted diseases significantly increases the risk of becoming infected with HIV. Additional risk factors are: genital ulcer disease, HIV disease stage, and exposure route (Powers *et al*., 2008; Boily *et al*., 2009). The HIV Modes of Transmission (MOT) model was developed to guide HIV prevention policies. This mathematical model divides the adult population into the following risk groups: female sex workers, men who have sex with men and people who inject drugs (Shubber *et al*., 2014).

Since its initial description, 75 million people have been infected with HIV and about 36.3 million people have died of HIVand HIV related diseases. The number of people living

with HIV worldwide has continued to increase till 2020, reaching an estimated number of 37.7 millions. Sub-Saharan Africa, accounting for 70% of the worldwide infections, remains the most heavily affected region (UNAIDS/WHO, 2021). Even though the number of people newly infected with HIV has significantly reduced from 60.000 to 35.000 between the year 2000-2016, Cameroon still suffers from a generalized HIV epidemic, with about 500.000 people living with HIV in 2020. The estimated average prevalence is 3% among the young adult population aged between 15–49 years (UNAIDS/WHO/UNICEF, Global AIDS Monitoring, 2020). The prevalence is not the same in all regions, ranging from 1.2% in the Far-North to 7.2% in the South (Cameroon Demographic and Health Survey (DHS). The most affected regions are: North West, South West, East and Center regions (Boyer *et al*., 2010). Although AIDS was first described in the United States, it has now been reported in almost all the countries around the world (Figure 2).

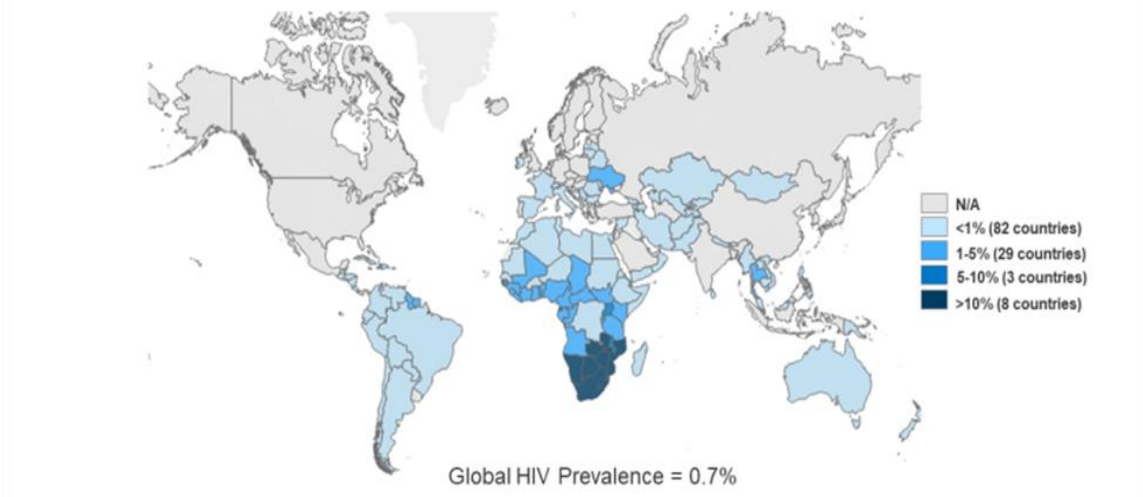

Figure 2*:* Estimated adult HIV Prevalence, 2020. (WHO/UNAIDS, 2021)

I.1.3. HIV Tropism and replication cycle

HIV tropism refers to the cell type that the virus infects and replicates in. The main cells targeted *in vivo* are T-cells, macrophages and dendritic cells (Wong *et al*., 2019). This narrow tropism is predominantly determined by the cell surface receptors required for the virus to attach to and gain entry into cells (Lin and Kuritzkes, 2009). Two different receptors, CD4+ T cell and a co-receptor, are usually needed for the efficient infection of cells. The chemokine co-receptor, CCR5, is predominantly used *in vivo*; however, variants that use another one, CXCR4, evolve during the disease in some AIDS patients (Agwu *et al*., 2016). The virus can be assigned to one of three classes based on its ability to utilize these two co-receptors.

Viruses that use CCR5 but not CXCR4 are R5, those that use CXCR4 but not CCR5 are X4 and those that can use either CCR5 or CXCR4 are referred to as R5X4 or dual/mixed tropism (Arif *et al*., 2017). The earliest classification was the T-tropic/M-tropic designation and was based on the ability of the cultured virus to efficiently replicate in T-cell or macrophage cultures. The T-tropic phenotype was later shown to be associated with the ability of the virus to form syncytia in cells. In contrast, M-tropic strains did not form syncytia in these cells. Thus the SI (syncytia-inducing)/NSI (non-syncytia-inducing) nomenclature was established (Berger *et al*., 1998).

HIV can replicate (make new copies of itself) only inside human cells. The replication cycle involved 13 steps (see figure 3). The process typically begins when a virus particle bumps into a cell that carries on its surface a special protein call Cluster of Differentiation Class 4 (CD4). The classical way for HIV-1 to infect CD4+ T cells involves interactions between glycoprotein gp120 and a complex made of cell-surface CD4+ T cells and an appropriate chemokine co-receptor (CXCR4 or CCR5), followed by fusion with the plasma membrane (Chikere *et al*., 2013; Maartens *et al*., 2014). The next one involves conformational changes in the gp41, which results in the insertion of a fusion peptide at the tip of gp41 into the cell membrane of the target T cell or macrophage (Mulinge *et al*., 2013). After fusion, the virus core containing the genome enters the cytoplasm of the cell. CCR5 is expressed in dendritic cells, macrophages, and T-cells while CXCR4 is found mainly in CD4+ T cells. M-tropic variants use the CCR5 while the T-tropic ones use the CXCR4 co-receptor (Waters *et al*., 2008).

The infection begins when envelope glycoprotein spikes engage the CD4 receptor and the membrane spanning co-receptor (step1), which leads to viral–cell membrane fusion and entry of the virus particle into the cell (step 2). Partial core shell uncoating (step 3) facilitates reverse transcription (step 4), which in turn yields the preintegration complex (PIC). Following import into the cell nucleus (step 5), PIC associated and orchestrates formation of the integrated provirus (step 6). Proviral transcription (step 7) yields different sizes of viral mRNAs (not shown), the larger of which require energy-dependent export to leave the nucleus (step 8). Genome-length mRNAs serve as a template for protein production (step 9) or viral particle assembly with protein components (step 10). ESCRT-mediated viral particle budding (step 11) and release (step 12) from the cell is accompanied or followed shortly thereafter by PR-mediated maturation (step 13) to create an infectious viral particle

(Engelman and Cherepanov, 2012). Each step in the HIV-1 lifecycle is a potential target for antiviral intervention.

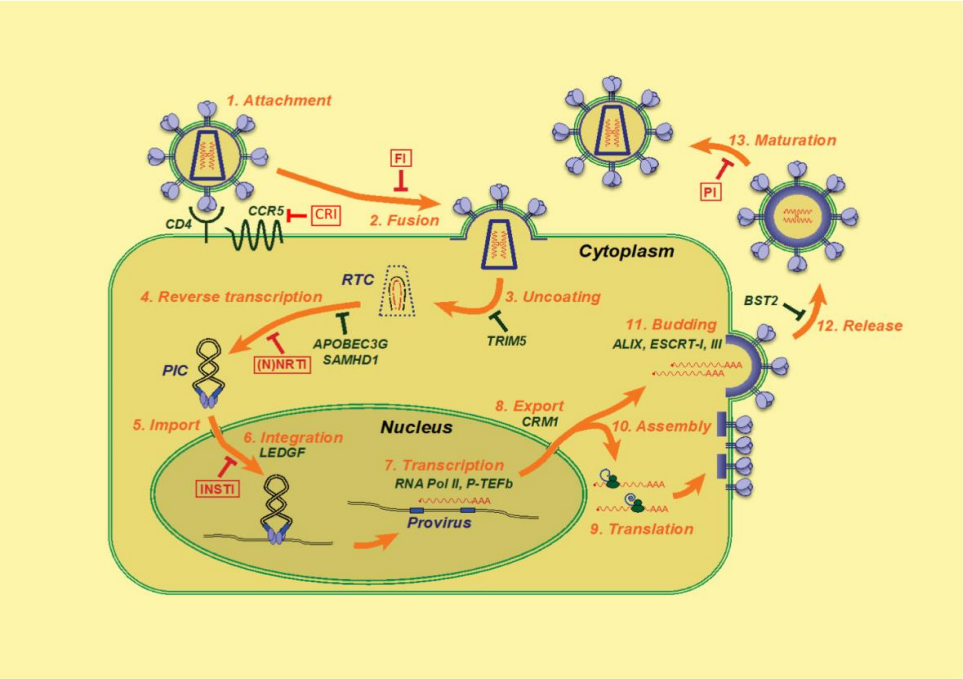

Figure 3: Schematic overview of the HIV-1 replication cycle. (Engelman and Cherepanov, 2012)

I.1.4. Serologic Profile and Clinical staging of HIV disease

HIV/AIDS occurs in discrete stages with specific symptoms. Figure 4 shows how CD4+ T cell count and plasma viral load change during the disease progression. In the absence of treatment, the progression is fairly slow taking a decade or more from infection to the development of severe immunodeficiency (NIAID, 2003). According to WHO, the course of infection is divided into four recognized stages based on clinical findings (Tüzüner *et al.*, 2016).

Stage 1: The primary HIV infection phase

This phase occurs immediately after infection, it is also referred to as the diagnostic window, acute conversion or serological latency. It is characterized by high viral production and activation of lymphocytes in lymphnodes. During this period, individuals may experience no symptoms or flu-like symptoms that can include: fever, chills, rash, night sweats, muscle aches, sore throat, fatigue, swollen lymph nodes and mouth (Lewthwaite and Wilkins, 2009).

At this stage, the viral nucleic acid and the p24 antigen are detectable in the host serum. However, no host antibodies can be detected and the host is said to be seronegative (Pankrac *et al*., 2017). During this phase, RNA levels in the blood spike at about 6 weeks post infection and then decline, while the CD4+ T-cell count drops rapidly until about 6 weeks post infection when it begins a modest increase (Rosenberg et al., 2015).

Stage 2: The asymptomatic phase

Antibodies to HIV are usually detected in the host serum about 6–8 weeks after infection, and it is unusual not to detect them by the third month (Gürtler, 1996). Their detection is referred to as seroconversion and it marks the beginning of the second stage known as the chronic phase. In this stage, an infected person displays no symptoms and may not even be aware that they are carrying the HIV. During this time, the host antibody response to the virus evolves and matures, resulting in an increase in their amounts over several years (Gray *et al*., 2011). In addition, RNA levels in the blood remain relatively stable and the host CD4+ T cell count begins a steady decline (Parekh *et al*., 1999). This phase of HIV infection is accompanied by clinical latency that may last from 1 to 20 years in untreated individuals (Merino *et al*., 2017).

Stage 3: The symptomatic phase

During this stage of infection, minor and early symptoms of HIV disease usually begin to manifest. Viral load is high and the immune system deteriorates and manifests itself by the occurrence of opportunistic infections. Some of these symptoms are : mild to moderate swelling of the lymph nodes in the neck, armpits and groin, occasional fevers, oral candidiasis (thrush), pulmonary tuberculosis, oral hairy leukoplakia, shingles (or Herpes zoster), skin rashes and nail infections, weight loss (up to 10%) and general feelings of tiredness (Braun *et al*., 2015).

Stage 4: Acquired Immune Deficiency Syndrome (AIDS)

The inception of clinical AIDS is usually marked by a CD4+ T cell count $\leq$ 200 cells/mm^3, which continues to decline as the number of HIV RNA copies in the blood increase. Major symptoms such as cancers (Kaposi's Sarcoma), and other severe opportunistic diseases namely, oral and esophageal candidiasis, proctitis, colitis, and hepatitis, pneumonia and

severe cryptosporidiosis can begin to appear as the immune system continues to deteriorate (Weston and Marett, 2009; Fanales-Belasio *et al*., 2010; Braun *et al*., 2015). Figure 4 shows a generalized graph of the relation between HIV copies (viral load) and the CD4+ T-cell counts over the average course of untreated HIV infection. It depicts that the higher the plasma viral load, the lower the number of health-protecting CD4+ T cells.

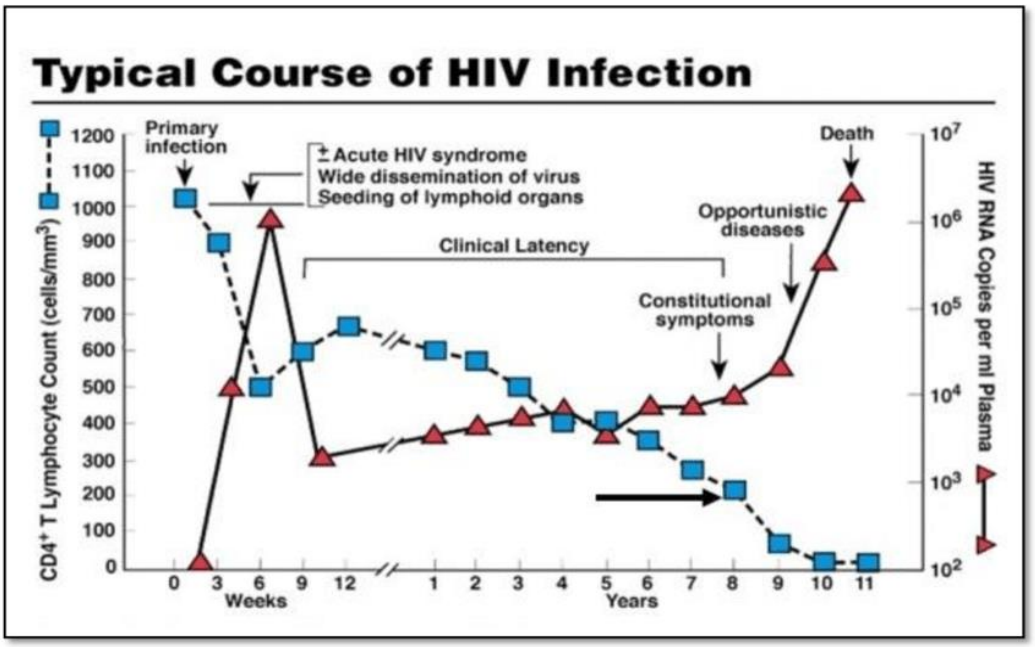

Figure 4: CD4+T-cells count, viral loads and HIV disease symtoms over time.
(Pantaleo *et al*., 1993)

I.1.5. HIV Phylogeny and Genetic Diversity

Since the initial transmission to human beings, HIV has extensively diversified through mutation and recombination (Lihana *et al*., 2012). Research has led the discovery of two subfamilies of the HIV: HIV-1 and HIV-2, both of which are transmitted by sexual contact, through blood, and from mother to child. They can cause clinically indistinguishable forms of AIDS, though HIV-2 seems to be less pathogenic in humans than HIV-1 (Fanales-Belasio *et al*., 2010). In fact, the vast majority of infected individuals carries HIV-1, and most AIDS cases result from its infection (Ngoupo *et al*., 2015). HIV-1 is related to viruses found in chimpanzees and gorillas living in West Africa, while HIV-2 is related to viruses found in sooty mangabeys, old monkeys found in forest from Senegal (Sharp *et al*., 2001).

HIV-2 is subdivided into 9 different groups (A-I) and four HIV-1 groups exist: namely, M (Major or Main), N (Non-M, Non-O), O (Outlier) and the recently described group P

(Putative)(Peeters *et al*., 2010; Kouanfack *et al*., 2019). This last group was discovered in a Cameroonian woman in 2009 and it is closely related to the gorilla Simian Immunodeficiency Virus (SIV) (Plantier *et al*., 2009) and it was later confirmed by another research team (Vallari *et al*., 2011). Group M represents the vast majority of HIV-1 strains found worldwide, and is responsible for the pandemic. Within group M, HIV-1 is also subdivided into 9 subtypes (A-D, F-H, J and K) and 40 CRFs (Soares *et al*., 2010). Cameroon is considered as an epicenter of the HIV pandemic because it hosts one of the broadest genetic arrays of the HIV with many circulating recombinant forms (CRFs) and unique recombinant forms (URFs) (Powell *et al*., 2010; Agyingi *et al*., 2014). Additionally, it is the only African country where group M, N, and O HIV-1 strains co-circulate, and group O and N were first discovered there, respectively in 1994 and 1998 (Gürtler *et al*., 1994; Simon *et al*., 1998).

I.1.6. Laboratory diagnosis

The goal of most HIV diagnostic tests is to detect the infection as early as possible, due to the high risk of transmission that precedes sero-conversion and also because it provides an opportunity to improve health outcomes with an early antiretroviral therapy (Smith *et al*., 2006). The definitive diagnosis of HIV infection at any age requires diagnostic testing that confirms the presence of HIV. Serological testing identifies HIV antigen and/or antibody generated as part of the immune response to infection with HIV (Lewis *et al*., 2015). HIV infection diagnosis is based on detecting host antibodies (IgG) against different proteins or by direct detection of the whole virus itself or its components (such as the p24 antigen and RNA) (Gürtler, 1996). Regardless of the purpose of the test or the types of molecules detected, dried whole blood spots, plasma, or serum are the preferred sample specimen. Other specimens like saliva and urines are also used.

I.1.6.1. Detection of specific host antibodies

The diagnostic tests that detect host antibodies (IgG) specific to the virus include the enzyme immunoassay (EIA, also commonly referred to as the Enzyme-Linked Immunosorbent Assay: ELISA), Western blot (WB), the Immunofluorescence Assay (IFA), rapid tests, salivary tests and urine tests (Gürtler, 1996). Rapid HIV assays can be based on several test formats; these tests are designed for use with individual specimen, and are quick and easy to perform, making them more cost-effective than EIAs in low-throughput laboratories (Aidoo *et al*.,

2001). Most EIAs have a high sensitivity and specificity, and are able to detect HIV-1/HIV-2 and HIV variants. The most recent advances in EIA technology have produced 'combination assays', which combine p24 Ag EIAs with traditional antibody EIAs, allowing for the simultaneous detection of HIV antigen and antibodies using a single test (Beelaert and Fransen, 2010). The WB assay consists of a multilayer process similar to that of the EIA. HIV antigens are laid out from the highest in molecular weight to the lowest on a strip of nitrocellulose. When a specimen is incubated with the strip, any existing HIV antibodies bind to these HIV antigens. Addition of enzyme leads to an antibody–enzyme complex. In a final step, a chemical is added that changes color when it comes into contact with the protein–antibody–enzyme layers (Urassa *et al*., 2002).

I.1.6.2. Direct detection of HIV

Diagnostic tests that directly detect the virus or parts of the virus are better than host antibody assays to identify an HIV-infected individual who has not seroconverted. These tests include HIV nucleic acid-based assays, p24 antigen detection, and peripheral blood mononuclear cell culture (Gürtler, 1996). There are currently two basic techniques used to amplify or increase the genomic copy number of HIV RNA extracted from clinical specimens in order to quantify and sequence them. The first technique involves reverse transcription followed by polymerase chain reaction (RT-PCR). In the second technique, HIV RNA is captured and quantified by hybridizing it to complementary oligonucleotide molecules (Iqbal *et al*., 2007). The tests that measure the free HIV p24 antigen in a serological specimen using EIA have been incorporated into a new generation of HIV screening assays often referred to as fourth generation assays (Aghokeng *et al*., 2004). Peripheral blood mononuclear cell culture is a method where a subset of the patient's blood cells (the mononuclear cells) is cultured in a controlled environment in order to isolate the HIV. This technique was used qualitatively to detect HIV in infants (Iweala, 2004). Quantitative PCR (qPCR) is equally used for viral load determination in early infant diagnosis. HIV diagnostic tests are still being developed and improved with the hope that the availability of simple/rapid and low-cost assays will facilitate access to diagnosis in resource-poor settings.

I.2. ANTIRETROVIRAL TREATMENT

There is currently no vaccine or cure for HIV/AIDS. The only method of prevention is avoiding exposure to the virus. Nevertheless the disease is controlled by the administration of antiretroviral therapy (Weissberg *et al*., 2018).

I.2.1. Treatment goals and effect on HIV burden

The introduction of highly potent drugs has transformed HIV from a deadly disease into a chronic manageable condition (Furrer, 2016). The goals of initial HIV therapy include restoration and preservation of immune function, control of HIV replication, prevention of drug resistance, reduction of HIV-related morbidity and mortality and improving quality of life (Cohen *et al*., 2016). The therapy has also been shown to give protection to the general population by reducing the rate of new infections. There was an estimated 25% reduction in new infections in sub-Saharan Africa in 2011 (total 1.8 million new infections) compared to 2001 (2.4 million new infections). Additionally, providing antiretroviral prophylaxis to pregnant women living with HIV prevented over 350 000 children from acquiring the infection between 1995 and 2010 (WHO, Global HIV/AIDS response, 2011). Unfortunately eradication of HIV cannot be achieved with current drugs available due to the pool of latently infected CD4 T cells established early during acute infection (Cihlar and Fordyce, 2016).

I.2.2. Current approach to treatment: Antiretroviral Therapy (ART) and when to start?

A variety of therapies has been developed since 1984 for persons infected with HIV. Bone marrow transplantation, lymphocyte transfusions, thymic transplantation, and therapeutic apheresis to remove virus-bearing cells were tried without significant success against HIV infection and are no longer employed (Kuritzkes, 2016). Today, ART is the acronym commonly used to describe HIV antiretroviral therapy and it was introduced since 1996 (Ejigu *et al*., 2018). Previous to this, cART (combination antiretroviral therapy) was the treatment paradigm established in the late 1990 (Atta *et al*., 2019) and previous to that the popular term HAART (Highly active antiretroviral therapy) was used (Cihlar and Fordyce, 2016). ART has become the standard of care for patients with HIV infection and involves the simultaneous administration of three or more antiretroviral drugs either taken individually or in fixed-dose combinations (Roshni *et al*., 2016). These therapies increase the survival time of the infected individuals, but does not lead to viral eradication within individuals meaning that

none of the agent used in these combinations can completely eliminate HIV from infected persons (Egger *et al*., 1997; Velasco-Hernandez *et al*., 2002).

In the past years, in most cases, treatment was limited to patients with advanced disease. The truth is that, ideal time to initiate ART is currently unknown (Hughes and Ribaudo, 2008). The Cluster of Difference 4 (CD4) count was the most common indicator of health status and immune function of patients infected with the human immunodeficiency virus (HIV) (Tinarwo *et al*., 2019). While guidelines for resource rich environments recommended initiating at CD4+ T-cell counts < 350, developing country guidelines recommended initiating ART at CD4+ T-cell counts ≤200 in the absence of clinical disease until November 2009 when the WHO recommended initiating treatment at CD4 < 350 (Grinsztejn *et al*., 2014). As new evidence from resource rich environments has accumulated showing that starting ART early, at higher CD4 counts is associated with better treatment outcomes, programs in resource limited settings were revised (Fox *et al*., 2010). Since 2019, Cameroon HIV treatment guidelines, recommend the implementation of therapy in all adults diagnosed with HIV, irrespective of CD4+ T-cells counts or stage of disease. The positive outcomes associated to this new strategy include the reduction in the risk of illnesses associated with HIV infection and the risk of HIV transmission (Cohen *et al*., 2016). ART has been particularly effective in high-income countries, where a large spectrum of antiretroviral drugs (ARVs) is widely available, along with early access to experimental drugs. However, HIV therapy options in low-middle and middle income countries remain limited (De Cock and El-Sadr, 2013). In contrast to high income settings, the choice of ART regimen mainly depends on which ARV drugs are available, regardless of those recommended in treatment guidelines (Dragovic *et al*., 2016).

I.2.3. Antiretroviral treatment coverage

Antiretroviral treatment benefits spurred a progressive improvement in the access to antiretroviral drugs. The Global Fund and United States President's Emergency Plan for AIDS Relief (PEPFAR) Fund among other donors ensured improved access of antiretroviral treatment to resource limited settings. In 2012, 9.7 million of the 34 million people living with HIV were estimated to be on ART in low and medium income countries, which is a six-fold increase from 2005 when only 1.3 million people were receiving ART (WHO, 2013). At the end of 2018, this number has increased reaching 23.3 million. Figure 5 below represents the

increase in ART coverage from 20% to 47% for low and mid-income countries, Cameroon inclusive.

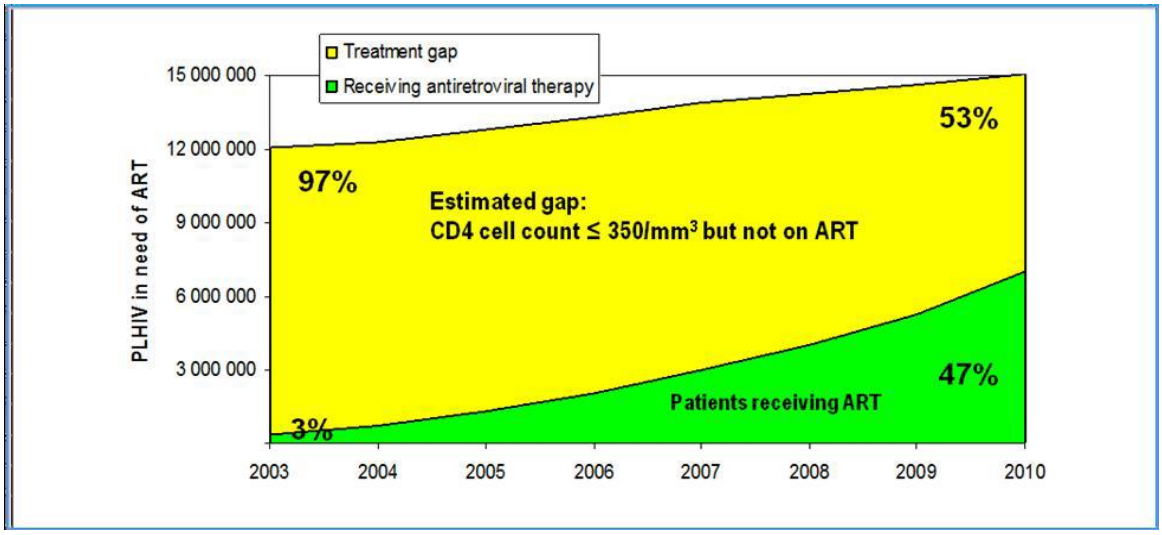

Figure 5: Progressive increase in ART coverage between 2003 and 2010. (WHO, UNAIDS, UNICEF, Global HIV/AIDS Response Progress Report 2011)

Cameroon has implemented an ART program since 1997 and the coverage has been extending gradually. Infected subjects receiving ART increased from 28,000 in 1998 to about 200,000 in 2013 (Kindzeka, 2013). In Cameroon, at the end of 2012, 168 accredited Treatment Centers and Management Care Units were operational for the dispensing of these medications to patients in its ten Regions (Kredo *et al*., 2013). The Cameroonian antiretroviral therapy program has reached one of the highest coverage in the eligible HIV infected population (58%) in Sub-Saharan Africa. It offers the opportunity to assess ART in the context of the decentralization of HIV care delivery (Boyer *et al*., 2010). Antiretroviral therapy coverage in Cameroon has continued to increase reaching 52 % in 2018 and the number of facilities dispensing ARV increased from 168 in 2013 to 481 in 2019.

Since the 90-90-90 targets were launched in 2014 by the Joint United Nations Programme on HIV/AIDS (UNAIDS) and partners, Global efforts were made to make ART more affordable and increase treatment access to more HIV-infected subjects (WHO, Global Health Observatory (GHO), 2015). The aim of this 90-90-90 strategy was to diagnose 90% of all HIV-positive persons, provide antiretroviral therapy (ART) for 90% of those diagnosed, and achieve viral suppression for 90% of those treated by 2020 (Bain *et al*., 2017).

I.2.4. Different classes of antiretroviral

The number of drugs and drug classes available to control HIV- 1 infection has greatly increased since 1989. Up to date, there are six classes of ARV drugs: nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), entry inhibitors (EIs), fusion inhibitors (FIs), and integrase inhibitors (INIs) (García-Blanco *et al*., 2019). Drugs in these classes are characterized by individual differences in absorption, metabolism, diffusion volume, toxicity and interactions (Capetti *et al*., 2015). These drugs target different stages in the HIV life cycle. With advances in research, several novel and experimental antiretroviral drugs including maturation inhibitors, uncoating inhibitors, transcription inhibitors and translation inhibitors are at various stages of development (Capetti *et al*., 2015).

Presently, there are more than 30 ARVs belonging to the above mentioned classes and several of these agents are no longer widely used due to their limited efficacy, inconvenient administration patterns and/or the development of severe adverse events (Apostolova *et al*., 2017).

I.2.4.a. Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

NRTIs are key components of ART regimens, and are often referred to as the "backbone" of HIV treatment. They interfere with the reverse transcriptase enzyme causing chain termination after they have been incorporated into viral DNA. These drugs are nucleoside analogue antiretroviral drug and its chemical structure constitutes a modified version of a natural nucleoside. NRTIs enter the host cells via endocytosis and require intracellular phosphorylation in order to produce an active triphosphate form. They inhibit viral replication of retroviruses by stopping extension of oligomer due to the absence of 3' hydroxyl group essential for addition of incoming new nucleotide (Maga *et al*., 2010; Tressler and Godfrey, 2012).

The most currently available NRTIs are: zidovudine(azidothymidine -AZT), didanosine (2'-3'-dideoxyinosine -ddI), stavudine (2'-3'-didéhydro-2'-3'-didéoxythymidine -d4T), lamividine (2',3'-didéoxy-3'-thiacytidine -3TC), tenofovir (ténofovir disoproxil fumarate -TDF), abacavir (ABC) and emtricitabine (2',3'-dideoxy-5-fluoro-3'-thiacytidine -FTC) (Kufel, 2016). Their mode of action is summarized in Figure 6 below:

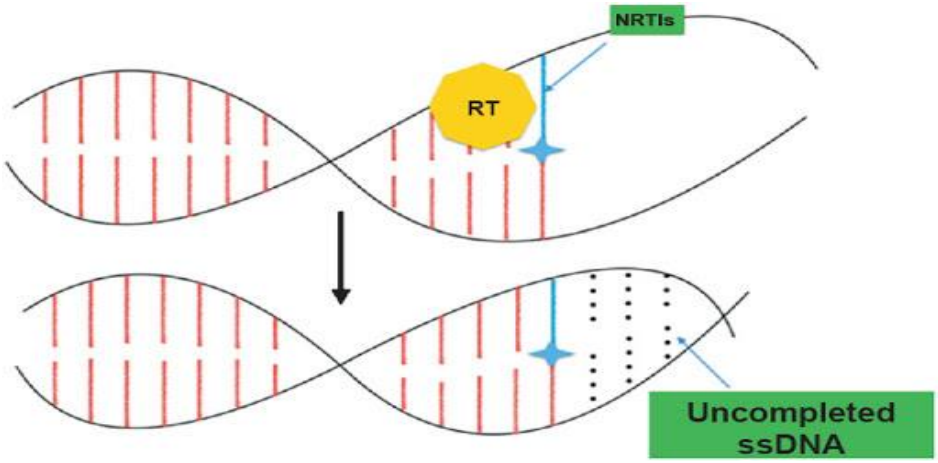

Figure 6: Mode of action of nucleoside reverse transcriptase inhibitor. (Dalal et al., 2015)

NRTIs= nucleoside reverse transcriptase inhibitor, ssDNA= single strand DNA; RT=Reverse Transcriptase

I.2.4.b. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Non-nucleosides are directly active drugs compared to NRTIs which are prodrugs. NNRTIs prevent HIV replication by targeting the enzyme reverse transcriptase, binding to a site near to but different from the active site for substrate. It decreases DNA synthesis drastically. However, unlike the NRTIs they do not act as false substrates or require transformation within the cell to form an active metabolite. Instead, the parent form binds non-competitively to the viral enzyme at a specific region known as the NNRTI pocket, located close to the substrate binding site (see figure 7). The resulting complex blocks the active site by allosteric interactions, so that fewer nucleosides can bind, thereby slowing the polymerase reaction significantly (Pauwels, 2004). Nevirapine (NVP), delavirdine (DEL), efavirenz (EFV) and etravirine (ETR), Nelfinavir (NFV) and Rilpivirine (RPV) are some of the NNRTIs already approved for clinical use (Maggiolo, 2009; Cihlar and Fordyce, 2016).

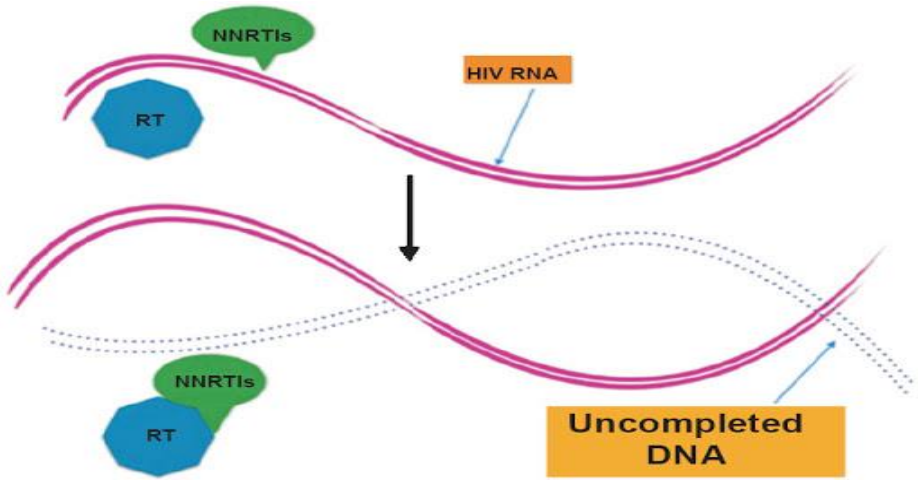

Figure 7: Mode of action of non-nucleoside reverse transcriptase inhibitor. (Dalal et al., 2015)
NNRTIs= non-nucleoside reverse transcriptase inhibitors, RT= reverse transcriptase

I.2.4.c. Protease Inhibitors (PIs)

The introduction of these drugs was done in 1996. HIV hijacks host genetic code on invading CD4+ T-cell and utilizes host cell machinery for its replication. The viral gag-pol polyprotein is excised into active protein particles of newly formed virus by viral protease, a molecular scissor. PIs act by blocking the active site of the viral enzyme thereby preventing a proteolytic splicing that produce shorter, functional proteins and results into immature non-infectious virus particles as illustrated in figure 8 (Monini *et al*., 2003; Titanji *et al*., 2013). There are ten HIV protease inhibitors approved by the FDA; those inhibitors include: saquinavir (SQV), indinavir (IDV), ritonavir (RTV), nelfinavir (NFV), amprenavir (APV), fosamprenavir (FPV), lopinavir (LPV), atazanavir (ATV), tipranavir (TPV), and darunavir (DRV) (Wang *et al*., 2015).

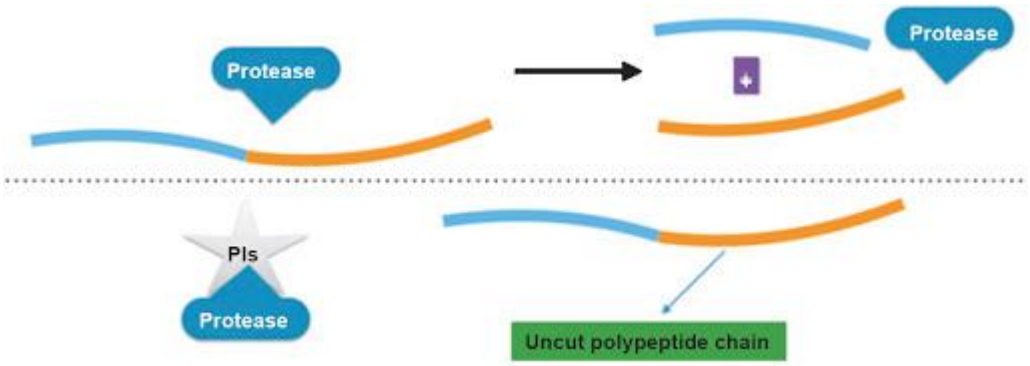

Figure 8: Mode of action of protease inhibitors. (Dalal *et al*., 2015)

PIs= protease inhibitors

I.2.4.d. Integrase Inhibitors (IIs)

HIV integrase is an important enzyme in viral replication through integrating viral DNA with host genome which is absent in human cells (Figure 9). Integrase binds to viral DNA and joins it with host DNA. Integrase inhibitors prevent the formation of covalent bonds with host DNA. This prevents incorporation of proviral DNA into the host genome. Raltegravir (RAL) is the best known (Tsiang *et al*., 2012).

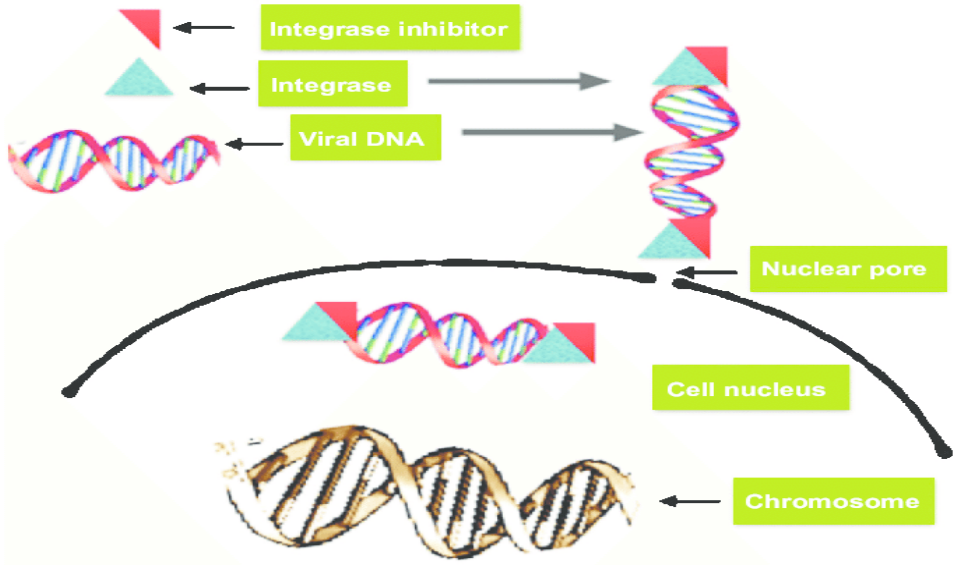

Figure 9: Mode of action of Integrase inhibitors (Dalal *et al*., 2015)

I.2.4.e. Fusion and attachment inhibitors (FIs)

This class of antiretroviral drugs works on the outside of the host CD4+ T-cell. One of the best known is enfuvirtide (T-20) similar to a portion of gp41, necessary for binding to heptad repeat 1 (HR1). It interferes with HR1 and HR2 (as shown in figure 10) thereby, inhibiting the conformational change of gp41 crucial for viral fusion to CD4+ T-cells (Eggink *et al*., 2010).

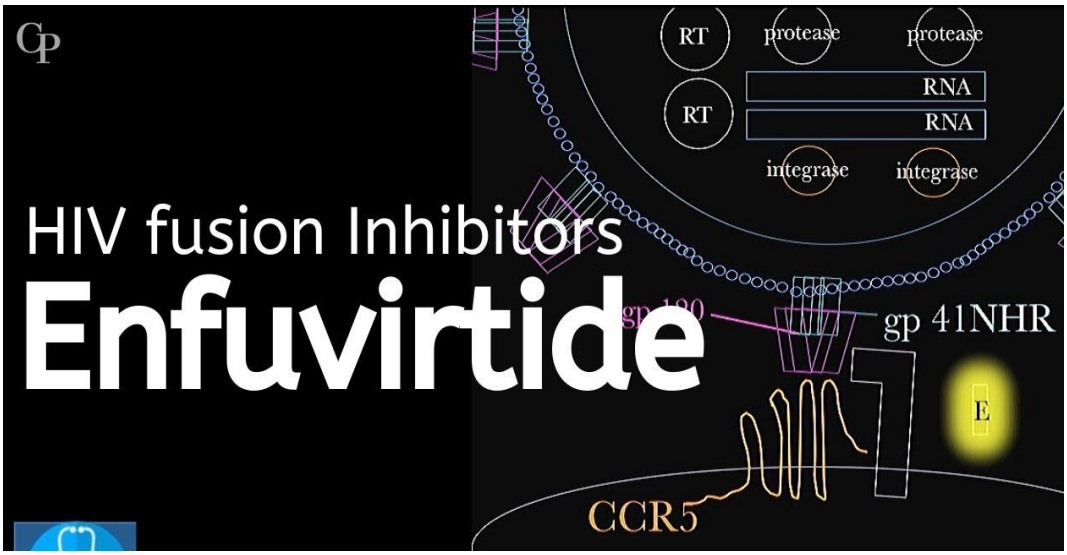

Figure 10: Mode of action of Fusion Inhibitor Enfuvirtide. (Eggink *et al*., 2010)

I.2.4.f. Entry Inhibitors (EIs)

HIV enters human body through fusion of viral envelope proteins with binding domain on host CD4+ T-cell. Theoretically inhibitors can interfere with every step of HIV entry into the host cell. EIs mainly target either the viral envelope glycoprotein gp120 or gp41 or the C-C chemokine receptor CCR5 or CXCR4 receptors on a CD4 cell surface. The result is restricting HIV from infecting a cell and multiplying. Maraviroc and Enfuvirtide are two EIs currently approved for clinical use (Henrich and Kuritzkes, 2013). The mode of action of EIs is presented in figure 11.

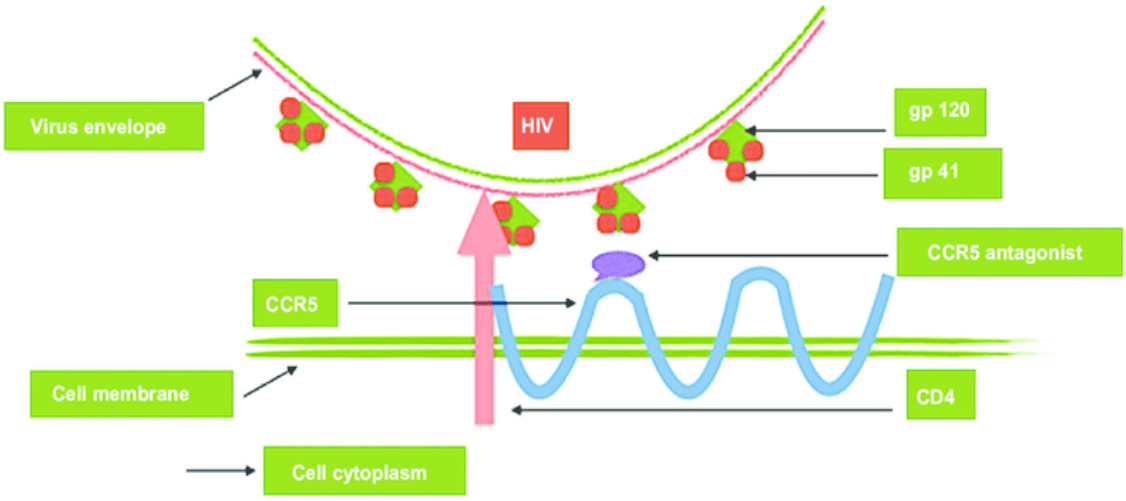

Figure 11: Mode of action of Entry Inhibitor. (Henrich and Kuritzkes, 2013)

Entry inhibitor prevents gp120 from binding to the CCR5 co-receptor and prevents the fusion peptide from gp41 inserting into the cell membrane, gp=glycoprotein, CCR5= chemokine co-receptor 5

I.2.5. ART in Cameroon

For effective care, antiretroviral treatment was introduced in 2003 and has been available free-of-charge since 2007 at all levels of the health system in Cameroon, meeting national and international therapeutic standards. The first line recommended by WHO in resource limited countries, including Cameroon, consists of two Nucleoside Reverse Transcriptase Inhibitors (NRTI) plus one Non-Nucleoside Reverse Transcriptase Inhibitor (NNTI) where Efavirenz (EFV) or Nevirapine (NVP) are the two most commonly used (Kouanfack *et al*., 2011). Wide differences across countries exist as far as ARVs availability is concerned. In fact, in some parts of the world we can find up to 120 different regimens, especially arising from switches from the first-line regimens due to toxicity or viral failure. Switching to 2nd line ART is as "patient being switched from a nucleoside reverse-transcriptase inhibitor and non-nucleoside reverse-transcriptase inhibitor regimen to a protease inhibitor based ART regimen" (Enoné *et al*., 2019). In general, the more regimens a patient have failed due to tolerability or viral failure, the more complex and expensive the regimen becomes, and drug interactions among antiretrovirals are frequent. In Cameroon third line ART is less available as compare to the first and second line.

I.2.6. Adverse drug reactions related to ART

One of the major obstacles to drug development and reasons for subsequent drug withdrawal from the market is toxicity issues (Hornberg *et al*., 2014). ART, which continues to be the mainstay of HIV management, prevents viral multiplication and reduces the viral load in the blood, resulting in improved immune function in an HIV-infected person and a decrease in the risk of transmitting the virus to sexual partners and children (Cohen *et al*., 2016). Unfortunately, alongside these gains, anti-retrovirals, like many other administered drugs, are reported to be associated with side effects. These adverse drug reactions (ADRs) have being identified as one of the main raison for treatment modification and a serious barrier to compliance which is a major determinant for the success of therapy (Elzi, 2010; Kim *et al*., 2015). As a consequence, there has been an increased risk of viral resistance and treatment failure. Anti-HIV-related adverse events have been particularly found to occur in higher proportions at the beginning of ART (Tadesse *et al*., 2014). According to the WHO definition, an ADR is any noxious, unintended, and undesired effect of a drug, which occurs at doses used in humans for prophylaxis, diagnosis or therapy. This definition excludes therapeutic

failures, intentional and accidental poisoning (overdose), and drug abuse (Ejigu *et al*., 2018). It is important to note that the outcome of ADR is observed both in adults and children (Oumar *et al*., 2012).

Many studies have been carried out around the world in developing and developed countries. Results from these studies showed that the incidence of ADR among patients on ART ranged between 4.3% to 90% (Shet *et al*., 2014; Roshni *et al*., 2016; Kindie *et al*., 2017; Mehari *et al*., 2017). Many risk factors have equally been identified. These include: age of the patient, gender, ART regimen, duration of treatment, opportunistic infection prophylaxis, WHO clinical stage, disease biomarkers (such as CD4 count and viral load) and Body Mass Index (BMI) (Bhatnagar *et al*., 2013; Lartey *et al*., 2014; Masenyetse *et al*., 2015; Kindie *et al*., 2017; Kumari *et al*., 2017; Ejigu *et al*., 2018; Raikar *et al*., 2018).

The risk of ADRs arises because of the effect of the disease on the immune system and the safety profile of the complex ART drugs (Hawkins, 2010). Some studies have described the association between ART use and a large spectrum of ADRs including lipodystrophy, fatigue, nausea and vomiting, abdominal pains, diarrhea, increase in liver function and liver failure, hypersensitivity syndrome reactions (rashes), central nervous system adverse events, pancreas and kidney toxicities (Reust, 2011; Neuman *et al*., 2012; Shubber *et al*., 2013). The severity of ADRs ranges from mild to life-threatening, and may occur following a single dose, a prolonged administration of medicine (Rolfes *et al*., 2016). The combination of two or more medicines may also aggravate the condition (Eluwa *et al*., 2012). Drug toxicities can add to the complexity of HIV management by impairing patient compliance to treatment. The plausible resulting consequences could be the inferior clinical outcomes associated with higher costs to the public health system (Rajesh *et al*., 2012; Hansana *et al*., 2013; Fagbami *et al*., 2015; Mehari *et al*., 2017). Generally about 6% of all admissions into medical hospital wards have been shown to be due to ADRs (Bushra, 2017). Some evidences showed that up to 25% of patients discontinued their initial ART regimen because of toxic effects (Lima *et al*., 2012; Kindie *et al*., 2017). Some clinical investigations went forth to demonstrate that there was an improvement in symptoms reported by more than 90% of the patients after drug discontinuation (Leutscher *et al*., 2013).

Efavirenz is associated with several central nervous system (CNS) side effects (To *et al*., 2009).

Various studies have documented an incidence of 60–90% of short term neuropsychiatric symptoms such as dizziness, hang-over sensation, difficulty concentrating, headache, vertigo,

euphoria, sleep disturbance (including somnolence, insomnia and vivid or abnormal dreams), irritability or mood changes, suicidal ideation and nervousness (Fumaz *et al*., 2005; Cavalcante *et al*., 2010; Abers *et al*., 2014). More severe neurological effects have also been registered (in fewer than 2% of the patients), including severe depression, delirium, paranoia, depersonalization, hallucinations, anxiety, aggressive behavior, abnormal thinking and mania (Scourfield *et al*., 2012). The management of HIV infection, however, is still complex and worrisome due to problems such as monitoring of therapy efficacy, chronic administration, drug toxicity, poor tolerability, drug resistance development, or therapy adjustment after treatment failures. For all these reasons, the search for new treatment approaches is still uptodate and will remain beneficial (Esposito *et al*., 2012).

I.3. Factors affecting response to ART

Despite the successful ART efficacy, a high percentage of patients do not achieve adequate suppression of plasma HIV RNA or experience drug toxicity. An individual's response to ART is dependent on many factors, which relate to the patient or 'host', the virus and the drug(s) administered. Thus, treatment failure can occur due to physiological, pathological, genetic and behavioral factors, and pharmacologically, due to poor pharmacokinetics, drug interactions, a lack of treatment efficacy or the development of viral resistance (Gonzalez-Serna *et al*., 2016).

I.3.1. Viral factors

High plasma viral load in a patient on ART often indicate treatment failure and it implies that the virus has acquired resistance to a drug, or a whole drug class (cross-resistance). Resistance occurs when mutations are acquired by viruses to become slightly different from the original wild- type population (Siliciano and Siliciano, 2013). The development of resistance in HIV is due to both the rapid and error prone replication, as the reverse transcriptase enzyme does not contain DNA proofreading properties like other retroviruses (Martinez-Picado and Martínez, 2008). These mutations accumulate when viral replication occurs in the presence of selective pressure from antiretrovirals and /or immune response. The potential for selection of drug resistant strains is, therefore, substantially higher in the presence of suboptimal antiretroviral concentrations, when the replicative capacity of the virus becomes greater (Gonzalez-Serna *et al*., 2016). This is the reason why effective monitoring of antiretroviral concentrations may be a suitable tool for evaluating or predicting the risk of resistance,

especially in patients with high viral loads and/or suspected adherence issues. The single point mutation K103N is one of the most predominant mutations that causes cross-resistance to all first generation NNRTI and does not change the replicative capacity of HIV (Cong *et al*., 2007). Consequently, a prompt change in therapy is vital to avoid NNRTI exclusion, and preserve future options.

I.3.2. Pharmacological factors

Not all virological failure can be attributed to the development of HIV drug resistance. Some drug-related or pharmacological factors which can explain therapeutic failure include poor drug pharmacokinetics, inadequate potency and a low genetic barrier to resistance, unfavorable toxicity profiles and poor penetration of antiretrovirals into viral sanctuary sites (Clevenbergh *et al*., 1999). Pharmacokinetics is the area of pharmacology which describes the Absorption, Distribution, Metabolism and Elimination (ADME) of drugs by physiological systems in the body (Banjoko, 2012). In the case of HIV treatment, acquiring and maintaining antiretroviral concentrations in the body fluids is crucial in ensuring that therapeutic drug concentrations reach their local receptor site (i.e. within CD4+ T-cells) in order to exert the desired pharmacological response (DiFrancesco *et al*., 2013). Most significantly, the NNRTIs (EFV in particular) have well defined pharmacokinetic/pharmacodynamic and pharmacokinetic/toxicity relationships in which, systemic (plasma) drug levels have been shown to correlate with observed virologic response, or to independently predict the risk of treatment failure/success (Fabbiani *et al*., 2010).

I.3.3. Host factors

External and internal factors relating to the patient or 'host' may influence on the observed inter-subject variation in drug concentrations and response to treatment. They can be psychological (life-style and adherence to therapy), pathological (the stage of HIV infection or presence of co-infections), physiological (age, gender, body weight and composition) and genetic (variations in genes involved in drug metabolism) (Sadée and Dai, 2005; Leger *et al*., 2016). Patient non-adherence to therapy has been cited as one of the main causes of treatment failure in HIV patients (Desta *et al*., 2020). Past studies have demonstrated that >95% adherence is essential for achieving viral suppression and treatment success. If the drugs are not taken appropriately, plasma concentrations may not be maintained above their therapeutic

thresholds (as consequences: suboptimal viral suppression and increased risk of viral rebound or development of resistance) (Desta *et al*., 2020). Several studies have described differences between men and women in their response to ART and drug pharmacokinetics. Additionally, there is evidence to suggest better clinical outcomes for women than men but women are likely more susceptible to adverse effects (Bersoff-Matcha *et al*., 2000). It is likely that differences in the level of drug exposure between males and females are mainly driven by differences in body weight and composition because the dosing of ARVs is not adjusted for body weight. Additionally, changes in drug metabolism, through differential expression and activity of CYP450 enzymes (fluctuations of reproductive hormones) also impact the sex-related differences in drug exposure (Bersoff-Matcha *et al*., 2000).

I.4. Overview on drug metabolism

I.4.1. Different phases of drug metabolism

ARVs are required for HIV patients to maintain a good health status but at the same time, they are foreign elements (xenobiotics) to the body. Hence, the human body tries to eliminate them at the earliest. It is highly preferable that the dugs get eliminated from the body immediately after exerting their action. The more the drug stays in the body, the greater are its side effects (Taxak and Bharatam, 2014). Drug metabolism involves reactions that modify the chemical structure of the drugs (Banjoko, 2012). During this, the conversion of drugs give compounds that are more water soluble and more easily excreted but can also be involved in the conversion of prodrugs into active compounds. These reactions may result in the generation and build up of harmful reactive metabolites that are more toxic than the parent drug (Corsini and Bortolini, 2013). There are three phases of metabolism: phase I reactions (oxidation, reduction and hydrolysis), phase II conjugation reactions (glucuronidation, acetylation, sulfation and methylation) and final phase III that involve further metabolism of conjugates of phase II reactions before being recognized by efflux transporters and pumped out of the cells (Guo *et al*., 2011). Phase I reactions include transformation of a parent compound to more polar metabolite(s) by unmasking of functional groups (e.g. -OH, -NH2, -SH). This is basically a functionalization reaction and the most common modifications in this phase is hydroxylation, a reaction catalyzed by an hepatic dependent mixed function oxidase system (Jancova *et al*., 2010).

I.4.2. Description/focus on the main phase I enzyme: CYP 450

The principal metabolic enzymes involved in this first phase are members of the cytochrome P450 (CYP450) superfamily, performing mainly hydroxylation and hence acting as monooxygenases, dioxygenases and hydrolases. The cytochromes P450 constitute a superfamily of heme enzymes responsible for the metabolism of a wide range of xenobiotics and endogenous compounds (Guengerich, 2018). The CYP450 enzymes are primarily located in the liver but can also be found in the small intestines, lungs, kidneys and even the heart (Chaudhary *et al*., 2009). Their etymology derives from their intracellular, membrane-bound localization (i.e., cyto-), with a heme pigment forming part of the protein (i.e., chrome). The heme portion of the enzymes absorbs light at a maximum wavelength of 450 nm when complexed with carbon monoxide in the reduced state (Fatunde and Brown, 2020). The superfamily is divided into families: CYP1, CYP2, CYP3, etc (the sequence identity of the members > 40%). Each family is divided into subfamilies: CYP1A, CYP1B, CYP1C, etc. (the sequence identity of the members > 55%). The individual members of each subfamily are numbered: CYP1A1, CYP1A2, CYP1A3, etc. The human CYP supergene family consists of 18 families, divided into 41 protein-coding subfamilies encoding 57 genes (Alzahrani and Rajendran, 2020).

I.4.3. Efavirenz and its metabolism

Efavirenz is a potent and effective non-nucleoside reverse transcriptase inhibitor that has become a cornerstone of antiretroviral combination regimens utilized in the management of patients with HIV infection (Gutierrez *et al*., 2005). It has been considered the NNRTI ''par excellence''. It acts by binding to reverse transcriptase non-competitively thus altering its function and preventing conversion of viral RNA to DNA (De Clercq, 2009). The drug forms part of the triple therapy regimen and is taken orally as a single dose of 600mg at night. Night time dosing is preferred in order to minimize the day time consequences of side effects common in patients (Apostolova *et al*., 2015). The general informations and characteristics of Efavirenz are summarized in Table 1 below.

Table 1*:* General and pharmacokinetic characteristics of Efavirenz

Marketing authorization	FDA approval in September 1998, making it the 14th approved antiretroviral drug
Chemical name	(4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one
Empirical formula	C14H9ClF3NO2
Molecular weight	315.68 g/mol
Administration	single daily dose
Bioavailability	50% following oral administration—much enhanced if taken with fatty food
Plasma half-life	45–50 h
Pharmacokinetic parameters of a 600mg dose	Cmax: 4.07 mg/L
	Cmin: 1.76 mg/L
	AUC: 57.9 mg h/L
	time to peak plasma concentration: 3–5 h
	time to steady-state plasma concentration: 6–10 days
Plasma protein binding	99% (particularly to albumin)
Volume of distribution	2.4 L/kg
Clearance of parent, unchanged drug	renal: <1%
	faeces: 16%–61%
Hepatic metabolism	metabolized mainly by: CYP2B6
	Inducer of: CYP3A4
	Inhibitor of: CYP3A4, CYP2C19, CYP2C9

(Apostolova *et al*., 2015)

AUC= area under the curve, FDA= Food and Drug Administration, L= liter, Kg= kilogramme, CYP= cytochrome, h= hour, mg= milligramme, c= concentration,

The fact that approximately 99% of EFV in the blood is protein-bound limits its extravascular diffusion and results in a high intracellular/ plasma concentration ratio, a characteristic that is specific to it. Efavirenz is metabolized primarily by isoenzyme 2B6 of cytochrome P450 (CYP2B6) through primary oxidative hydroxylation in the liver (Leger *et al*., 2016). This

major route of EFV metabolism generates 8-hydroxyefavirenz (8-hydroxy-EFV) predominately (Figure 12). Other enzymes namely, CYP3A4, CYP3A5, CYP1A2 and CYP2A6 may play minor contributing roles in the metabolism of EFV (Ward *et al*., 2003) . CYP2B6 is also the main enzyme involved in formation of the secondary metabolite 8,14-dihydroxy-EFV. EFV can be hydroxylated to 7-hydroxy-EFV by CYP2A6, a minor pathway of EFV metabolism accounting for around 23% of overall EFV metabolism *in vitro* (Ogburn *et al*., 2010). EFV can also go through a direct N-glucuronidation by UDP glucuronosyl transferase (UGT) 2B7(Ji *et al*., 2012). Some studies have shown that, 8-OH-EFV was more toxic than EFV or 7-OH-EFV. These findings demonstrate that the 8-OH metabolite of EFV is a potent neurotoxin (Tovar-y-Romo *et al*., 2012). EFV and its metabolites are largely (60%) eliminated via bile through faeces. Few studies have examined the influence of renal impairment on Efavirenz levels. However the study revealed that less than 1% of Efavirenz is excreted unchanged in the urine (Apostolova *et al*., 2015).

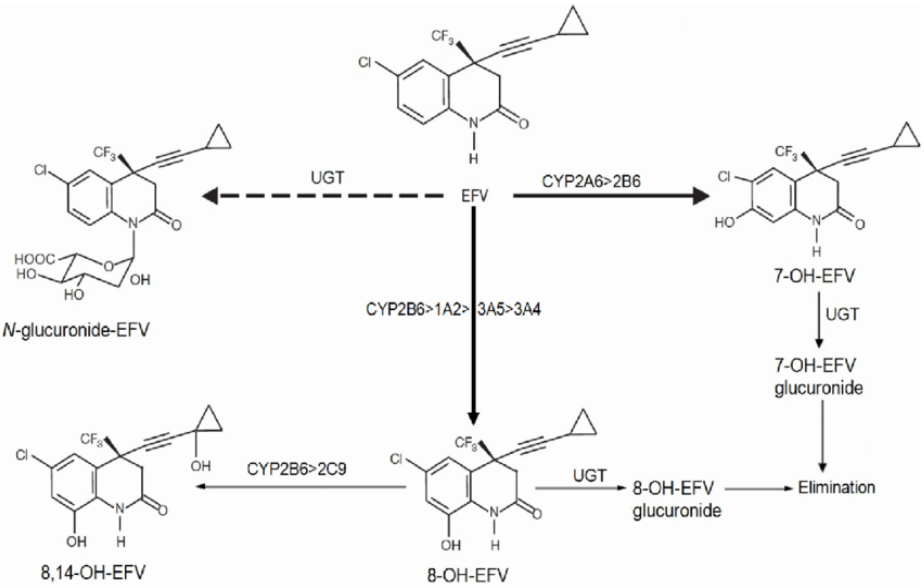

Figure 12: Suggested metabolic pathways for Efavirenz and its catalytic hepatic enzymes (Bienvenu *et al*., 2015). *UGT= Uridine 5'-diphospho-glucuronosyltransferase, CYP= cytochrome, EFV= Efavirenz*

Though very effective at suppressing replication of the virus that causes AIDS, a standard dose of Efavirenz is known to carry a significant risk for CNS-mediated neuropsychiatric adverse events (Muñoz-Moreno *et al*., 2009; Mills *et al*., 2013). However, little is known on the mechanisms taking place in neuropsychiatric toxicity caused by Efavirenz and which CNS off-targets might be involved. There are suggestions that exposure to supra-therapeutic levels

due to slower metabolism of EFV could explain why neuropsychiatric disturbances are common (Sarfo *et al*., 2016). 8-Hydroxy-efavirenz, the main metabolite of EFV, has been shown to be toxic to neuron cultures at concentrations found in the cerebrospinal fluid (Decloedt and Maartens, 2013). A study in rats suggests that EFV readily accumulates in the brain to levels that exceed 4.6 times the plasma levels within 1 hour of an intraperitoneal dose of 15 mg/kg (Dirson *et al*., 2006). By assuming that a similar level of brain accumulation occurs in humans, then EFV plasma levels >2.74 μg/mL would correspond to a brain concentration >40 μg/mL. The rapid accumulation of relatively high concentrations of EFV in the brain and its very high propensity for protein binding in combination with its narrow therapeutic window seem to be key contributing factors responsible for its CNS-related adverse events (Dalwadi *et al*., 2016).

I.5. Importance of pharmacogenetics of EFV in HIV treatment

I.5.1. History of pharmacogenetics

Arno Motulsky in 1957 (Motulsky, 1957) postulated that inheritance might explain variability in drug efficacy and toxicity. Two years later, the word "pharmacogenetics" was then coined and published for the first time by the German Pharmacologist Friederich Vogel in 1959 to define a new science applying genetic and pharmacology to study the influence of inheritance on drug response (Pirmohamed, 2011). With advancements in molecular biology's techniques (as gene cloning advanced to sequencing of the entire human genome), the term pharmacogenomics, which was first used by Andrew Marshall in 1997, started to be used in addition to pharmacogenetics (Daly, 2017). Pharmacogenomics and its predecessor pharmacogenetics study the contribution of genetic factors to the interindividual variability in drug efficacy and safety. One of their major goals is to tailor drugs to individuals based on their genetic makeup and molecular profile (Charlab and Zhang, 2013). Essentially the two terms are now used interchangeably though the scope of pharmacogenomics is broader. In fact, most of pharmacogenetics studies investigate on the pharmacological consequences of single gene mutations. Pharmacogenomic investigations require an increased use of methods designed to study many genes or gene patterns, that is to look simultaneously at the structure and expression of whole sets of genes namely the entire genome (Kalow, 2006).

It is important to note that, the individual variation in drug response is attributed to the complex interplay of multiple factors (figure 13). These include differences in genetic

makeup, environmental factors, co-morbidities, age, sex, race, organ dysfunction, disease characteristics, co-medications and drug–drug interactions (Evans and Johnson, 2001). Sometimes genetic variability is solely responsible for these variations (Dalal *et al*., 2015).

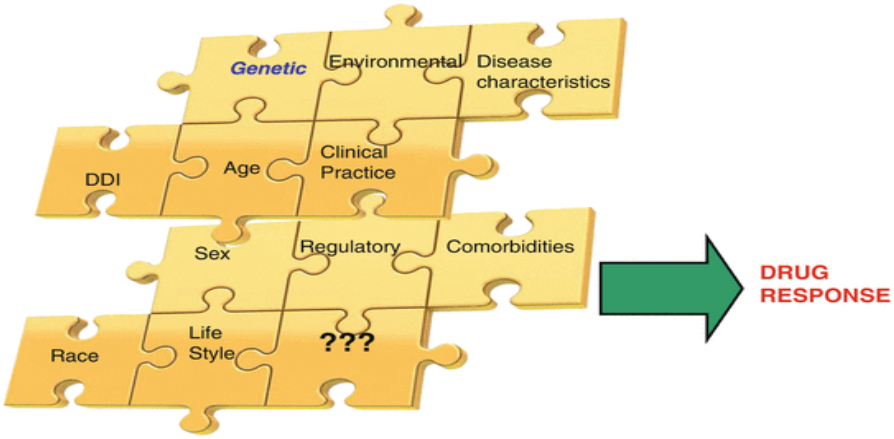

Figure 13: The drug response "puzzle". (Charlab and Zhang, 2013)

DDI= drug–drug interactions

Genetic makeup is just one of many factors that contribute to interindividual variability in drug response. The contribution of each factor can be different for different patient (Charlab and Zhang, 2013).

It is crucial to assess the contribution of the various genetic and non-genetic components to the overall response. It has been shown that genetic factors were by far the more important because it was estimated that this component accounts for 20–95 % of variability in drug response (Evans and McLeod, 2003). Unlike other factors influencing drug response, inherited determinants generally remain stable throughout a person's lifetime. In recent years, Pharmacogenetics and pharmacogenomics have moved beyond candidate gene approaches and genome wide association studies (GWASs) toward personalized genomics (Rodríguez-Vicente *et al*., 2016).

I.5.2. Basis of pharmacogenetics

Variation in drug metabolism and drug response among individuals can be due to temporary causes such as transient enzyme inhibition, induction or permanent causes such as genetic mutation, gene deletion or amplification (Banjoko and Akinlade, 2010). Pharmacogenetics affects pharmacokinetics, pharmacodynamics, or both, which are two major determinants conferring the interindividual differences in drug responses. Pharmacokinetics deals with how much of a drug is required to reach its target site in the body, while pharmacodynamics deals

with how well the targets such as receptors, ion channels, and enzymes respond to various drugs (Ahmed *et al*., 2016).

Within the area of pharmacogenetics, findings of genetic variation influencing drug levels have been more prevalent, and variation in the cytochrome P450 (CYP) enzymes is one of the most common causes. Much of the work concerning sequence variations in CYPs aims at finding biomarkers of use for individualized treatment, thereby increasing the treatment response, lowering the number of side effects and decreasing the overall cost of treatment regimens (Sim and Ingelman-Sundberg, 2010). However, recent data indicate that pharmacogenetic mechanisms are complex, appear on several levels of gene expression from the initial mRNA transcript to splice variants (pre-mRNA splicing and mRNA expression) to altered proteins, and affect function in various ways (Zanger and Klein, 2013). A genetic mutation frequency exceeding 10% of a population is considered a genetic polymorphism (Banjoko, 2012). Genetic variations are the result of multiple mechanism such as insertion, deletion, variable tandem repeats and microsatellites but the most frequent polymorphism are point mutation or single nucleotide polymorphism (SNPS). A transitional SNP involves the substitution of a purine such adenine (A) or guanine (G) for another purine or one pyrimidine such as cytosine (C) or thymidine (T) for another. A transversional SNP involves a change of a purine (A, G) for a pyrimidine (C, T) and vice versa. By nomenclature G>A means G which is the wild type has been substituted by the mutant A. G>A and C>T transitions account for approximately 25% of all SNPs in the human genome (Zanger and Klein, 2013) .

Some of the polymorphism are without consequences but others cause synthesis of altered proteins, truncated proteins, unstable proteins or affect the level of expression (Banjoko, 2012). Variability in CYP450 expression and function is found to contribute to four clinical phenotypes: poor (PM), intermediate (IM), extensive (EM) and ultra-rapid metabolizers (UMs) (Johansson and Ingelman-Sundberg, 2010). PMs are individuals with mutation or deletion of both alleles and therefore prone to accumulation of drug substrates in their systems with high susceptibility to adverse drug reactions. IMs are heterozygous for one copy of a null allele and a functional allele of a certain CYP450. This results in a diminution in enzyme activity and they may need lower than average drug dose for optimal therapeutic response (Banjoko, 2012). EMs are homozygous for two functional alleles and are call normal drug metabolizers (characteristic of the normal population), and they often require higher concentrations of an administered drug than IMs and PMs. UMs carry more than two active gene copies. Ultra-rapid metabolism is a result of gene duplication (i.e. copy number variation

(CNV) of a gene's coding and regulatory regions). They possess enhanced drug metabolism capabilities due to gene amplification and are prone to drug failure because drug concentrations at normal doses are expected to be too low for therapeutic effects (Bains, 2013). It has been reported that various cytochrome P450 isozymes and drug transporters, might contribute to the variability in drug response, thereby influencing an individual's response to drugs, both in terms of response and adverse reactions (Preissner *et al*., 2013).

I.5.3. Overview on CYP 2B6 gene

CYP2B6 (Cytochrome P450 Family 2 Subfamily B Member 6) is a protein coding gene. Importantly, this gene is highly polymorphic and harbor a large repertoire of single nucleotide polymorphisms and copy number variations (Zhou *et al*., 2017). Thus, SNPs in CYP genes are major determinants of drug pharmacokinetics and toxicity and constitute pharmacogenetic biomarkers for drug dosing, efficacy, and safety. Strikingly, the distribution of CYP alleles differs considerably between populations with important implications for personalized drug therapy and healthcare programs (Zhou *et al*., 2017). The cytochrome P450 enzyme CYP2B6 is one of about a dozen human CYPs that are primarily involved in the biotransformation of drugs (Zanger and Klein, 2013).

Localization and structure of the CYP 2B6 gene

The CYP2B6 gene has been mapped to chromosome 19 (see figure 14), in a cluster of genes of the CYP2 sub-family and in close proximity to the CYP2B7 pseudogene, which shares more than 90% sequence homology with CYP2B6. It is 28 kb long and consists of 9 exons and 8 introns (Zanger and Schwab, 2013). The CYP2B6 enzyme is expressed in the liver, nasal mucosa, trachea, lung and brain. It catalyzes the metabolism of xenobiotics and several therapeutic drugs, such as bupropion, cyclophosphamide, Efavirenz and Nevirapine (Alessandrini *et al*., 2013).

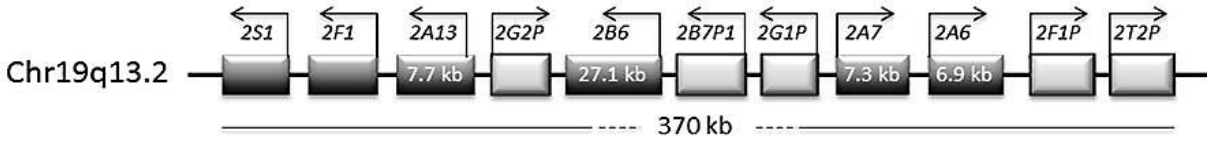

Figure 14: Structure of genomic regions on chromosome 19 and localization of CYP 2B6 gene. (https://ghr.nlm.nih.gov/gene/CYP2B6)

Regulation of the CYP 2B6 gene

CYP2B6 is the major biotransformation enzyme for a large number of substrate such as clinically used drugs and many diverse environmental toxins and chemicals, and it is highly variable due to the existence of strong inhibitors and inducers (Turpeinen and Zanger, 2012). The major discovery in this field was the identification of orphan nuclear receptors (NR1I3) and (NR1I2) as the major regulators of CYP2B6 by drugs and other xenobiotics via a phenobarbital-responsive enhancer module (PBREM) and a distal xenobiotics-responsive enhancer module (XREM) at -1.7 kb and -8.5 kb respectively of the CYP2B6 promoter (Sueyoshi *et al*., 1999; Wang *et al*., 2003). CYP2B6 inducers are typically ligands of either of these receptors, such as rifampicin and barbiturates, or substances that activate both receptors. Several structurally unrelated drugs and certain non-pharmaceutical compounds have been shown to inhibit CYP2B6 and many of them do that in a mechanism-based, irreversible manner (Turpeinen and Zanger, 2012). In human hepatocytes inducibility of CYP2B6 was reported for cyclophosphamide, artemisinine, carbamazepine, efavirenz and nevirapine (Wang and Tompkins, 2008).

CYP2B6 alleles

CYP2B6 is thus the second most variable CYP-gene of the superfamily, second only to CYP2D6. More than 500 single nucleotide polymorphisms (SNPs) have been identified in the CYP2B6-coding (exonic) and intronic regions as well as the 5'- and 3'-flanking regions according to NCBI (http://www.ncbi.nlm.nih.gov/). To date, a total of 28 alleles have been described with CYP2B6*1A being the wild-type allele and over 50 determined haplotypes documented and listed on the CYP allele nomenclature website (http://www.imm.ki.se/CYPalleles). No copy number variation has been described for *CYP2B6* (Leger *et al*., 2009). Lang and collaborators performed the first systematic analysis of the CYP2B6 genetic polymorphisms on the nine exons in the coding region (Lang *et al*., 2001). At the end of their study, they identify nine mutations (five nonsynonymous amino acid alterations and 4 silent mutations). These SNPs alone or in association generated six different CYP2B6 alleles designated as CYP2B6*2 (64C>T), CYP2B6*3(777C>A), CYP2B6*4 (785A>G), CYP2B6*5 (1459C>T), CYP2B6*6 (516G>T and 785A>G) and CYP2B6*7 (516G>T, 785A>G and 1459C>T). Through the dedicated works of many research groups, 19 more alleles in the coding region of CYP2B6 have been defined (Lang *et*

al., 2004; Hesse *et al*., 2004). These newly characterized alleles included CYP2B6*16 (785A>G and 983T>C), *18(983T>C), *27(593T>C) and *28(1132C>T) as well as four phenotypic null alleles *8 (415A>G), *11 (136A>G), *12 (296G>A), and *15 (1172T>A). Even though all these alleles are resulting from amino acid changes, the functional consequences of each on the gene expression or function have varied widely.

I.5.4. Genetic variability of CYP2B6 gene and clinical relevance in Efavirenz-based treatment

The CYP2B6 gene is involved in human physiological responses to a wide range of xenobiotic compounds, including many clinically useful drugs among which those used for HIV treatment and a variety of exogenous chemicals present in the diet and the environment (Gross *et al*., 2017). The genetic variations of CYP2B6 and its potential clinical relevance have only been discovered in recent years. Nevertheless, along with the realization of the inter-individual variability in CYP2B6 expression and activity, the number of pharmacogenetic studies on this isoenzyme has been growing rapidly (Atta *et al*., 2019). Genetic polymorphisms on this gene influence individual variation in responses to environmental toxins, and the effectiveness of prescribed medications (Ahmed *et al*., 2016). The relationships between genotypes and phenotypes have been elucidated in several pharmacogenetic studies. A number of single nucleotide polymorphisms (SNPs) of CYP2B6 that influence the gene activity have been systematically classified and applied in the human clinical studies (Oluka *et al*., 2015). Functionally, these SNPs translate into a variety of phenotypic outcomes including proteins with significantly reduced catalytic activity or a complete loss of CYP2B6 expression (Rotger *et al*., 2007). Some CYP2B6 alleles are associated with no detectable function or expression (CYP2B6*8, *12, *15, *18, *21), reduced function or expression (CYP2B6*5, *6, *11, *14, *19, *20, *21) or increased expression (CYP2B6*22) (Rotger *et al*., 2007; Cummins *et al*., 2015). Some analysis of human liver mRNA discovered that aberrant splicing of the CYP2B6 gene due to mutations was the cause of the reduced function of mRNA and protein (Hofmann *et al*., 2008).

Ethnic differences exist in the frequencies of specific CYP2B6 polymorphisms. It was reported 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). Two other functional alleles of CYP2B6, namely *16 (983T>C and 785A>G) and *18 (983T>C), may also be considered as African

specific, as the common 983T>C SNP has been reported in several African populations (Ghana, Ivory Coast Senegal, South Africa, Tanzania, Sierra Leone, Cameroon and Guinea) and in African–Americans, while it is largely absent in the majority of Caucasian and Oriental populations assessed to date (Alessandrini *et al*., 2013).

Reduced enzyme activity resulting in decreased metabolism of Efavirenz was associated with some SNPs of CYP2B6, such as the 516G>T SNP reported to have a major impact on the pharmacokinetics and pharmacodynamics (Marzolini *et al*., 2001; Saitoh *et al*., 2007; Ramachandran *et al*., 2009; Mahungu *et al*., 2009; Lindfelt *et al*., 2010; Maganda *et al*., 2016; Müller *et al*., 2017). Additionally, there are 785 A>G, 983 T>C, 593 T>C and 1132 C>T substitutions in CYP2B6 reported to result in slow metabolism of EFV (Dalal *et al*., 2015; Gross *et al*., 2017). Several clinical studies have reported that mutations associated with decreased metabolism of Efavirenz (primarily 516 G>T (rs3745274) and 983 T>C (rs28399499)) result in increased substrate plasma concentrations, decreased drug clearance and higher susceptibility to central nervous system side effects (Haas *et al*., 2009; Damronglerd *et al*., 2015; McDonagh *et al*., 2015; Carr *et al*., 2014; Dhoro *et al*., 2015; Gallien *et al*., 2017). Ribaudo and colleagues (2006) equally revealed a possible link to drug resistance in the presence of a homozygous 516TT genotype (Ribaudo *et al*., 2006). Other reports in HIV-infected Thai patients showed that the heterozygous/homozygous mutation associated with low EFV concentrations was 18492T>C, and those associated with high EFV concentrations were 516G>T, 785A>G, and 21563C>T(Sukasem *et al*., 2012; Sukasem *et al*., 2014). Another SNP, CYP2B6 15582C>T (rs4803419), predicts modestly increased plasma Efavirenz exposure with both White and African ancestry, and is most frequent in Whites (Leger *et al*., 2016).

Many studies have been carried out to investigate the impact of CYP2B6 polymorphisms on virological and immune response in HIV patients and there are many controversial results. Some of the studies revealed associations (Ribaudo *et al*., 2006; Habtewold *et al*., 2011; Queiroz *et al*., 2017) while others say there was none (Saitoh *et al*., 2007; Haas *et al*., 2014; Müller *et al*., 2017; Gross *et al*., 2017; Chang *et al*., 2018; Cheng *et al*., 2019). Individuals harboring slow metabolizer genotypes were shown to have significantly higher Efavirenz concentrations and were prone to high rate of treatment discontinuation due to reported CNS symptoms. There are suggestions that exposure to supra-therapeutic levels mostly among African Americans for instance could explain why neuropsychiatric disturbances are more common among them than in European American or Hispanic patients (Leger *et al*., 2016).

The mechanisms for neuropsychiatric toxicity caused by Efavirenz have been elucidated. 8-Hydroxy-efavirenz, its main metabolite has been shown to be toxic to neuron cultures at concentrations found in the cerebrospinal fluid (Sarfo *et al*., 2014). Proposed mechanisms for this neurotoxicity include alterations in calcium homeostasis, increases in brain pro-inflammatory cytokines, involvement of the cannabinoid system as well as mitochondrial damage. Others have postulated that the neuropathic effects of HIV itself, the effect of Efavirenz on cytokine homeostasis, previous predisposition towards neuropsychiatric disturbances and sleep disturbances may all contribute to neuropsychiatric toxicity (Sarfo *et al*., 2016).

The effects of genetic polymorphism in the CYP2B6 gene on EFV metabolism led to investigations of CYP 2B6 SNPs as a promising genetic marker to be used toward the prediction of EFV toxicity. It also allows the recognition of patients being slow EFV metabolizers and who are at risk of higher plasma exposure, central nervous system (CNS) side effects, and possibly EFV resistance after drug withdrawal (Bushyakanist *et al*., 2015). All this provide sufficient motivation for the use of CYP2B6 genotyping in clinical practice where and whenever possible (Barco, 2013).

I.5.5. Methods used for the detection of single nucleotide polymorphisms

The evolution of SNP detection technology is characterized by the clever adoption of new biological methods, fluorescent and other reporters, computational algorithms, and highly sensitive analytical instruments. Genotypic analysis of the CYP2B6 gene typically involves polymerase chain reaction (PCR) with either restriction fragment length polymorphism or sequencing analysis of PCR products**.** A number of methods for determining mutations have been published; the Polymerase Chain Reaction coupled to Restriction Fragment Length Polymorphism (PCR-RFLP) method (Rotger *et al*., 2005), the real-time PCR allelic discrimination method and the direct sequencing method (Mardis, 2008) . A study was carried out in order to compare three genotyping methods: gene re-sequencing, real time PCR allelic discrimination and PCR-RFLP for the detection of a genetic variation (516G>T) in the CYP2B6 gene (Dhoro *et al*., 2013)**.** Results revealed a 100% correlation with all three methods. Comparison for cost of equipment and reagents required for each method revealed an order of: sequencing > real time-PCR > PCR-RFLP. This study demonstrates the reproducibility of these three methods (Dhoro *et al*., 2013). Newer genotyping methods include genotyping by matrix-assisted laser desorption/ionization–time of flight mass

spectrometry (MALDI-TOF/MS) that utilizes a soft ionization technique and allows for the analysis of various large organic molecules, proteins and polypeptides. This technique combines high throughput with high accuracy and may be used for rapid screening for SNPs, quantitative allele studies and for discovery of new polymorphisms.

I.5.6. Importance of Therapeutic Drug Monitoring (TDM) in Efavirenz-based treatment

Therapeutic drug monitoring is defined as the clinical laboratory measurement of the levels of drugs in plasma, serum or blood of patients that, with appropriate medical interpretation, will directly influence drug prescribing procedures (Astuti, 2015). TDM is an approach to optimize and individualize drug administration. It has a great importance in the treatment of HIV because it enable the increase of treatment efficiency and possibly, lower the adverse drug reactions associated to ART (Ma *et al*., 2011). EFV is a suitable candidate for TDM because it is well known that a correlation exist between its plasma concentraions and the pharmacological response, as measured by disease markers (CD4 cell counts and viral load) as well as toxicity. The correlation is more visible with toxicity than with efficacy (Cabrera *et al*., 2009). Some single nucleotide polymorphisms in CYP2B6 are associated with lower rate of EFV metabolism and lead to high exposure (very high plasma concentrations) as well as a higher risk of central nervous system toxicity, particularly the homomutant (harboring a slow metabolizer phenotype) variant (Maganda *et al*., 2016). These associations led to suggestions of Efavirenz dose reduction. In Japan, it was found that, CYP2B6*6 carriers who receiving EFV 600 mg had a very high plasma drug concentration, their EFV dose was reduced from 600 to 200 mg to remain in normal therapeutic range and minimize adverse neuropsychological events (Damronglerd *et al*., 2015). Patients with EFV concentrations of > 4000 μg/L may be exposed to CNS adverse effects more frequently, while those with plasma concentrations < 1000 μg/L were shown to have a higher risk of developing selective drug resistance and treatment failure (Marzolini *et al*., 2001).

Different chromatographic methods have been used to measure EFV. These include High Performance Liquid Chromatography (HPLC) with ultraviolet or fluorescence detection, Reversed-phase Liquid Chromatography (RP-HPLC) and Liquid Chromatography Tandem Mass Spectrometry (LS/MS/MS), High Performance Thin Layer Chromatography (HPTLC) and capillary electrophoresis (Marzolini *et al*., 2001; Haas *et al*., 2004; Rotger *et al*., 2007; Hamrapurkar *et al*., 2009; Pereira *et al*., 2005; Panchagiri *et al*., 2018; Srivastava *et al*., 2013). These previously cited methods use a complex and time consuming sample pre-treatment and instrument set-up. Additionally, the equipment are costly and require highly

skilled personnel for manipulations (Mogatle and Kanfer, 2009; Ramachandran *et al*., 2006). This makes them unsuitable in resource limited countries. Other methods such as automated immunoassays were developed that require less expertise and no sample pre-treatment and at the same time demonstrate good performance to distinguish suboptimal, normal and high EFV concentrations (Abdissa *et al*., 2014). A part from plasma, other types on sample specimens were tested for possibility of use in EFV quantification such as dried blood spots and hair (Hoffman *et al*., 2013; Johnston *et al*., 2018). Generally there is a possibility to simultaneously measure parent drug and significant metabolites and other anti-retroviral drugs with these various methods.

CHAPTER TWO: MATERIALS AND METHODS

II.1. DETERMINATION OF THE GENERAL PROFILE OF ADVERSE DRUG REACTIONS AND THE PREVALENCE OF THOSE ASSOCIATED WITH EFAVIRENZ IN HIV POSITIVE CAMEROONIANS ON ANTIRETROVIRAL THERAPY

II.1.1. Study area and design

A hospital-based observational retrospective study was conducted in the outpatient ART center (day care hospital) of the Central Hospital of Yaoundé, the second largest city and political capital of Cameroon. This center is one of the largest in the country, with approximately 200 HIV-positive individuals getting enrolled in the program every month. The data for this study were obtained by reviewing the medical records of patients enrolled in the ART center for one year period.

II.1.2. Study population

The study population consisted of all HIV-positive patients aged 18 years and more who initiated follow up at the ART center between 1st January, 2013 and 31st December, 2013 and who had at least one follow-up clinical visit after commencing treatment. According to the standard procedure at the Center during that period, once the HIV status was confirmed positive, medical information was obtained by health workers during the initial visit through a standardized interview (including detailed socio-economic and demographic data), and entered into the patient's medical file. The patient then underwent a first clinical consultation by a medical doctor to determine his/her WHO HIV clinical stage. After this, a set of baseline laboratory tests (Hematology, biochemistry, CD4+ T-cell count) was required from the patients. The test results were then analyzed by the physician and the patient was classified as eligible or not eligible for ART initiation. Once declared eligible, the patients were initiated on a combination antiretroviral therapy consisting of two nucleosides reverse transcriptase inhibitors (NRTIs) (Zidovudine (AZT)/Lamivudine (3TC), or Tenofovir (TDF)/3TC) plus one non-NRTI (NNRTI) (either Nevirapine (NVP) or Efavirenz (EFV). All patients were generally given a 30-day supply at initiation. Thereafter, they were received once more and interviewed by the physician to evaluate early response to treatment and adherence. After the first prescription pick-up, a maximum of 3 months of treatment at a time was dispensed to the patient depending on his tolerance and compliance with the medication. Follow-up laboratory tests (CD4+ T-cell count, HIV viral load and others) were repeated every 6 months unless an

earlier evaluation was medically indicated. All patient data and test results were documented and kept in their medical records.

II.1.3. Data collection

ADRs were identified based on patient complaints and/or observations made and reported in the patient's record by physicians during routine clinical examination. Physicians considered that an adverse effect was associated with ART if it was absent prior treatment initiation, and to which other causes could not be attributed. ADRs were considered as mild if the patient continued with the same medicines or severe if his/her treatment was changed to another. Data for this study were retrieved from available patient medical records and reported in a standardized data collection forms (Appendix 4). Baseline demographic characteristics (age, marital status, sex, employment status, geographic site of residence and educational level) and clinical factors (WHO HIV clinical stage, ART regimen, hemoglobin level, weight, Tuberculosis (TB) co-infection, Cotrimoxazole prophylaxis, poor compliance history and CD4+ T-cell count) were collected. Baseline was defined as date obtained at the time of ART initiation. The onset time of ADRs was evaluated by estimating the time between the treatment initiation date and the date of complaints reported or the date on the test results for laboratory-based ADRs (full blood count to detect the presence or not of anemia).

- **Inclusion criteria**

Were included in this study, all patients who initiated ART in the center during the study period.

- **exclusion criteria**

Were not included, patients who never initiated ART, who were transferred-in and those with missing files.

II.1.4. Ethical considerations

Direct patient identifiers, like names, were not collected during the research; in their place, unique codes were used. Since only patient data was extracted, this was considered low risk research. All information regarding participants (e.g. identity) were kept confidential during data collection, analysis and reporting by replacing names with assigned codes and all records containing participant data were kept in a secured cupboard always locked with keys and only accessible to the investigators of the project. Ethical clearance was obtained from the National

Ethics Committee for Research in Human Health of Cameroon ($N^0$2014/12/670/CE/CNERSH/SP).

II.1.5. Data analyses

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 25 to relate variables and/or compare groups in terms of variables. Descriptive and univariate analyses were performed on quantitative data. Age, marital status, sex, employment status, geographic site of residence and educational level) and clinical factors (WHO HIV clinical stage, ART regimen, hemoglobin level, weight, Tuberculosis (TB) co-infection, Cotrimoxazole prophylaxis, poor compliance history and CD4+ T-cell count) were collected. The cut-off for statistical significance was set at a p value of <0.05.

II.2. EVALUATION OF THE PREVALENCE OF CYP2B6 516 G>T AND 983 T>C POLYMORPHISMS AND INVESTIGATION OF THEIR ASSOCIATION WITH THE DEVELOPMENT OF ADVERSE DRUG REACTIONS ASSOCIATED WITH EFAVIRENZ IN CAMEROONIAN HIV INFECTED INDIVIDUALS AND DEVELOPMENT OF THE MANUAL OF PROCEDURES.

II.2.1. Study area

The pharmacogenetic study was conducted at two different sites: the outpatients ART Centers of the Yaoundé Central Hospital (YCH), which is one of the largest in Cameroon and the Bertoua Regional Hospital (BRH) located in the East Region of Cameroon. These ART centers of YCH and BRH were created in 1988 and 2003 respectively. The services provided include full consultation by devoted physicians and psycho-social counselors, a pharmacy for drug refills, and laboratory testing (CD4+ T-cell count, HIV viral load and others) for biological follow-up. They are opened from 8:00 a.m. – 3:30 p.m from Monday to Friday. HIV patients who visit these treatment centers come from different urban and rural areas of the Center and East Regions of Cameroon.

II.2.2. Study design

This was a case-control study. HIV-infected individuals already under ART, with or without ADRs associated to Efavirenz, were selected retrospectively, based on information documented in their medical records by the consulting clinician. Participants were prospectively recruited after contacting them through phone calls.

II.2.3. Participants enrollment

A list of eligible patients with their telephone numbers was thus constituted. ADRs were determined based on patient complaints during follow-up visit as reported in the registers by the physician. Central nervous system toxicities or events were said to be associated with Efavirenz when absent before treatment and present after EFV-based treatment initiation. Controls (individuals with no reported central nervous system ADRs) were recruited from the same Health Center and were selected based on the absence of ADR development during at least 2 years of treatment regimen containing EFV. The following inclusion and exclusion criteria were used to select eligible patients for enrollment:

- **Inclusion criteria**

A patient was included into this study if he or she was: (i) Under ART, (ii) age ≥ 18 years, (iii) willing to provide a signed informed consent form, (iv) under follow up, (v) not under concomitant drug.

- **exclusion criteria:**

Meanwhile a patient was excluded after decision of withdrawal from the study.

II.2.4. Ethical considerations

The study was conducted in accordance with the principles laid down in the Helsinki Declaration of 2008. Ethical clearance was obtained from the National Ethics Committee for Research in Human Health of Cameroon (Nº 2014/12/670/CE/CNERSH/SP). Prior to inclusion in the study, patients were given detailed verbal and written information in the presence of a witness for those unable to read. This was with regards to the nature of the study, confidentiality issues and it was made clear that participation was voluntary. Upon agreement to participate they were given an informed consent form to sign. All information regarding participants (e.g. identity) were kept confidential during data collection, analysis and reporting by replacing names with assigned codes and all records containing participant's data were kept in a secured cupboard always locked with keys and only accessible to the investigators of the project. Soft data were also kept confidentialy.

II.2.5. Data collection

Demographic, clinical and therapeutic data were obtained from the clinical records of the HIV/AIDS patients taking ART using a questionnaire. Such data included: sex, age, weight,

ART regimen and treatment initiation date, complaints after treatment initiation, ADR onset time, treatment modification and information on treatment observance or adherence.

II.2.6. Sample Collection and DNA extraction

Venous blood (5ml) was collected by venepuncture into EDTA tubes and used to prepare dried blood spots (DBS) on filter paper that were stored until genomic analysis. The rest of blood was sepatated as plasma and blood cells and stored at -20°C. DNA was extracted from DBS using the Chelex method. The final supernatant (DNA) was transferred into a fresh tube and stored in TE buffer at -20 ºC for further pharmacogenetic analyses.

DNA was extracted from dried blood spot on filter papers (Protein Saver Card)) by chelex boiling method as previously described by Plowe *et al*., (1995). This method involves boiling a sample in a 5% suspension of deionized water and Chelex. This process disrupts the cell membranes, destroys cell proteins. The suspension was then centrifuged, to separate the resin and cellular debris from the supernatant containing DNA for amplification. All materials and reagents were sterilized using the autoclave (SANO clav, Germany) at a maximum temperature of 144ºC. Using a scissors sterilized by flaming on a bursen burner, each blood spot on the filter paper was carefully excised and transferred into a 1.5 mL microfuge tube into which 1 mL of 0.5% saponin in 1× PBS was added. The scissors was flamed in between samples, dipped into distilled water and wiped with a clean tissue paper. The tubes were then inverted 4 times to completely submerge the filter paper and kept at 4°C overnight. The following day the solution was discarded and the filter papers washed with 1mL of 1× PBS and incubated at 4°C for 15-30 min. During this period, 50 µL of 20% chelex (sterile) were added into 150 µL of nuclease free water in a 1.5 mL microfuge tube and placed on a heat block set at 100°C. After incubation, the filter papers were transferred into 1.5 mL microfuge tubes containing hot 20% chelex-100 and nuclease free water using a sterilized forceps. The forceps were sterilized in between samples by flaming and cleaned as described above. During the incubation period of 10 minutes in the heat block, each tube was vortexed twice for 30 s, centrifuged at 10,000 rpm for 2 minutes and 150 µL of the supernatant transferred into a fresh tube. Centrifugation was repeated again at 10000 rpm for 2 minutes and 100 µL of the final supernatant (DNA) transferred into a fresh tube and stored at -20ºC for PCR analysis.

II.2.7. Determination of CYP2B6 516 G>T and 983 T>C genetic polymorphisms

Two alleles: CYP2B6 516G>T (rs3745274) and CYP2B6 983T>C (rs28399499) were investigated using polymerase chain reaction-restriction fragment length polymorphism

(PCR-RFLP) method. This included: PCR amplification and enzymatic digestion followed by agarose gel electrophoresis.

II.2.7.1. PCR amplification

PCR (polymerase chain reaction) is a molecular technique used to generate a large amount of DNA from a specific sequence. The desired DNA is amplified using specific primers. The primer sequences used for amplification of the CYP2B6 gene to detect the 516G>T and 983T>T polymorphisms were CYP 516 F: 5'-GGT CTG CCC ATC TAT AAA C-3' and CYP 516 R: 5'-CTG ATT CTT CAC ATG TCT GCG-3', CYP 983 F: 5'-AGG AAT CCA CCC ACC TCA AC-3' and CYP 983 R: 5'-GAT AAG GCA GGT GAA GCA ATC A-3' respectively. The amplification was done in a T3 thermal cycler (Biometra, UK). The reaction mixture was prepared to a total volume of 25 µL, containing 17.75 µL of nuclease free water (NFW), 2.5µL of 10X thermopol buffer from New England Biolabs (NEB), 0.5µL of 10mM dNTPs (200µM of each deoxyribonucleotide), 0.5 µL of each primer and 0.25 µL of 5U/ µL *Thermophillus acquaticus* (Taq) polymerase (NEB) and 3 µL of DNA extract.

For 516 G>T, after initial denaturation at 95 °C for 10 min, 35 cycles of amplification were carried out with denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 60 s, followed by a final extension at 72 °C for 10 min and then conservation at 4-8 °C for 48 h. For 983T>C, the conditions were different at the level of annealing (59°C for 25 sec) and the total number of cycles was 45. To confirm the presence of CYP 2B6 gene, the expected amplicon sizes of the PCR products (526bp and 759bp respectively) were verified by separating 8µL of PCR products by electrophoresis on 2% agarose gel as described in section II.2.5.3 before proceeding with restriction fragment length polymorphism (RFLP) when bright bands were obtained compared to the standard DNA ladder. Figure 15 below summarises the different steps involved in PCR amplification.

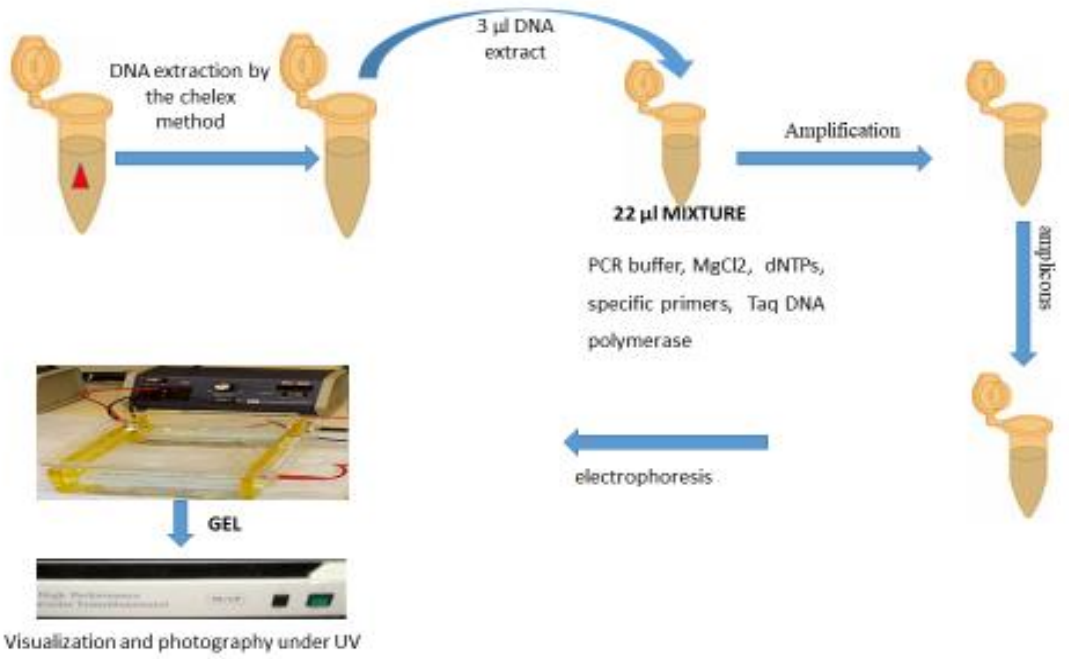

Figure 15: Summary of the different steps involved in PCR amplification

II.2.7.2. Restriction Fragment Polymorphism: Digestion by restriction enzymes

The process of restriction digestion involved cutting DNA molecules into smaller pieces with the aid of special enzymes called restriction endonucleases (often referred to as restriction enzymes or RE's). These special enzymes recognize specific base sequences in the DNA molecule. Restriction enzymes usually digest DNA at specific sites containing palindromic sequences. This is a critical step in mutation detection based on the fact that a SNP can either create or destroy a restriction site. PCR products were digested with a restriction enzyme which the recognition site includes the targeted SNPs. Depending on the presence of the SNP, the amplicon is either digested or left intact (undigested).

Specific restriction enzymes were used following the manufacturer's instructions. Separation of the resulting fragments on agarose gels gives rise to a fragment pattern which facilitates differentiation between genotypes. The RFLP reaction conditions for digestion with *BsrI* (for 516 G>T) and *BsmAI* (for 983 T>C) (New England Biolabs, USA) were set at 65 ºC and at 55 ºC respectively, both for 16 h each. The PCR product was digested in a total reaction mixture of 20µL consisting of 8µL of PCR product, 8 µL of nuclease free water (NFW), 3µL of 10X buffer, and 1µL of each enzyme. The products of the digestion reaction were separated on a 2% agarose gel stained with ethidium bromide and visualized under UV light against a

standard DNA ladder. Polymorphisms were determined according to specific expected fragment patterns as follows:

(516 G/G): 268 bp, 236 bp, 22 bp

(516 G/T): 504 bp, 268 bp, 236 bp, 22 bp

(516 T/T): 504 bp, 22 bp

(983 T/T): 759 bp

(983 C/T): 759 bp, 637 bp, 122 bp

(983 C/C): 637 bp, 122 bp

II.2.7.3. Gel electrophoresis

Electrophoresis is a method for separation and analysis of macromolecules and their fragments, based on their size and charge by applying an electric field to move the negatively charged molecules through a matrix (agarose, polyacrylamide). When the migration is completed, the molecules in the gel are stained followed by direct visualization under ultraviolet light. Photographs are taken for further analysis to detect specific mutations based on the different fragment patterns obtained after restriction digestion.

In this study, digestion products were separated on 2% agarose gel (Seakem, Nusieve). Briefly, 2g of agarose gel was weighted into a 250 mL bottle and 100 mL of 1×TBE buffer added. The mixture was allowed to boil on a bursen burner for 25 minutes then allowed to cool at room temperature (25ºC) to about 60°C and 2.5 µL of ethidium bromide (EtBr) added. The mixture was gently swirled to avoid air bubbles and the solution then poured into a clean-leveled casting tray with combs already inserted and allowed to solidify at 25ºC for at least 45 minutes. After setting, the combs were carefully removed and the casting tray inserted into the electrophoresis chamber filled with sufficient amount of running buffer (1×TBE). With a micropipette adjusted to 10 µL, the PCR product was gently mixed up with 2.5 µL of loading buffer and gently loaded into samples' wells. DNA ladder standard (50 or 100bp) was also loaded in the first well. The power leads were then connected to the electrophoresis system (Fisher Biotech) set at 100 volts and the system was allowed to run for 30 minutes. The distance migrated was visually monitored by tracking dyes (bromophenol blue and xylene cyanol). The gel was later on visualized under high performance UV transilluminator and

photographed using a digital camera and the distance migrated calculated against the standard DNA ladder from the standard curve (Appendix 7). Figure 16 below summarises the different steps involved in RFLP.

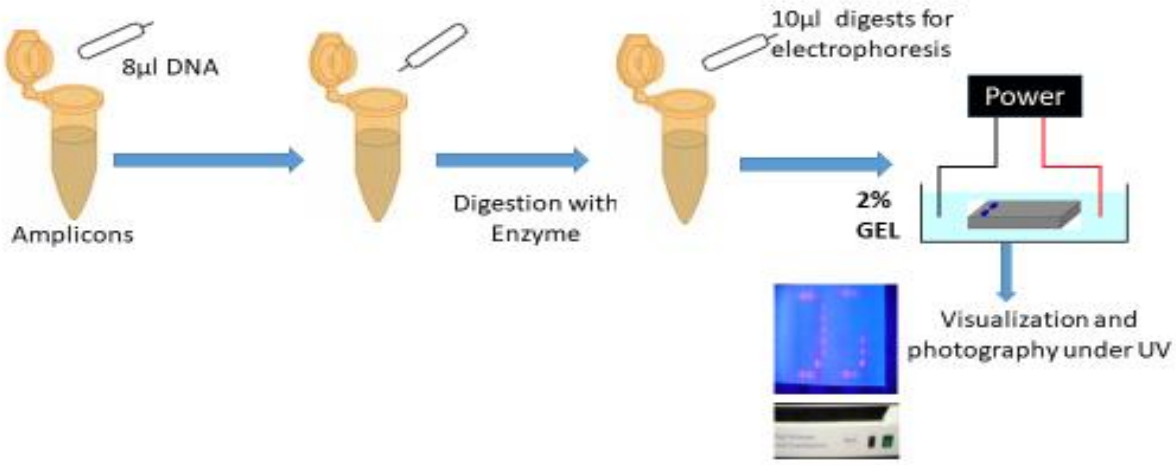

Figure 16: Summary of the different steps involved in RFLP

II.2.8. Data analysis

Allele frequencies were calculated by Hardy-weinberg, where f_x= (2nx/x + nx/-)/2n, f_x= the allele frequency of SNP x; nx/x= the number of patients homozygous for x; nx/-= the number of patients heterozygous for x; n= the total number of patients.

All data were entered into Excel files and analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0 (SPSS Inc., USA). Descriptive statistics, percentage rate and frequencies were used to describe the socio-demographic and clinical data. Frequencies of the CYP2B6 516G>T and 983T>C genotypes in the study population were obtained by descriptive statistics. The association between the genotype/phenotype of the CYP2B6 516G>T and 983T>C and adverse drug reactions associated to EFV in the study population was evaluated using the Chi Square test. The Odds Ratios (ORs) at 95% Confidence Intervals (CIs) were also calculated and the cut-off for statistical significance was set at a p value of <0.05.

II.2.8. Writing up of the manual of procedures

The development of the manual was inspired from experience on the field and in the laboratory, and so took into account all the challenges faced. The manual will be structured in chapters and standard operating procedures (SOPs), which provide amendments to these

challenges. A standard operating procedure, is a step-by-step set of instructions to guide researcher to perform tasks in a consistent manner (APPENDIX 9). SOPs are particularly important for complex tasks that must conform to regulatory standards and are also critical to ensuring efficient effort with little variation and high quality in output. In the laboraratory each protocol was tested and standardized.

CHAPTER THREE: RESULTS AND DISCUSSION

III.1. Specific objective 1: To determine the general profile of ADRs and the prevalence of EFV-related Central Nervous System adverse events.

III.1.1. Profile and Baseline Characteristics of the Study Population

A total of 2,108 patients were enrolled into the ART program of the Day Care Unit of Yaoundé Central Hospital between January and December 2013. Only 1,996 complete medical files were available and were screened; the other 112 files were either missing or unavailable. Among the files reviewed, 645 patient's files were excluded because they were never initiated on ART treatment and 97 belonged to patients transferred from other treatment centers (who initiated treatment elsewhere and were transferred to the Central Hospital for diverse reasons). This last category was excluded from the analysis because most of the time, the data on their past clinical/medical history was incomplete. For this study therefore, a total of 1,254 patient's records were considered eligible for inclusion into the sudy (Figure 17).

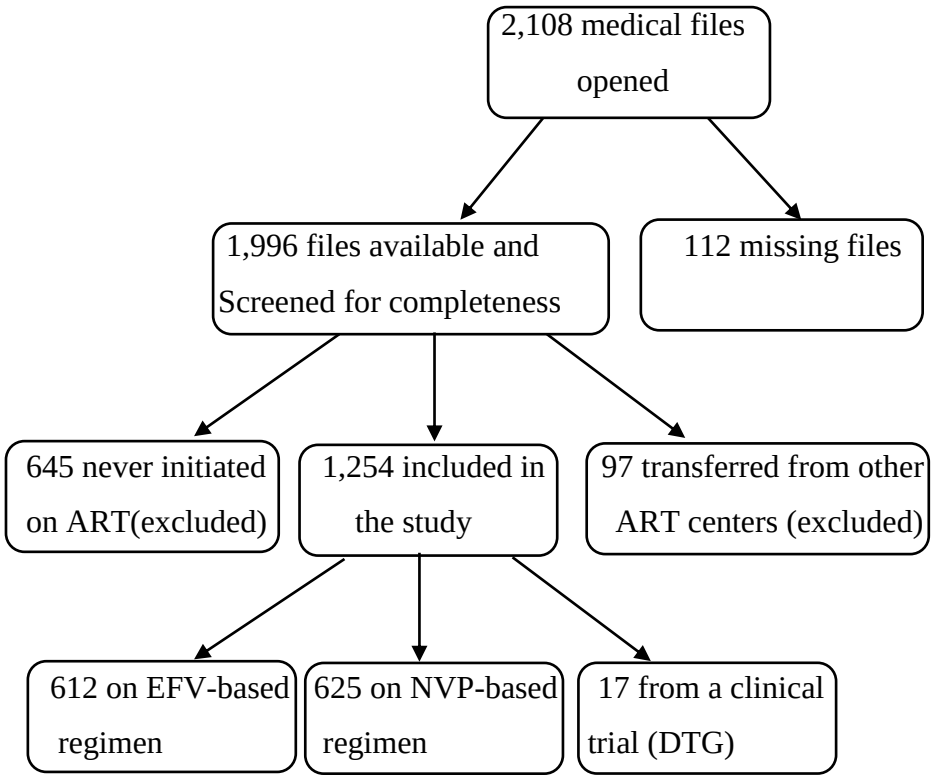

Figure 17: Flow diagram showing medical files selection procedure.

EFV= Efavirenz, NVP= Nevirapine, DTG=Dolutegravir

Table 2 shows the socio-demographic characteristics of the study population. They were twice more females (845; 67.4%) than males (409; 32.6%). The mean age was 38 years ± 10. The age group with the highest number of patients was that between 25 and 34 years, with 430 (34.3%) participants. Six hunded and fifty four (52.2%) were of secondary school education. The number of people who reported to be single were 547(43.6%); 469 individuals (37.4%) were unemployed, and 1,106(88.2%) reported to be living in Yaoundé.

Table 2: Socio-demographic characteristics of patients at treatment initiation

Variables	Category	Number	Percentage (%)
Sex	Female	845	67.4
	Male	409	32.6
Age	15-24	90	7.2
	25-34	430	34.3
	35-44	398	31.7
	≥45	336	26.8
Marital status	Married	294	23.4
	Cohabiting	225	17.9
	Single	547	43.6
	Widowed	145	11.6
	Divorced	43	3.4
Employment status	Employed	361	28.8
	Self-employed	424	33.8
	Unemployed	469	37.4
Site of residence	In Yaoundé	1106	88.2
	Out of Yaoundé	148	11.8
Educational level	None	40	3.2
	Primary	438	34.9
	Secondary	654	52.2
	Tertiary	122	9.7

III.1.2. Clinical and immunological characteristics

More than half (55.7%; 699) of the patients had a body weight of more than 60Kg. One-third (33.2%) of them were at WHO stage III at the moment of treatment initiation. Over half of them (57.7%) had CD4+ T-cell counts less than 200 cells/µL. The number of patients who had received cotrimoxazole prophylaxis was 463(36.9%) and 7.5% had TB co-infection. The two predominant ART regimens initially prescribed for the patients during the data collection period were a combination of Zidovudine, Lamivudine and Nevirapine (AZT/3TC/NVP) 46.6% (584) followed by Tenofovir, Lamivudine and Efavirenz (TDF/3TC/EFV) 40.9% (513). Individuals classified under "Others" for the ART regimen were those included in a clinical trial research that was on going at the Day Care Hospital during that period. One-fifth (21.9%) of the patients had a history of self-reported poor compliance in their medical files (Table 3).

Table 3: Clinical and immunological characteristics of the study population

Variables	Category	Number	Percentage (%)
Weight (Kg)	<50	122	9.7
	50-60	433	34.5
	>60	699	55.7
WHO stage	I	407	32.5
	II	351	28.0
	III	416	33.2
	IV	80	6.4
CD4 cell count (cells/µL)	≤200	724	57.7
	201-350	411	32.8
	>351	119	9.5
TB at initiation	Yes	94	7.5
	No	1160	92.5
Cotrimoxazole	Yes	463	36.9
	No	791	63.1
ART regimen at initiation	AZT/3TC/EFV	99	7.9
	AZT/3TC/NVP	584	46.6
	TDF/3TC/EFV	513	40.9
	TDF/3TC/NVP	41	3.3
	Others	17	1.4
Self-reported poor compliance history	Yes	274	21.9
	No	980	78.1

AZT= Zidovudine, 3TC= Lamivudine, EFV= Efavirenz, NVP= Nevirapine, TDF= Tenofovir, TB= tuberculosis, WHO= World Health Organization, ART= Antiretroviral Therapy

III.1.3. General Profile of ADRs in the study population

The profile of ADRs in this study is shown in Table 4. A broad variety of specific ADRs was reported and they were classified into 8 different biological systems or groups namely: gastro-intestinal (GI), dermatological (DMT), central nervous system (CNS), peripheral nervous system (PNS), musculoskeletal (MSK), hematological (HMT), hepatic and renal (HR) and systemic symptoms (SS).

III.1.4. Occurrence of ADRs

Among the 1,254 patients who initiated treatment at the Day Care Unit of the Yaoundé Central Hospital during the study period, a total number of 306 (24.40%) individuals had at least one ART-associated ADR reported. Of these, 55.2% (169/306) reported only one ADR, 43.8% (134/306) reported at least 2 ADRs and 1% (3/306) of the study patients reported 3 ADRs. The most common systems affected by the first adverse reactions were hematological, systemic, gastro-intestinal, dermatological and the central nervous system with 37.58%, 12.75%, 12.75%, 12.75% and 10.78% respectively. The highest numbers of ADRs were observed with AZT/3TC/NVP (65.3%) followed by TDF/3TC/EFV (20.91%). A cross-tabulation between the ADRs in different biological systems by ART regimen showed that there was an association between the distribution of ADRs according to initial ART regimen and system affected (p=0.000) (Table 5).

Table 4: General profile of specific ADRs by systems or groups

System	Gastro-Intestinal (GI)	Dermato-logical (DMT)	Central Nervous (CNS)	Musculo Skeletal (MSK)	Systemic Symptom (SS)	Hemato-logical (HMT)	Hepatic and renal (HR)	Peripheral Nervous (PNS)
Specific ADRs	-abdominal pains -diarrhea -nausea/ -vomiting -anorexia	-rash -pruritus -blue nail -hyper-pigmentation -Steven Johnson syndrome	-headache -dizziness -insomnia -drowsiness -nightmares -vision reduction -memory loss -hallucination	-myalgia -arthralgia -parathesia	-fatigue -asthenia -dysnoea	-mild to severe anemia	-increased ALAT/ ASAT -increased Creatinemia	-tingling -numbness

ADRs= adverse drug reactions

Table 5: Frequency distribution of the number of study patients with ADRs according the ART regimen

ART Regimen	Number of patients with ADR by system								Total (%)
	GI	DMT	CNS	MSK	SS	HMT	HR	PNS	
Others	0	0	0	0	0	1	0	0	**1(0.34)**
AZT/3TC/EFV	3	3	9	0	0	16	0	2	**33(10.78)**
AZT/3TC/NVP	24	27	6	1	18	98	13	12	**199(65.03)**
TDF/3TC/EFV	10	7	17	1	19	0	5	5	**64(20.91)**
TDF/3TC/NVP	2	2	1	1	2	0	0	1	**9(2.94%)**
Total (%)	**39 (12.75)**	**39 (12.75)**	**33 (10.78)**	**3 (0.98)**	**39 (12.75)**	**115 (37.58)**	**18 (5.88)**	**20 (6.53)**	**306 (100)**

P=0.000

AZT= Zidovudine, 3TC= Lamivudine, EFV= Efavirenz, NVP= Nevirapine, TDF= Tenofovir, GI= gastro-intestinal, DMT= dermatological, CNS= central nervous system, PNS= peripheral nervous system, MSK= musculoskeletal, HMT= hematological, HR= hepatic and renal, SS= systemic symptoms

III.1.5. Time of onset of ADRs

Based on the clinical records, onset of ADR for each patient was determined as the time that elapsed between ART initiation and the date of complaint or date reported on test results. Table 6 shows the time of onset of ADRs of individuals who reported ADRs. In 88.56%, ADRs had occurred between the first three months after ART initiation.

Table 6: Frequency distribution of the time of onset of ADRs from ART initiation

Onset of ADRs	Number	Percentage (%)
0-3 months	271	88.56
4-6 months	23	7.52
7-12 months	8	2.61
> 12 months	4	1.31
Total	306	100

ADRs= adverse drug reactions

III.2. Specific objective 2: To evaluate the prevalence of CYP 2B6 516G>T SNP and investigate its association with EFV related CNS.

III.2.1. General Characteristics of the Study population

III.2.1.1. Profile and socio-demographic characteristics of the study population

A total of 22,500 medical files were screened at the Yaoundé Central Hospital (YCH) and at the Bertoua Regional Hospital (BRH). A repertoire of 600 patients' phone numbers from each study site was generated from the records. We were able to enroll a total of 425 participants from the two study sites, 174 participants (78 who reported ADRs and 96 who did not) from the YCH and 251 (160 who reported ADRs and 91 who did not) participants from the BRH. Table 7 shows the socio-demographic characteristics of the study population. They were more females than males: 281 (66.1%) versus 144 (33.9%). The mean age was 39 years ± 12. The age group with the highest number of patients was that between 25 and 34 years, with 153 (36%) study participants. One hundred and ninety nine (46.8%) were of secondary school education. The number of people who reported to be single was 133(31.3%) and 170 individuals (40%) were self-employed.

Table 7: Socio-demographic characteristics of the study population

Variables	Category	Number	Percentage (%)
Sex	Female	281	66.1
	Male	144	33.9
Age	15-24	35	8.23
	25-34	153	36.00
	35-44	130	30.59
	≥45	107	25.18
Marital status	Married	103	24.2
	Cohabiting	87	20.5
	Single	133	31.3
	Widowed	80	18.8
	Divorced	22	5.2
Employment status	Employed	174	40.9
	Self-employed	170	40.0
	Unemployed	81	19.1
Educational level	None	50	11.8
	Primary	133	31.3
	Secondary	199	46.8
	Tertiary	43	10.1

III.2.1.2. Clinical characteristics of the general study population

More than half (51.75%; 220) of the patients had a body weight of more than 60 Kg. About half of the individuals (48.7%) did the HIV test as a result of clinical presentation. The predominant ART regimen initially prescribed for the patients was a combination of Tenofovir, Lamivudine and Efavirenz (TDF/3TC/EFV) 68.5% (291). About one-fifth (18.35%) of the patients had a history of self-reported poor compliance due to ADRs in their medical files. These ADRs was the cause of treatment modification in 35.71% (85) of the study participants (Table 8).

Table 8: Clinical characteristics of the study population

Variables	Category	Number	Percentage (%)
Weight (Kg)	<50	43	10.13
	50-60	162	38.12
	>60	220	51.75
Reason for test	Voluntary testing	81	19.1
	Clinical presentation	207	48.7
	Partner dead or positive	68	16.0
	Pregnancy	69	16.2
ART regimen at	AZT/3TC/EFV	134	31.5
initiation	TDF/3TC/EFV	291	68.5
Self-reported poor	Yes	78	18.35
compliance due to	No	347	81.65
ADRs			
Duration on ART	1-3	195	45.88
	3-6	141	33.18
	6-9	65	15.30
	>9	24	5.65
Treatment modification due to	Yes	85	35.71
ADRs	No	153	64.29

ART= antiretroviral therapy, ADRs= adverse drug reactions, AZT= Zidovudine, 3TC= Lamivudine, EFV= Efavirenz, TDF= Tenofovir

III.2.1.3. Pharmacovigilance following ART Administration

Central nervous system adverse reactions reported following ART initiation and during follow- up were: insomnia, hallucinations, nightmares, headache, dizziness, drowsiness, memory lost, drunk like sensation and only one participant reported madness-like sensation. Dizziness, drunk like sensation and insomnia were the most reported representing 28.99%, 23.53% and 10.92% respectively (Table 9).

Table 9: Frequency distribution of the CNS adverse drug reactions reported during follow-up following ART administration in Yaoundé and Bertoua

Adverse drug reactions	Number	Percentage (%)
Hallucinations	14	5.88
Dizziness	69	28.99
Drowsiness	23	9.66
Drunk like sensation	56	23.53
Headache	23	9.66
Insomnia	26	10.92
Madness like sensation	1	0.42
Nightmares	16	6.72
Vision reduction	6	2.52
Memory lost	4	1.68
Total	238	100

III.2.1.4. Time of onset of Central Nervous System ADRs

Table 10 shows the time of onset of ADRs after ART initiation. In the vast majority of participants (68.06 %), ADRs occurred within the first week after treatment initiation.

Table 10: Onset of Central Nervous System ADRs from ART initiation in Yaoundé and Bertoua.

Onset of ADRs	Number	Percentage (%)
0-1 week	162	68.06
1-2 week	52	21.85
2-3 week	8	3.36
3-4 week	7	2.94
>4 week	9	3.78
Total	238	100

ADRs= adverse drug reactions

III.2.1.5. Duration of CNS adverse drugs reactions

Table 11 reveals that, the vast majority of participants had suffered from ADRs for about 0-3 months (70.18%).

Table 11: Percentage distribution of the duration of CNS ADRs among study participants

Duration of ADRs	Number	Percentage
0-3 months	167	70.18
4-6 months	32	13.44
7-12 months	22	9.24
> 12 months	17	7.14
Total	238	100

ADRs= adverse drug reactions

III.2.2. Allelic and Genotypic (phenotypic) frequencies of CYP2B6 516G>T SNP among the study participants

Electrophoresis of PCR products showed bands of 526 bp for all the samples revealing the presence of the CYP 2B6 gene (Figure 18).

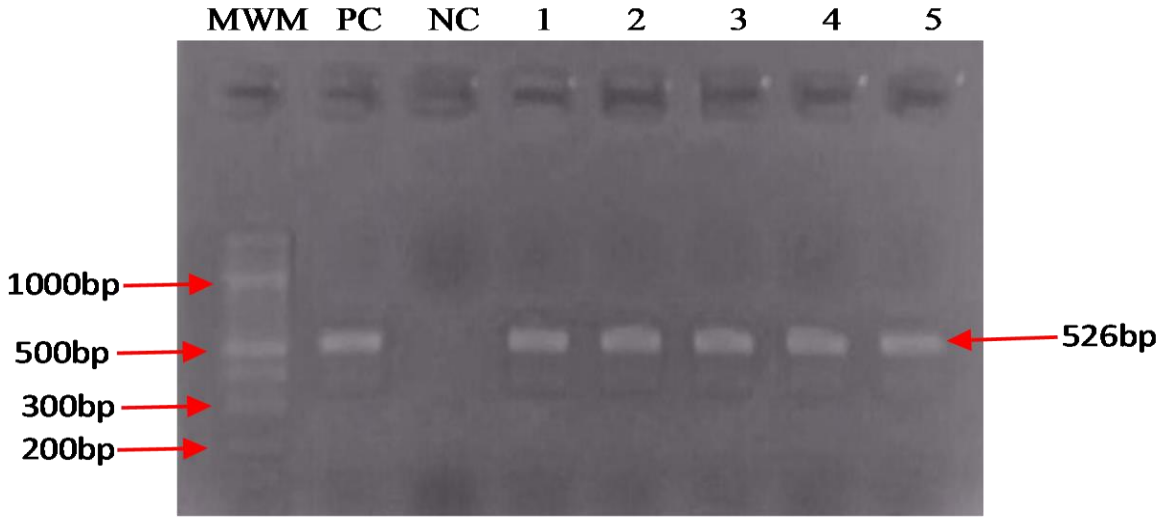

Figure 18: Electrophoregram of PCR products after amplification of CYP 2B6 (516 G>T).

Lane 1-5= 526 bp (successful amplification of the gene), MWM= molecular weight marker (100 bp ladder), PC=positive control, NC=negative control, bp= base pair

The genotyping of the CYP2B6 516G>T SNP was done successfully for all the 425 study participants. Band sizes combination of 268bp and 236bp; combination of 504bp, 268bp and 236bp; and 504bp alone were identified as 516 G/G (wild type), 516 G/T (heterozygous) and 516 T/T (mutants) respectively (Figure 19). These identified genotypes corresponded to Extensive (EM), Intermediate (IM) and Slow metabolizers (SM) phenotypes respectively.

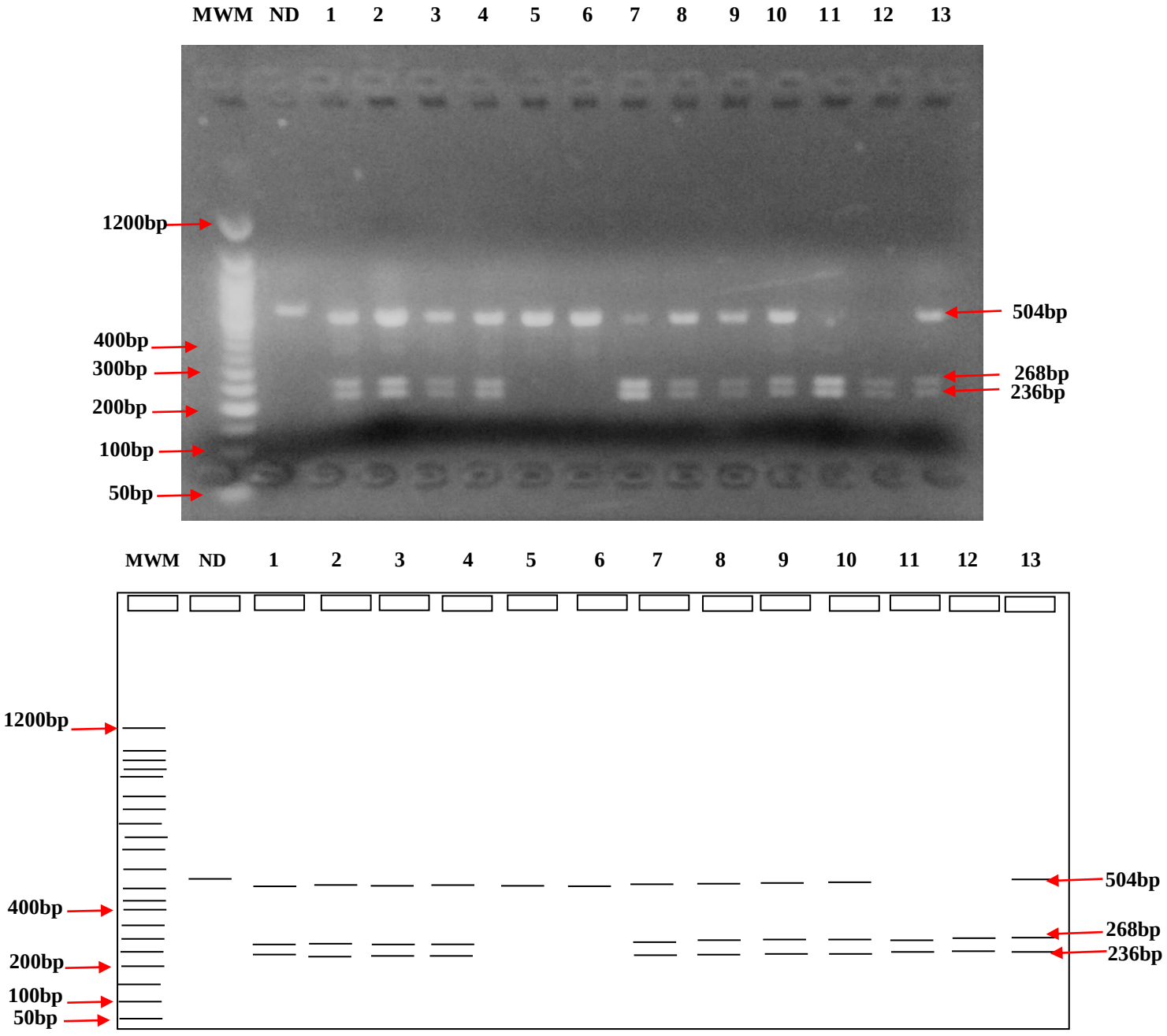

Figure 19: Electrophoregram of RFLP products of CYP 2B6 gene after digestion with *BsrI*.

Lanes 1-4, 7-10 and 13 (504bp, 268bp, 236bp and 22bp) correspond to 516G/G genotype; Lanes 5 and 6 (504bp and 22bp) correspond to 516T/T genotype, Lanes 11 and 12 (268bp, 236pb and 22bp)

correspond to 516G/G genotype, ND= undigested product, MWM=molecular weight marker (50bp ladder), bp=base pair

The number of individuals presenting the CYP2B6 516G/G wild-type genotype (extensive metabolizers) in Yaoundé and Bertoua was 13 (7.47%) and 22(8.76%) respectively. One hundred and twenty-one (69.54%) participants had the heterozygous G/T genotype (intermediate metabolizers) in YCH and 206 (82.07%) in BRH. The number of participants expressing the homozygous T/T genotype considered as poor or slow metabolizers was 40 (22.98%) and 23 (9.16%) in YCH and BRH respectively (Figures 20 and 22). Allele frequencies for the CYP2B6 516G>T SNP in YCH were 42.24% for the G allele and 57.76% for the T allele (Figure 21). In BRH we obtained 49.80% for the G allele and 50.20% for the T allele (Figure 23).

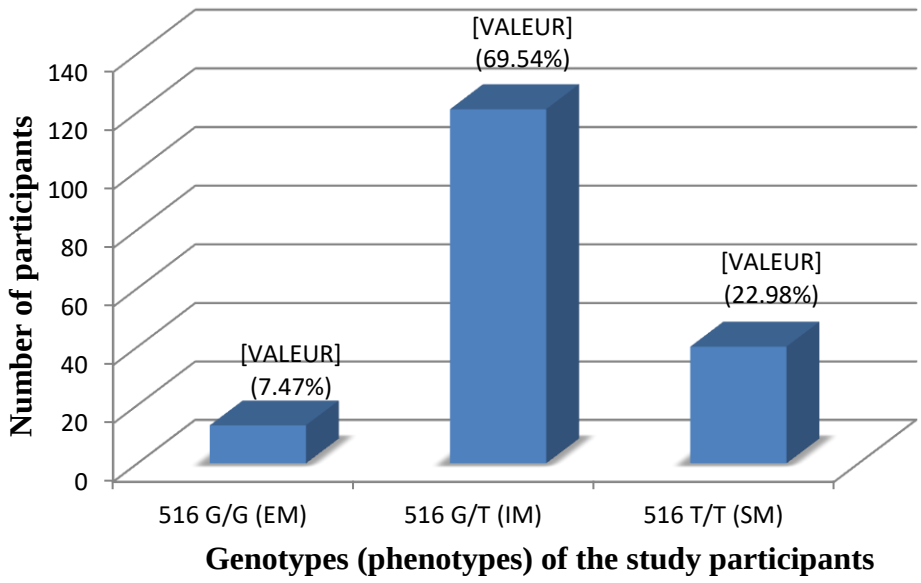

Figure 20: Frequency distribution of the CYP 2B6 516 G>T Genotypes (phenotypes) among study participants from Yaoundé.

G/G= wild type, G/T= heterogyzous, T/T = mutant genotype, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer

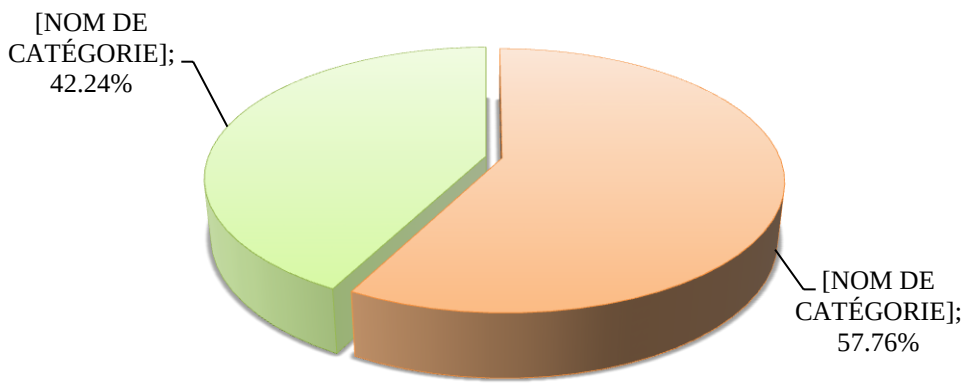

Figure 21: Frequency distribution of the CYP 2B6 516 G>T Alleles among study participants from Yaoundé.

T=T allele, G=G allele.

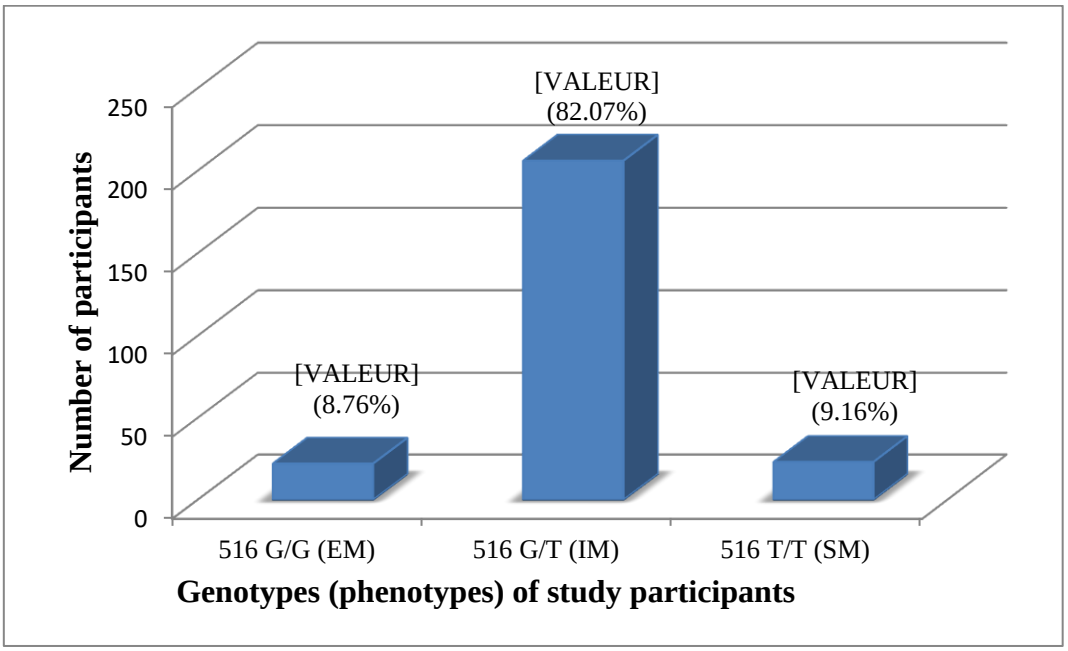

Figure 22: Frequency distribution of the CYP 2B6 516 G>T Genotypes (phenotypes) among study participants from Bertoua.

G/G= wild type, G/T= heterogyzous, T/T = mutant genotype, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer

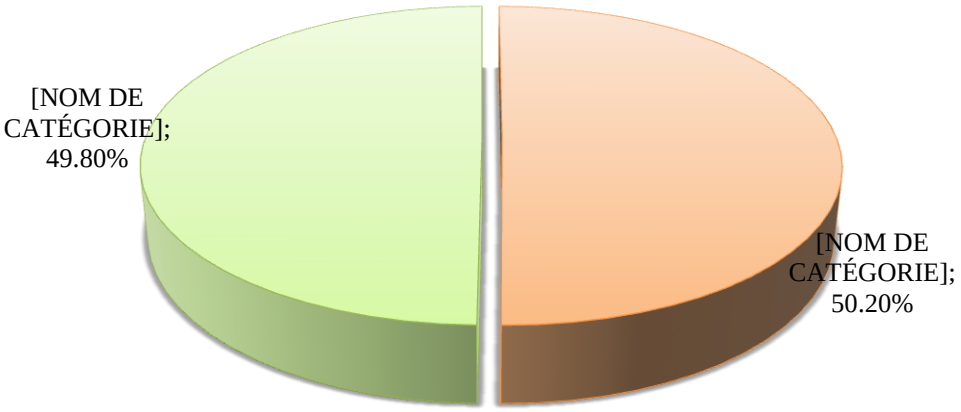

Figure 23: Frequency distribution of the CYP 2B6 516 G>T Alleles among study participants from Bertoua.

T=T allele, G=G allele.

When the data from Bertoua and Yaoundé were combined, the predominance of the Heterozygous genotype (GT) representing 76.94% (Figure 24) was still evident.

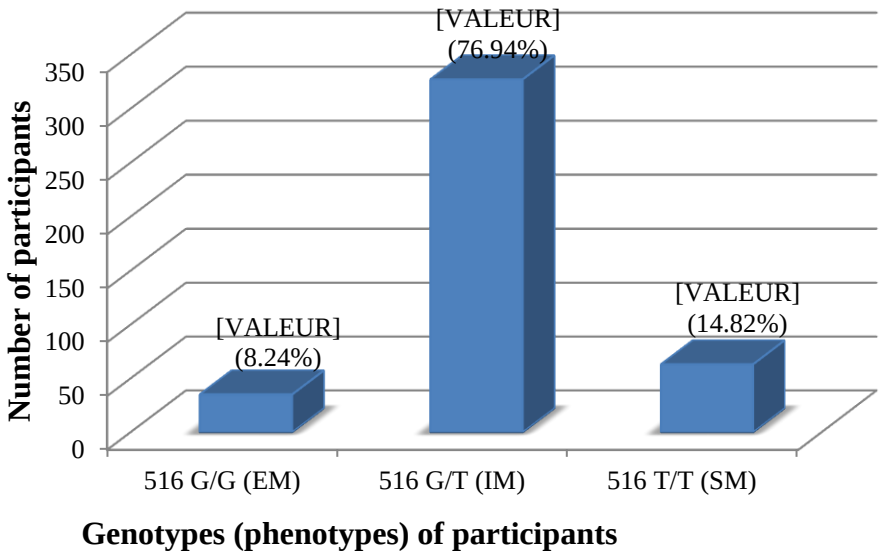

Figure 24: Frequency distribution of the CYP 2B6 516 G>T Genotypes (phenotypes) among study participants from Yaoundé and Bertoua.

G/G= wild type, G/T= heterogyzous, T/T = mutant genotype, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer

In the global study population (Yaoundé and Bertoua) the wild type G allele was the less predominant (Figure 25).

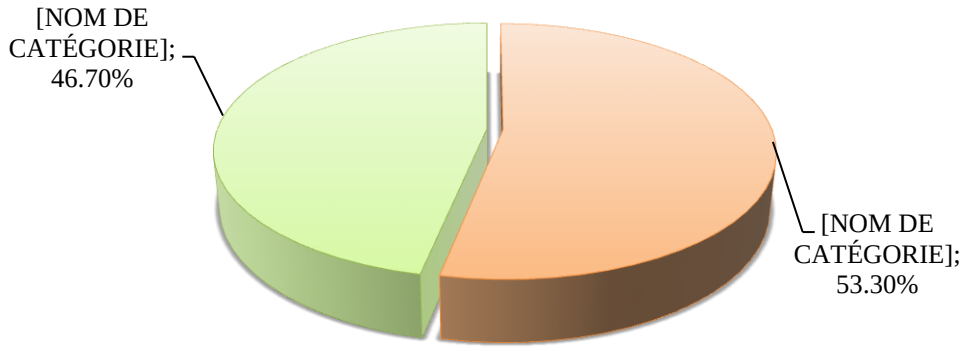

Figure 25: Frequency distribution of the CYP 2B6 516 G>T Alleles among study participants from Yaoundé and Bertoua.

T=T allele, G=G allele.

III.2.3. Association between CYP 2B6 516G>T genotypes / phenotypes and CNS adverse drug reactions to Efavirenz.

III.2.3.1. Association between CYP 2B6 516G>T genotypes / phenotypes and susceptibility to any CNS adverse events to Efavirenz.

Association analysis of the CYP2B6 516G>T SNP showed that individuals homozygous for the wild type allele (GG) were likely to have some degree of protection against ADRs with a statistically significant difference when compared with heterozygous and the mutants (OR=0.865, P=0.000) in Yaoundé (Table 12). Additionally, it was observed that individuals homozygous mutants (slow metabolizers) were about 7 times at risk of developing CNS ADRs with a statistically significant difference in Bertoua (OR=6.723, p=0.002) (Table 13). When the data from Yaound**é** and Bertoua were combined, it was still observed that individuals with the GG genotype were more likely to have some degree of protection against ADRs (OR=0.379, p=0.006) (Table 14).

Table 12: Association between CYP 2B6 516G>T genotypes (phenotypes) and susceptibility to any CNS adverse events in study participants from Yaoundé.

SNP	Genotype (Phenotype)	Any CNS adverse drug reactions		OR	95% CI	P-value
		Yes	No			
516 G>T	GG (EM)	0	13	0.865	0.799-0.936	0.000*
	GT (IM)	59	62	1.703	0.876-3.311	0.079
	TT(SM)	19	21	1.150	0.567-2.335	0.417
	Total	78	96			

*SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, *= statistically significant p value, CI= confidence interval, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer, n= number, %= percentage, G/G= wild type, G/T= heterogyzous, T/T = mutant genotype*

Table 13: Association between CYP 2B6 516G>T genotypes (phenotypes) and susceptibility to any CNS adverse events in study participants from Bertoua.

SNP	Genotype (Phenotype)	Any CNS Adverse Drug Reactions		OR	95% CI	P-value
		Yes	No			
516 G>T	GG (EM)	12	10	0.657	0.272-1.586	0.237
	GT (IM)	127	79	0.585	0.285-1.198	0.094
	TT(SM)	21	2	6.723	1.539-29.375	0.002*
	Total	160	91			

*SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, *= statistically significant p value, CI= confidence interval, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer, n= number, %= percentage, G/G= wild type, G/T= heterogyzous, T/T = mutant genotype*

Table 14: Association between CYP 2B6 516G>T genotypes (phenotypes) and susceptibility to any CNS adverse events in study participants from Yaoundé and Bertoua.

SNP	Genotype (phenotype)	Any CNS Adverse Drug Reactions		OR	95% CI	P-value
		Yes	No			
516 G>T	GG (EM)	12	23	0.379	0.183-0.783	0.006*
	GT (IM)	186	141	1.167	0.742-1.836	0.290
	TT(SM)	40	23	1.440	0.829-2.504	0.123
	Total	238	187			

*SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, *= statistically significant p value, CI= confidence interval, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer, n= number, %= percentage, G/G= wild type, G/T= heterogyzous, T/T = mutant genotype*

III.2.3.2. Association between CYP 2B6 516G>T genotypes (phenotypes) and susceptibility to specific CNS adverse events to Efavirenz.

There was a significant association between CYP 2B6 516G>T slow metabolizers and susceptibility to develop hallucinations and drunk-like sensation (Table 15). However, no significant difference was observed between the different genotypes/phenotypes and susceptibility to develop insomnia, nightmares, headache, dizziness, drowsiness, memory lost, and madness like sensation. Generally, slow metabolizers were about 4 times more at risk to develop hallucinations (OR=4.191, p=0.017) and about 3 times more at risk to develop drunk like sensation (OR=2.626, p=0.017).

Table 15: Association between CYP 2B6 516G>T genotypes (phenotypes) and susceptibility to specific CNS adverse events to Efavirenz in the global study population.

Specific CNS adverse events	516G>T Genotype (Phenotype)	Yes	No	OR	95% CI	P value
Hallucinations	G/G (EM)	0	12	0.938	0.907-0.970	0.475
	G/T (IM)	8	178	2.683	0.974-7.387	0.059
	T/T(SM)	6	34	4.191	1.368-12.841	0.017*
Dizziness	G/G (EM)	2	10	0.475	0.101-2.225	0.271
	G/T (IM)	56	130	1.292	0.641-2.606	0.297
	T/T(SM)	11	29	1.065	0.616-1.842	0.492
Drowsiness	G/G (EM)	2	10	0.558	0.148-2.106	0.326
	G/T (IM)	15	171	1.908	0.856-4.250	0.098
	T/T(SM)	6	34	1.879	0.691-5.108	0.167
Drunk like sensation	G/G (EM)	4	8	0.690	0.300-1.591	0.303
	G/T (IM)	48	138	1.913	0.841-4.351	0.080
	T/T(SM)	4	36	2.626	1.007-6.849	0.017*
Headache	G/G (EM)	0	12	0.898	0.860-0.939	0.286
	G/T (IM)	19	167	1.365	0.443-4.205	0.406
	T/T(SM)	4	36	1.047	0.336-3.260	0.564
Insomnia	G/G (EM)	3	9	0.407	0.142-1.168	0.131
	G/T (IM)	18	168	1.590	0.733-3.447	0.178
	T/T(SM)	5	35	1.204	0.425-3.408	0.452
Madness like sensation	G/G (EM)	0	12	0.996	0.115-5.538	0.950
	G/T (IM)	1	185	1.005	0.995-1.016	0.782
	T/T(SM)	0	40	0.995	0.985-1.005	0.832
Nightmares	G/G (EM)	1	11	0.796	0.155-10.580	0.575
	G/T (IM)	13	173	1.227	0.336-4.480	0.522
	T/T(SM)	2	38	1.414	0.334-5.981	0.476
Vision reduction	G/G (EM)	0	12	0.973	0.953-0.995	0.731
	G/T (IM)	4	182	1.788	0.337-9.494	0.390
	T/T(SM)	2	38	2.553	0.451-14.436	0.266
Memory loss	G/G (EM)	0	12	0.982	0.965-1.000	0.812
	G/T (IM)	4	182	1.022	1.000-1.044	0.370
	T/T(SM)	0	40	0.980	0.960-1.000	0.477

*SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, *= statistically significant p value, CI= confidence interval, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer, n= number, %= percentage, G/G= wild type, G/T= heterogyzous, T/T = mutant genotype*

III.3. Specific objective 3: To determine the prevalence of CYP 2B6 983T>C SNP and investigate its association with EFV related CNS.

III.3.1. Allelic and Genotypes (phenotypes) frequencies of CYP2B6 983T>C SNP among the study participants

Electrophoresis of PCR products showed bands of 759 bp revealing the presence of the CYP 2B6 gene (Figure 26).

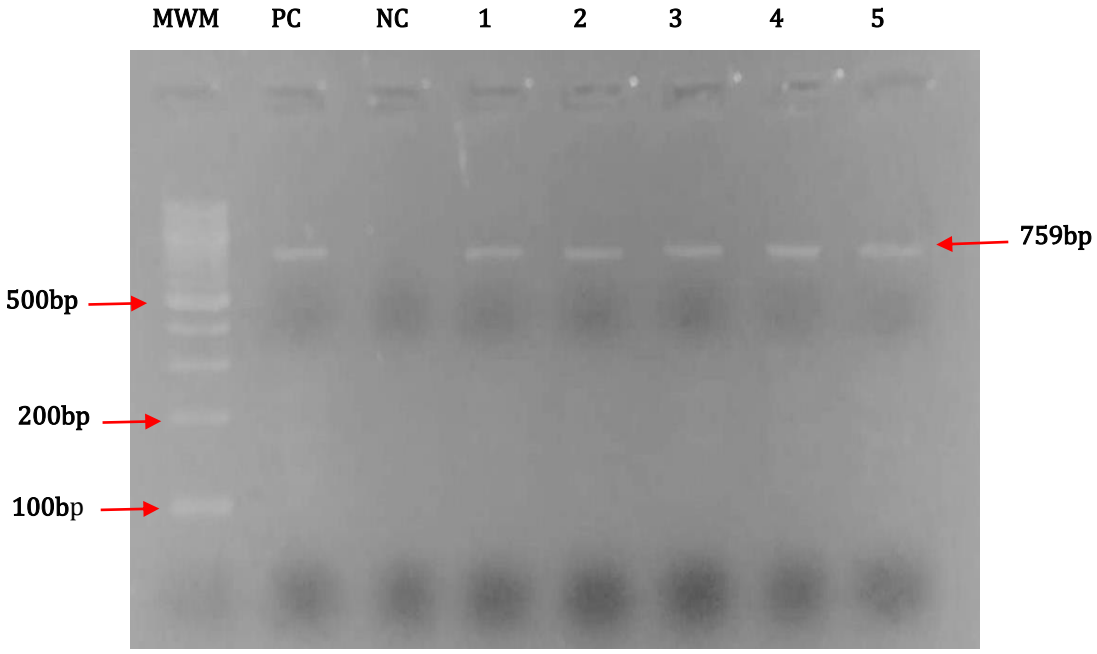

Figure 26 : Electrophoregram of PCR products after amplification of CYP2B6 (983T>C) *Lane 1-5= 759 bp (successful amplification of the CYP2B6 983T>C gene), MWM= molecular weight marker (100bp ladder), PC=positive control, NC=negative control, bp=base pair*

The genotyping of the CYP2B6 983T>C SNP was done successfully for 409 participants. Band sizes of 759bp combination of 759bp, 637bp and 122bp; combination of 637 and 122bp were identified as 983 T/T (wild type), 983 C/T (heterozygous) and 983 C/C (mutants) respectively (Figure 27). The so identified genotypes correspond to Extensive (EM), Intermediate (IM) and Slow metabolizers (SM) phenotypes respectively.

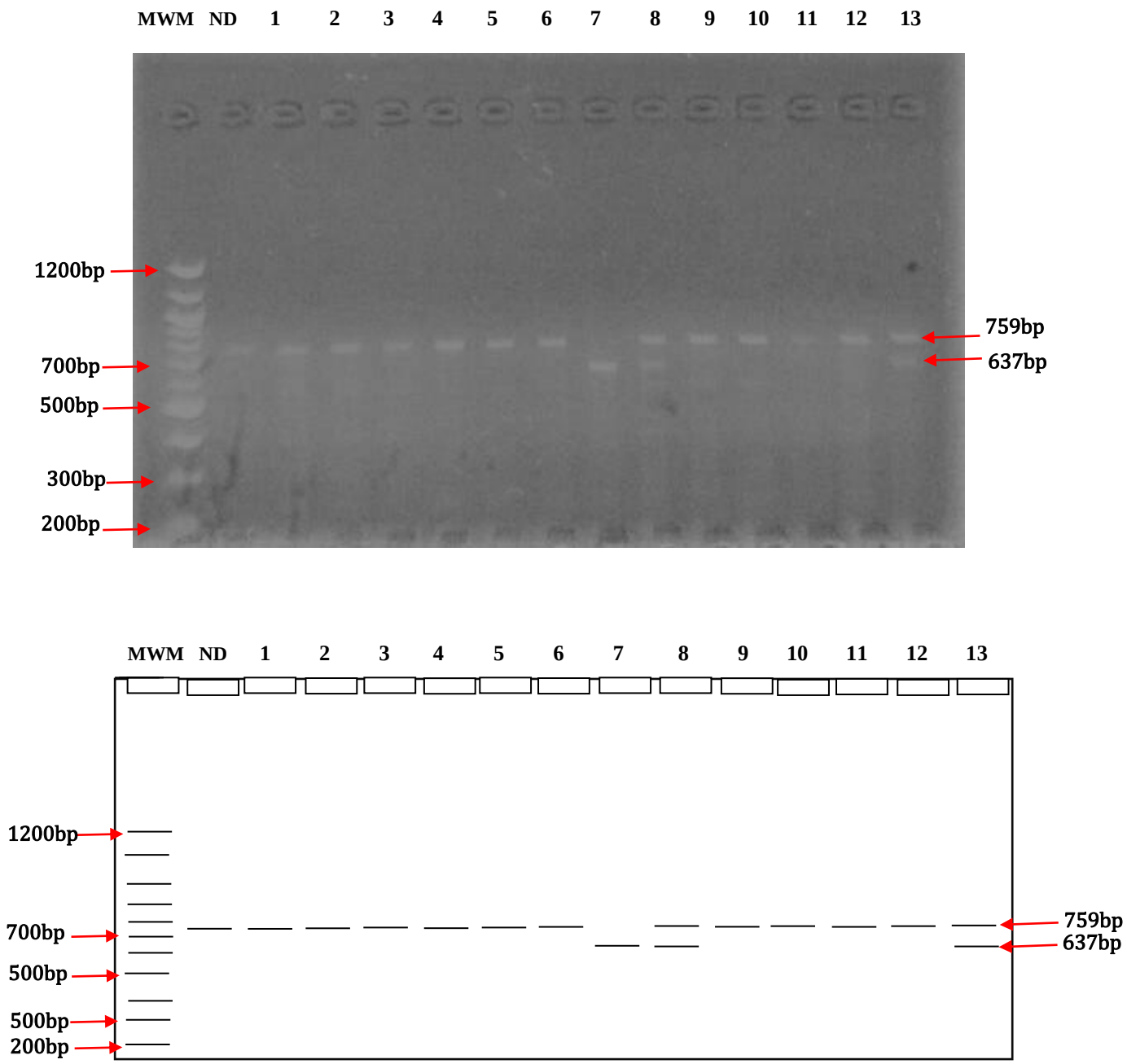

Figure 27: Electrophoregram of RFLP products of CYP 2B6 gene after digestion with *BsrI*. *Lane 1= Undigested (759bp), Lanes 2-8, 11-14= 983T/T (759bp); Lanes 10 and 15= 983T/C (759bp, 637bp and 122bp), Lane 9= 983C/C (637pb and 122bp); MWM=molecular weight marker (100bp ladder), bp= base pair*

The detection of the 983T>C polymorphism was successful among all the 174 enrolled study participants from Yaoundé and on 235/251 participants from Bertoua (detection unsuccessful for 16 participants). Three types of metabolizers were identified: extensive (T/T), intermediate (T/C) and slow (C/C). Prevalences of 88.50% T/T, 5.75% T/C and 5.75% C/C were obtained in Yaoundé (Figure 28). Allele frequencies for the CYP2B6 983T>C SNP in Yaoundé were

91.38% for the T allele and 8.62% for the C allele (Figure 29). Values of 84.68% T/T, 14.47% T/C and 0.85% C/C were obtained in Bertoua (Figure 30) and 92% for the T allele and 8% for the C allele (Figure 31).

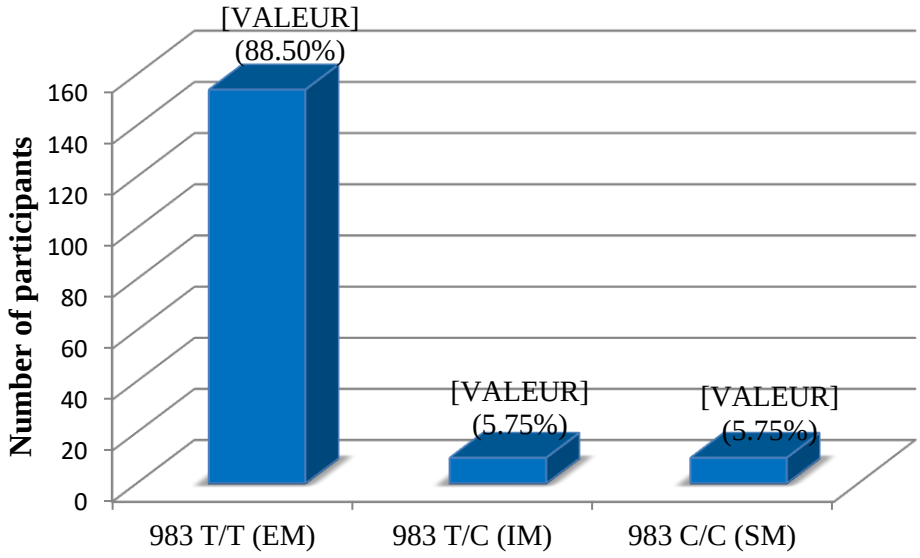

Figure 28: Frequency distribution of the CYP 2B6 983T>C Genotypes (phenotypes) among study participants from Yaoundé.

T/T= wild type, T/C= heterozygous, C/C= mutant genotype, EM= Extensive, IM= Intermediate, SM= Slow Metabolizer

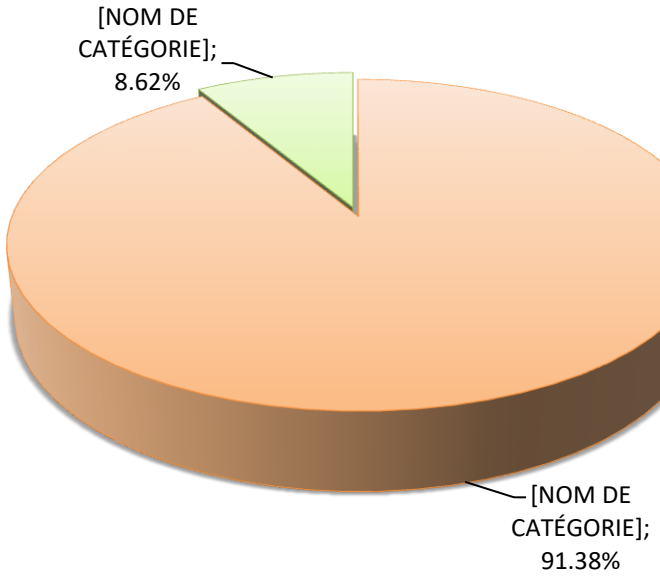

Figure 29: Frequency distribution of the CYP 2B6 983T>C alleles among study participants from Yaoundé

T=T allele, C=C allele

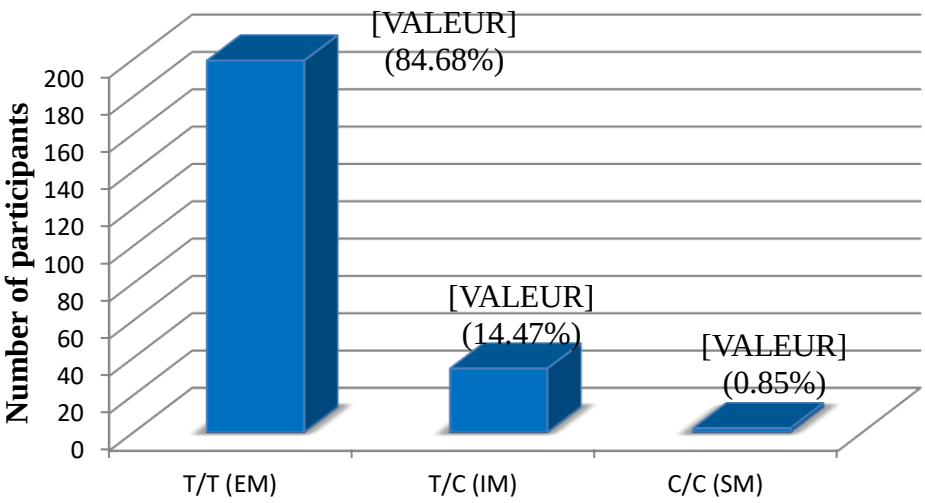

Figure 30: Frequency distribution of the CYP 2B6 983T>C Genotypes (phenotypes) among study participants from Bertoua.

T/T= wild type, T/C= heterozygous, C/C= mutant genotype, EM= Extensive, IM= Intermediate, SM= Slow Metabolizer.

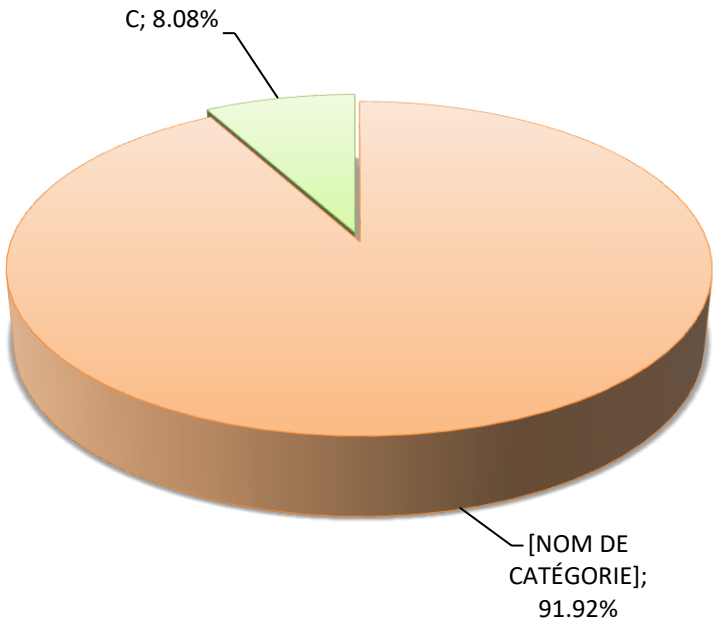

Figure 31: Frequency distribution of the CYP 2B6 983T>C alleles among study participants from Bertoua.

T=T allele, C=C allele

When the data from Bertoua and Yaoundé were combined, the predominance of the wild type genotype (TT) representing 86.31% (Figure 32) could still be observed.

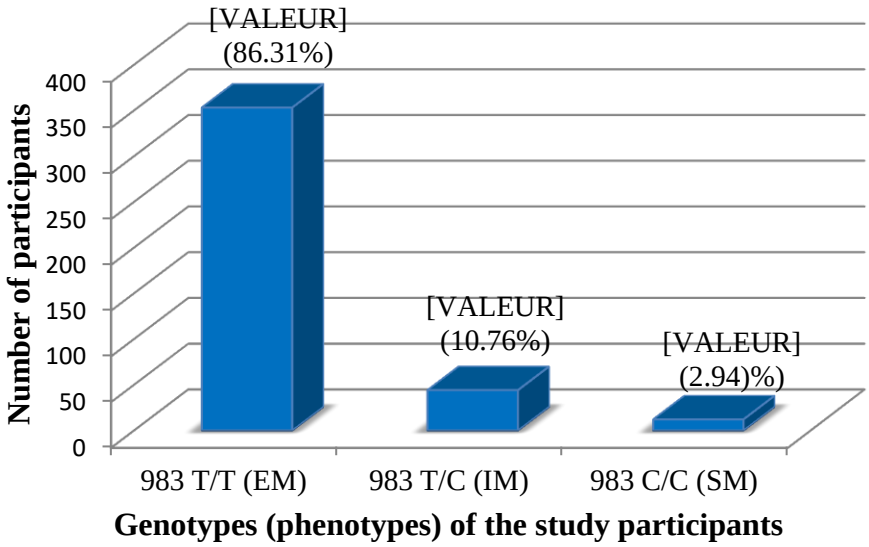

Figure 32: Frequency distribution of the CYP 2B6 983T>C Genotypes (phenotypes) among study participants from Yaoundé and Bertoua.

T/T= wild type, T/C= heterozygous, C/C= mutant genotype, EM= Extensive, IM= Intermediate, SM= Slow Metabolizer

In the global study population the wild type T allele was the most predominant (Figure 33).

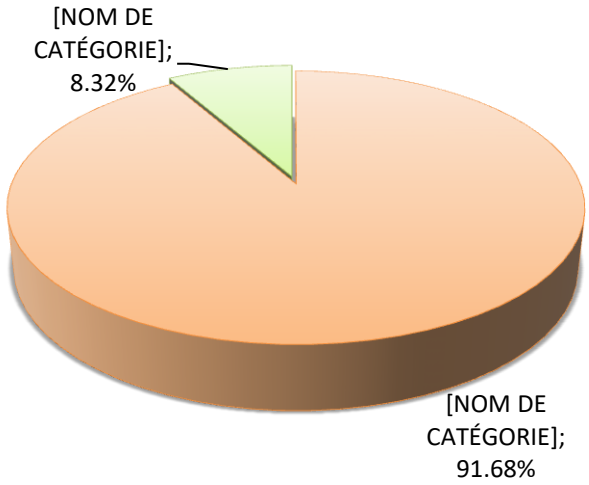

Figure 33: Frequency distribution of the CYP 2B6 983T>C alleles among study participants from Yaoundé and Bertoua.

T=T allele, C=C allele

III.3.2. Association between CYP 2B6 983T>C genotypes (phenotypes) and CNS adverse events to Efavirenz.

III.3.2.1. Association between CYP 2B6 983T>C genotypes (phenotypes) and susceptibility to any CNS adverse events to Efavirenz.

No significant difference was observed between the different CYP 2B6 983T>C genotypes/phenotypes and susceptibility to develop any CNS ADRs in Yaoundé (Table 16).

Table 16: Association between CYP 2B6 983T>C genotypes (phenotypes) and susceptibility to any CNS adverse events in Yaoundé.

SNP	Genotype (phenotype)	Any CNS Adverse Drug Reactions		OR	95% CI	P-value
		Yes	No			
983 T>C	T/T (EM)	67	87	0.630	0.247-1.608	0.231
	T/C (IM)	5	5	1.247	0.348-4.471	0.491
	C/C (SM)	6	4	1.917	0.521-7.048	0.252
	Total	78	96			

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, CI= confidence interval, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer, %= percentage, T/T= wild type, T/C = heterogyzous, C/C = mutant genotype

No significant difference was observed between the different CYP 2B6 983T>C genotypes/phenotypes and susceptibility to develop any CNS ADRs in Bertoua (Table 17).

Table 17: Association between CYP 2B6 983T>C genotypes (phenotypes) and susceptibility to any CNS adverse events in Bertoua.

SNP	Genotype (phenotype)	Any CNS Adverse Drug Reactions		OR	95% CI	P-value
		Yes	No			
983 T>C	T/T (EM)	138	62	0.946	0.836-1.070	0.228
	T/C (IM)	21	13	1.321	0.700-2.492	0.253
	C/C (SM)	1	1	2,133	0.135-33.646	0.537
	Total	160	76			

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, CI= confidence interval, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer, %= percentage, T/T= wild type, T/C = heterogyzous, C/C = mutant genotype

No significant difference was observed between the different CYP 2B6 983T>C genotypes/phenotypes and susceptibility to develop any CNS ADRs in the overall study population (Table 18).

Table 18: Association between CYP 2B6 983T>C genotypes (phenotypes) and susceptibility to any CNS adverse events in Yaoundé and Bertoua.

SNP	Genotype (phenotype)	Any CNS Adverse Event		OR	95% CI	P value
		YES	NO			
983 T>C	T/T (EM)	205	149	0.959	0.541-1.700	0.503
	T/C (IM)	26	18	1.042	0.552-1.969	0.516
	C/C (SM)	7	4	1.006	0.314-3.225	0.618
	Total	238	171			

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, CI= confidence interval, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer, %= percentage, T/T= wild type, T/C = heterogyzous, C/C = mutant genotype

There was a trend toward significant association between CYP 2B6 983T>C slow metabolizers and susceptibility to develop hallucinations and a significant association with vision reduction and memory loss (Table 19). However, no significant difference was observed between the different genotypes/phenotypes and susceptibility to develop insomnia, nightmares, headache, dizziness, drowsiness, drunk like sensation and madness like sensation. Slow metabolizers were about 7 times more at risk to develop hallucinations (OR=7.3, p=0.057) and about 22 times more at risk to develop vision reduction (OR=22.7, p=0.011). Extensive metabolizers were showing a degree of protection against the development of memory loss (OR=0.049; p=0.009).

Table 19: Association between CYP 2B6 983T>C genotypes (phenotypes) and susceptibility to specific CNS adverse events to Efavirenz in the global study population.

Specific CNS adverse events	Genotype (phenotype)	Yes	No	OR	95% CI	P value
Hallucinations	T/T (EM)	12	193	0.964	0.206-4.515	0.605
	T/C (IM)	0	26	0.934	0.901-0.968	0.189
	C/C(SM)	2	5	7.3	1.282-41.580	0.057
Dizziness	T/T (EM)	59	146	0.929	0.417-2.012	0.503
	T/C (IM)	10	16	1.621	0.696-3.774	0.183
	C/C (SM)	0	7	0.701	0.645-0.763	0.088
Drowsiness	T/T (EM)	21	184	0.592	0.145-2.406	0.352
	T/C (IM)	0	26	0.892	0.851-0.934	0.061
	C/C (SM)	2	5	4.000	0.731-21.897	0.139
Drunk like sensation	T/T (EM)	51	154	0.609	0.262-1.413	0.158
	T/C (IM)	5	21	1.251	0.549-2.850	0.394
	C/C (SM)	0	7	0.758	0.704-0.815	0.149
Headache	T/T (EM)	21	184	0.592	0.145-2.406	0.352
	T/C (IM)	2	24	1.288	0.320-5.181	0.527
	C/C (SM)	0	7	0.900	0.863-0.940	0.486
Insomnia	T/T (EM)	21	184	0.639	0.233-1.832	0.282
	T/C (IM)	5	21	2.166	0.740-6.341	0.136
	C/C (SM)	0	7	0.887	0.848-0.929	0.440
Madness like sensation	T/T (EM)	1	237	1.005	0.995-1.015	0.861
	T/C (IM)	0	26	0.995	0.986-1.005	0.891
	C/C (SM)	0	7	0.996	0.987-1.004	0.971
Nightmares	T/T (EM)	14	191	0.887	0.211-3.728	0.613
	T/C (IM)	1	25	1.840	0.253-13.362	0.458
	C/C (SM)	1	6	2.4	0.271-21.247	0.390
Vision	T/T (EM)	4	201	0.308	0.054-1.756	0.196

reduction	T/C (IM)	0	26	0.972	0.950-0.994	0.496
	C/C (SM)	2	5	22.700	3.345-154-027	0.011*
Memory loss	T/T (EM)	1	204	0.049	0.005-0.487	0.009*
	T/C (IM)	3	23	27.522	2.749-275.533	0.004*
	C/C (SM)	0	7	0.983	0.966-1.000	0.887

*SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, *= statistically significant p value, CI= confidence interval, EM= Extensive Metabolizer, IM= Intermediate Metabolizer, SM= Slow Metabolizer, %= percentage, T/T= wild type, T/C = heterogyzous, C/C = mutant genotype*

III.3.3. Haplotypes' distribution and their association with CNS ADRs in the study population.

III.3.3.1. Frequency distribution of the different haplotypes among the study population.

Haplotypes were constructed with the two variants explored in this study. The most frequent ones were the 516GT/983TT, 516TT/983TT and 516GT/983TC with 66.75%, 13.20% and 8.31% respectively. The 516GG/983CC was totally absent in the study population (Table 20).

Table 20: Frequecy distribution of the different haplotypes among the study participants from Yaoundé and Bertoua

Haplotypes	Number	Frequency (%)
516GG/983TT	26	6.35
516GG/983TC	6	1.47
516GG/983CC	0	0
516GT/983TT	273	66.75
516GT/983TC	34	8.31
516GT/983CC	6	1.47
516TT/983TT	54	13.20
516TT/983TC	4	0.97
516TT/983CC	6	1.47
Total	**409**	**100%**

III.3.3.2. Association between haplotypes and susceptibility to CNS adverse events to Efavirenz.

Our study revealed that individuals harboring the wild type genotype for the two polymorphisms demonstrated a significant degree of protection against the development of any CNS ADRs (OR=0.356, p=0.011) (Table 21). The other identified haplotypes did not have any association to any of the CNS adverse event.

Table 21: Association between different haplotypes and susceptibility to any CNS adverse events in the study population.

Haplotypes	Any CNS adverse events		OR	95% CI	P value
	Yes	No			
516GG/983TT	9	17	0.356	0.155-0.819	0.011*
516GG/983TC	3	3	0.715	0.143-3.585	0.493
516GT/983TT	164	109	1.261	0.832-1.910	0.162
516GT/983TC	20	14	1.029	0.504-2.099	0.544
516GT/983CC	2	4	1.757	0.565-5.463	0.203
516TT/983TT	32	22	1.052	0.588-1.883	0.493
516TT/983TC	3	1	2.170	0.224-21.043	0.444

**= statistically significant p value, CI= confident interval*

III.4. DISCUSSION

The aim of the present study was to study the pattern of the occurrence of adverse drug reactions to antiretroviral drug and assessed pharmacogenetics data in a Cameroonian population on CYP 2B6 gene coding for proteins involved in the metabolism of Efavirenz.

Pharmacovigilance in Cameroon is still properly constituted and consequently reporting of ADRs due to ART is very poor. This retrospective study carried out becomes even more important because the development of ADRs among HIV patients undergoing ARV treatment is one of the most limiting factors that compromises compliance and adherence to treatment. Furthermore, they are the major cause of treatment changes as shown by many studies and constitute one of the reasons for loss to follow-up of ART patients and deaths as well. The most common regimen used was a combination of Zidovudine, Lamivudine, and Nevirapine (AZT/3TC/NVP), accounting for 46.6% of the patients, followed by a combination of Tenofovir, Lamivudine, and Efavirenz (40.9%). This was in accordance with the guidelines being followed during that period.

The results from prior studies regarding the prevalence of ADRs showed a lot of variation. In our study, out of 1254 patients, 306 of them experienced at least one ADR, which puts the prevalence of ADRs at 24.50%. Among these 306 individuals, 134 (43.8%) reported two ADRs and 3 reported 3 ADRs. The prevalence reported in this study was lower than that obtained in many African and non-African countries. Several studies in India have reported higher prevalences of 75.65% (Bhatnagar *et al*., 2013), 81.42% (Kumari *et al*., 2017) and 90% (Shet *et al*., 2014). Such high values were equally found in Iranian and Ethiopian study populations with 87.6% and 89.8% respectively (Khalili *et al*., 2009; Tadesse *et al*., 2014). In contrast, some research groups in Ghana, Nigeria and Ethiopia reported values of 9.4%, 4.6%, 6.4% and 4.3% respectively, which were considerably lower than what was found in the present study population (Eluwa *et al*., 2012; Lartey *et al*., 2014; Lorío *et al*., 2014; Kindie *et al*., 2017). Some studies carried out in India and Brazil reported prevalences very close to what was found in this study, with 21% and 20.2% respectively (Roshni *et al*., 2016; Santini-Oliveira *et al*., 2014). A Cameroonian study carried out at the Douala Reference Hospital found the prevalence of ADR to be 19.5% (Luma *et al*., 2012), which was also close to what we found at the Yaoundé Central Hospital. It is possible that not all patients' complaints were reported as adverse reactions, thus generating an underestimation of their true incidence in the population studied. These variations could be due to study design, sample size, or demographic variations, hormonal effects, immunological status, drug susceptibility, drug

metabolism and elimination, or genetic constitutional differences at the levels of various enzymes amongst others.

The spectrum of adverse events is wide and varied affecting different biological systems. In our study, most of the ADRs were observed from individuals under AZT/3TC/NVP regimen (65.03%) followed by TDF/3TC/EFV combination (20.91%). The reason for this could probably be that these two combinations were the most frequently administered first line drugs among the various regimens employed. Hence the number of ADRs observed towards these two combinations was also higher.

From the study population, it was observed that the hematological system was most frequently affected, with a prevalence of 37.58% recorded from 115 cases of ADRs that consisted mainly of mild to severe anemia associated with AZT. These results are in line with many other studies which also found that anemia was the most frequently reported ADR (Bhatnagar *et al*., 2013; Lartey *et al*., 2014; Malalur *et al*., 2016; Roshni *et al*., 2016). On the other hand, these results were in contrast with others that reported gastro-intestinal disorders as the most frequent ADRs (Khalili *et al*., 2009; Kumari *et al*., 2017; Raikar *et al*., 2018). Yet other studies still regarding the most common side effects reported pains (Eluwa *et al*., 2012), peripheral neuropathy (Luma *et al*., 2012) and central nervous system ADRs (Lorío *et al*., 2014). There is a need for intense laboratory monitoring to diagnose drug toxicity early, besides the clinical diagnosis. This discrepancy might be explained by possible variations in the study settings, regimen type and time gap during which the studies were conducted.

The prevalence of CNS adverse drugs reactions in this study was 10.78% which was very close to 9.9% previously reported in another study carried out in Cameroon (Luma *et al*., 2012). Several studies reported higher prevalences of 16.8%, 27.16% and 30% (Khalili *et al*., 2009; Kumari *et al*., 2017; Raikar *et al*., 2018). Lower incidences of 6.55% and 7.6% were reported in India and Ghana (Roshni *et al*., 2016; Sarfo *et al*., 2016). A study carried out in South Africa revealed that in their cohort no patient under EFV- based regimen developed CNS adverse events (Gaida *et al*., 2016). This variation can be due to differences in study populations in terms of genetic variation of genes involved in the metabolism of Efavirenz.

We observed that 21.9% of patients had in their medical files reported history of poor adherence to ART. This result is lower than that reported by Olowookere *et al*. and Pefura-Yone *et al*. who reported non-adherence in 37.1%% and 34.9% of Indian and Cameroonian patients respectively (Olowookere *et al*., 2008; Pefura-Yone *et al*., 2013). Other reports also showed lower non-adherence rate of 9%-17% in Kenya, Ethiopia, Brazil and United Kingdom

(Filho *et al*., 2008; Wasti *et al*., 2012; Mukui *et al*., 2016; Jima and Tatiparthi, 2018). The differences can be attributed to variation in ART combination or in the adherence assessment method. Our current findings showed that nonadherence to ART treatment is common among people living with HIV and efforts should be made to address this problem.

It was observed that 91 cases (29.74%) out of the 306 ADRs necessitated a modification of ART combination due to the negative impact of ADRs on the quality of life (daily tasks), whereas in the remaining 215 cases (70.26%) the regimen was not modified and ADRs were managed. Others studies have reported modification rates due to ADRs ranging from 16%-58.5% (Elzi, 2010; Cicconi *et al*., 2010; Shet *et al*., 2014; Malalur *et al*., 2016). The ART regimen used could have influenced the variations in this ART modification prevalence.

Most of the adverse reactions occurred during the first 3 months of initiating treatment (88.56%), and 96.18% took place within the first 6 months as confirmed by some previous studies (Eluwa *et al*., 2012; Shet *et al*., 2014; Kindie *et al*., 2017) who found 38%, 59% and 60% respectively. An explanation was proffered that early occurrence of ADRs is an expression of a mechanism of intrinsic intolerance rather than a time-dependent toxic accumulation process. It is important to monitor patients within this time frame to prevent the occurrence of severe ADRs and avoid failure in compliance. This goes with the need to improve documentation of ADR occurrences (Duval *et al*., 2004).

There was an association between the initial ART regimen and the occurrence of ADRs both in general and according to the distribution in different systems ($p=0.000$). This result is similar to those reported in many studies (Eluwa *et al*., 2012; Luma *et al*., 2012; Shet *et al*., 2014; Masenyetse *et al*., 2015). EFV was mainly associated with the central nervous system ADRs, ZDV to anemia, NVP to dermatological problems and liver toxicity, and TDF to renal toxicity. Similar associations were found elsewhere (Kumari *et al*., 2017; Ejigu *et al*., 2018). Despite the results obtained, the main limitation of this study was its retrospective nature that may have led to the underestimation of the actual prevalence of ADRs in the health facility.

In this study, we were able to assess the profile of CNS ADRs. In our cohort, dizziness, drunk like sensation, insomnia, drowsiness, headache, nightmares, hallucinations, vision reduction, memory loss and madness like sensation were reported at different frequencies, dizziness being the most predominant with 28.92%. Our results are in contrast with those reported in Spain and Nigeria where nighmare was reported as the most predominant with 48% and 6.8% respectively (Fumaz *et al*., 2005; Abah *et al*., 2015). Meanwhile a study carried out on a Ghanaian cohort instead mentioned insomnia as the most predominant neuropsychiatric

toxicity (Sarfo *et al*., 2016) with a prevalence of 50%. Some results have been published in Iran reporting headache as the most prevalent representing 7.3% of the study population (Khalili *et al*., 2009).

Our data showed that, in the majority of participants (68.06 %), central nervous system ADRs had occurred within the first week after treatment initiation. These results are in agreement with those reported by other studies (Khalili *et al*., 2009; Sarfo *et al*., 2016) which revealed that, Efavirenz-related neurotoxicity occur most of the times within the first 4 weeks after initiating therapy. In a study carried out in Nigeria, it was reported that, in one-third of the patients, CNS adverse events occurred within 12 months of ART initiation and in 34.1% these symptoms appear very lately after greater than 36 months of treatment (Abah *et al*., 2015). There are also some studies showing that, in about 50% of patients the development of neuropsychiatric disorders may be delayed (Gutierrez *et al*., 2005).

In our study it was observed that, in the majority of patients (70.18%) neuropsychiatric symptoms was resolved within the first three months after their appearance. This finding agrees with other results indicating that in most of the cases neuropsychiatric toxicity ceased after 6-8 weeks (Arendt *et al.*, 2007). Additionally, we observed that in some patients (7.14%) even after 12 months of treatment they were still presenting Efavirenz-related neurotoxicity. Our results are in line with some studies reported elsewhere which demonstrate that, some cases of ADRs may persist for several months and even years (Hawkins *et al*., 2005; Dhoro *et al*., 2015; Vo and Gupta, 2016).

We noted that the initial EFV-containing regimen was modified in 35.71% of patients experiencing CNS disorder. The proportion of patients who had their ART regimen modified (substitution of EFV) due to CNS ADRs was higher compared to that obtained in Haiti ,Côte d'Ivoire and Spain (4%, 10% and 8.3% respectively) (Severe *et al*., 2005; Bartlett *et al*., 2007). A study in Nigeria reported treatment modifications in up to 50% of patients (Abah *et al*., 2015). The fact that treatment was not changed in all the patients experiencing neurotoxicity may be attributed to their tolerance or poor availability of replacement drugs.

Data obtained on the distribution of CYP 2B6 genetic variations in populations are valuable in understanding inter-individual differences in response to treatment. The association of the CYP 2B6 516 G>T and 983 T>C polymorphisms with Efavirenz metabolism is well established. The frequencies of these SNPs were determined and their association with the development of adverse drug reactions to Efavirenz investigated. For the CYP 2B6 516 G>T polymorphism, individuals having the GG, GT and TT genotypes in Yaoundé and Bertoua

represented 7.47%, 69.54%; 22.98% , 8.76% and 82.07% ,9.16% respectively. Results from this study are comparable to the reports of only two previous studies existing to the best of our knowledge on the prevalence of these genotypes in Cameroonian cohorts. The first report was on a Bantu-speaking population from Yaoundé made of 72 healthy unrelated individuals. While the second study enrolled 168 subjects from the Western region of Cameroon (Dschang). For GG, GT and TT they obtained 42%, 42% and 16% and 34.5%, 42.3% and 23.2% respectively (Swart *et al*., 2012; Paganotti *et al*., 2015). The prevalences of GG (wild type genotype) found in our study were very low compared to previous reports from Cameroon. The prevalences of TT genotype (slow metabolizer's phenotype) in this study was lower than in West Africa, Papua New Guinea, Spain and Japan (Rodriguez-Novoa *et al*., 2005; Mehlotra *et al*., 2007; Gatanaga *et al*., 2007)). In Europeans, Caucasians and Taiwanese, considerably lower values of 5.9% and 1.8% were reported (Blievernicht *et al*., 2007; Huang *et al*., 2017). Some studies on Indian, Cambodian and Burundese cohorts reported values very closer to our results (Ramachandran *et al*., 2009; Chou *et al*., 2010; Varshney *et al*., 2012; Calcagno *et al*., 2012) . All this variability can be due to differences in sample sizes or to differences in ethnicity of studied populations.

Regarding the other SNP 983 T>C, we obtained 88.50% TT, 5.75% TC and 5.75% CC in Yaoundé. The distribution in Bertoua revealed: 84.68% TT, 14.47% TC and 0.85% CC. The prevalence of slow metabolizers (CC genotype) in Bertoua was very low compared to that reported in Yaoundé. Our results are in coroboration with that reported by Calgano and collaborators in Burundi, who found 1.5% CC which is very close to what we obtained in Bertoua. Additionally, their reported prevalence (87.6%) of the TT(wild type genotype) was very close to the values we obtained in Yaoundé and Bertoua (Calcagno *et al*., 2012). This finding is contradictory from one reported on a Cameroonian cohort revealing the total absence of the 983CC genotype in their study population (Paganotti *et al*., 2015). Another research team found that, this mutation was completely absent among individuals of white European origin (Blievernicht *et al*., 2007). There are some others studies that instead reported the total absence of the slow metabolizers in their cohorts. One of them was in Kenya and the other was a cohort constituted by individuals of diverse origins: Whites, Blacks, Hispano-American and Asians (Rotger *et al*., 2007; Oluka *et al*., 2015). All these differences may be due to various geographic and ethnic differences of the populations studied.

In our study, the predominant and minor allele frequencies in the global population (Yaoundé and Bertoua) were 0.53 (T) and 0.47 (G) respectively for 516G>T. The allele frequencies for the wild type (T) and mutant allele (C) at position 983 were 0.92 and 0.8 respectively. The results obtained for minor alleles were close to the 0.34 and 0.07 obtained in Germans (Wyen *et al*., 2008). But other studies on Cameroonians and Papua Guineans reported a very low frequency (0.01) of this C allele (Mehlotra *et al*., 2007; Paganotti *et al*., 2015). The C allele frequency obtained in the present work was a bit higher than that found in Ghanians, Senegalese and Ivoriane 0.07, 0,06 and 0.04 respectively (Mehlotra *et al*., 2007). The prevalence of the T allele (516G>T) which was observed in this study, was higher (0.53) than the 0.35 reported in Cambodia (Chou *et al*., 2010). This discrepancy can be due to the differences in the geographic/ethnic distribution of the various genotypes related to the two SNPs.

Genetic functional polymorphisms of xenobiotic metabolizing enzymes have been associated with therapeutic response differences both in terms of efficacy and toxicity (Wilkinson, 2005).

It was observed that normal/extensive metabolizers (individuals homozygous for the wild type allele 516 GG) were more likely to be protected from CNS ADRs susceptibility (OR=0.86, P=0.000) when compared to heterozygous and mutants. This could be due to the fact that extensive metabolizers eliminate drugs from the system more rapidly, thus such people are not exposed to high drug levels in the plasma. Our results equally showed that homozygous mutants for the 516G>T (TT) were about 7 times at risk of developing any ADRs namely insomnia, hallucinations, nightmares, headache, dizziness, drowsiness, memory lost, drunk like sensation and only 1 participant had reported madness like sensation due to the presence of the non-functional alleles (OR=6.72, P=0.002). For CYP2B6 SNP, T983C, participants with the homozygous genotypes (CC) were more susceptible (two fold higher risk OR=1.92 in Yaoundé and OR=2.13 in Bertoua) to develop any neurotoxicity symptom compared to those with other genotypes, although the difference was not significant. This could be due to the fact that slow metabolizers eliminate drugs from the system slowly, thus leading to drug persistence thus resulting in toxicity.

Relative to other CNS adverse reactions, hallucinations, drunk like sensation, vision reduction and memory loss were observed to be significantly associated with slow metabolizers. The lack of significance difference between the other adverse events and the phenotype might have been due to the sample size. Results from this study confirm previous reports from

Uganda, South Africa and France on the association between the presence of 516G>T or 983T>C, high plasma EFV concentrations and susceptibility to related ADRs (Gounden *et al*., 2010; Ribaudo *et al*., 2010; Mukonzo *et al*., 2013; Gallien *et al*., 2017). However, our results are not in agreement with some reports from Brazil and Ghana where no association was found between various genotypes and the susceptibility to EFV-related neuropsychiatric adverse events (Sarfo *et al*., 2014; Müller *et al*., 2017). The lack of significant difference between the presence of the adverse events and the genotypes might have been due to the sample size. This study demonstrated that genetic variability in a metabolizing enzyme gene can also be correlated with susceptibility to ADRs, a condition that should be considered. This is the first study carried out on ART-treated HIV infected patients in Cameroon that has considered associating the presence of polymorphisms on the CYP 2B6 gene with the susceptibility to develop ADRs. CYP 2B6 polymorphisms may also be a good genetic determinant for safety assessment of inter-individual variability to adverse reactions of Efavirenz in this population.

CONCLUSIONS AND PERSPECTIVES

CONCLUSIONS

In this study, we assessed the pharmacovigilance and pharmacogenetic profiles of HIV patients under ART and the following conclusions could be drawn:

1-The spectrum of adverse drug reactions in this study was wide and varied. The overall prevalence of adverse drug reactions was 24.40%. The prevalence of EFV associated ADRs was 10.78%.

2- The CYP 2B6 516G>T SNP was strongly associated with susceptibility to adverse drug reactions induced by Efavirenz in HIV/AIDS-infected Cameroonians. The GG genotype was protective while the risk of developing ADRs with TT genotype was about seven times higher.

3-Individuals homozygous for the 983 T>C mutation (983CC) defined as slow metabolizers (CC) and were shown to be about two times more at risk to develop CNS adverse events.
Additionally, we showed that haplotypes was also associated with the susceptibility of developing CNS adverse effects.
The manual developed and presented take into account all the challenges in the context of the standards and means available in our country, to guide healthcare professionals, program managers and others researchers in systematically conducting further pharmacogenetic studies.

PERSPECTIVES

- To include participants from others regions, for purposes of capturing the different genetic representation of the country.
- To study the relationship between EFV plasma drug levels and the presence of SNPs using stored plasma samples.
- To study the implication of others CYP 2B6 polymorphisms on the treatment outcomes.

RECOMMENDATION

Pharmacogenetics and pharmacokinetics combined with therapeutic drug monitoring should be used to guide EFV dosages. Thus, CYP2B6 genotyping could be introduced in routine clinical practice because it would help the patients to avoid or reduce the adverse neuropsychiatric events after the initiation of EFV-based antiretroviral regimen. Additionally archiving procedures should be improved in our health centres.

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APPENDICES

APPENDIX 1: INFORMATION NOTICE

The Laboratory for Public Health Biotechnology

The Biotechnology Centre, University of Yaoundé I

TITLE: PHARMACOGENETIC STUDY OF HIV PATIENTS UNDER EFAVIRENZ EXPERIENCING ADVERSE DRUG REACTIONS IN CAMEROON: DEVELOPMENT OF A DIAGNOSTIC PROCEDURE

1. AIMS OF STUDY

Sir, Madame, I am …….working at……

This document was prepared to explain the research study that you are being invited to join.

The objectives of this study are as follows:

The general objective of this study was to obtain pharmacovigilance and pharmacogenetic data in a Cameroonian population regarding the CYP2B6 gene coding for an enzyme involved in the metabolism of Efavirenz and propose a procedure manual and for the better management of HIV patients in Cameroon.

Specifically, we sought to:

1- Determine the general profile of anti-retrovirals associated adverse drug reactions and the prevalence of those associated to Efavirenz;

2- Determine the prevalence of CYP2B6 516G>T polymorphism and investigate its association with Efavirenz related central nervous system toxicities;

3- Determine the prevalence of CYP2B6 983T>C polymorphism and investigate its association with Efavirenz related central nervous system toxicities;

4- Develop a manual of procedures to detect CYP2B6 516G>T and CYP 2B6 983T>C polymorphisms.

2. Procedures

If you agree to participate in this study, you will be asked some few questions. A small amount (5 ml) of blood will be drawn from your veins and it will hurt a little. There may be a small bruise at the site where we take the blood. Your blood sample will be submitted to pharmacogenetic testing. All the data collected will be registered in a data base for further analyses. The results of this study will be of a great impact in the public health domain and by

participating you will bring your one contribution to this. If you agree your blood samples will be stored for a maximum of 20 years period for further studies.

3. Voluntary participation

The participation in the study is voluntary and you are free to withdraw at any time. If you do not accept to participate in this study, you will continue to receive the same care and attention in this institution. The staff of this project will be happy to give you more information if needed. Please ask any question about the study before you agree to join, or at any time after joining the study.

4. Expected benefits

The results of this study will be of a great impact in public health. The impact will then be to administer suitable alternative dose of antiretroviral with greater efficiency and less adverse drug reactions to these patients based on their genetic profile. Equally the results of the study will help to develop an algorithm for the better management of HIV patients in Cameroon.

5. Confidentiality

We plan to keep records which contain all your information, locked up in cupboard and to guarantee maximum confidentiality, individual demographic and clinical information of the study participants will be retained in a confidential manner. All blood sample taken will be given a code through which all other records can be traced. All information will be compared and only the research team can have access to the information when required.

6. SAFETY

Nothing will be done to harm you in any way. Everything that will be done to you will be medically correct and not harmful. You will have a minor pain where the needle will pricked your skin and vein while taking blood. In the event of physical injury resulting from the research procedures, medical treatment will be available and provided free of charge.

7. Compensation

A fixed amount of 2000 frs will be given to compensate your transportation fees from your house to the health care center.

8. Contact body

Comité National d'Ethique de la Recherche pour la Santé Humaine au Cameroun (CNERSH**), sis à Hygiène mobile, quartier Messa, Yaoundé**. **Tel :** 00 237 699 87 47 65 / 00 237 699 76 74 27 **E-mail:**
cnersh.cameroun@gmail.com

APPENDIX 1: NOTICE D'INFORMATION

Laboratoire de Biotechnologie de Santé Publique

Centre de Biotechnologie, Université de Yaoundé I

TITRE: Etude pharmacogénétique des patients HIV positifs sous Efavirenz ayant presenté des éffets secondaires au Cameroun : dévelopement d'une procédure de diagnostique

1. Objectifs de l'étude

Monsieur, Madame, je suis .....travaillant à.....

Ce document a été redigé pour vous expliquer l'étude à laquelle vous êtes invité à participer.

Les objectifs de cette étude sont les suivants :

L'objectif général de cette étude était d'obtenir des données de pharmacovigilance et de pharmacogénétique dans une population camerounaise concernant le gène CYP2B6 codant pour une enzyme impliquée dans le métabolisme de l'Efavirenz et de proposer un manuel de procédure pour une meilleure prise en charge des patients VIH au Cameroun.

Plus précisément, nous avons:

1- Déterminer le profil général des effets indésirables associés aux antirétroviraux et la prévalence de ceux associés à l'Efavirenz ;

2- Déterminer la prévalence du polymorphisme 516G>T du CYP2B6 et son association avec les toxicités du système nerveux central liées à l'Efavirenz ;

3- Déterminer la prévalence du polymorphisme 983T>C du CYP2B6 et son association avec les toxicités du système nerveux central liées à l'Efavirenz ;

4- Développer un manuel de procédures pour détecter les polymorphismes CYP2B6 516G>T et CYP 2B6 983T>C.

2. Procédures

Si vous acceptez de participer à cette étude, on vous posera quelques questions. Une petite quantité (5 ml) de sang sera prélevée de vos veines et cela sera un peu douloureux. Il est possible que vous ayez une petite ecchymose à l'endroit où nous prélevons le sang. Votre échantillon de sang sera soumis à un test pharmacogénétique. Toutes les données recueillies seront enregistrées dans une base de données pour des analyses ultérieures. Les résultats de cette étude auront un grand impact dans le domaine de la santé publique et en participant, vous y apporterez votre contribution. Si vous acceptez, vos échantillons de sang seront conservés pendant une période maximale de 20 ans pour des études ultérieures.

3. Participation volontaire

La participation à l'étude est volontaire et vous êtes libre de vous retirer à tout moment. Si vous n'acceptez pas de participer à cette étude, vous continuerez à recevoir les mêmes soins et la même attention dans cette institution. Le personnel de ce projet sera heureux de vous donner plus d'informations si nécessaire. N'hésitez pas à poser toute question sur l'étude avant d'accepter d'y participer, ou à tout moment après avoir rejoint l'étude.

4. Avantages attendus

Les résultats de cette étude auront un grand impact sur la santé publique. Ils permettront d'administrer à ces patients des doses alternatives d'antirétroviraux plus efficaces et présentant moins d'effets indésirables, en fonction de leur profil génétique. De même, les résultats de l'étude permettront de développer un algorithme pour une meilleure prise en charge des patients VIH au Cameroun.

5. Confidentialité

Nous prévoyons de conserver les dossiers qui contiennent toutes vos informations, enfermés dans une armoire et pour garantir une confidentialité maximale, les informations démographiques et cliniques individuelles des participants à l'étude seront conservées de manière confidentielle. Tous les échantillons de sang prélevés recevront un code grâce auquel tous les autres dossiers pourront être retrouvés. Toutes les informations seront comparées et seule l'équipe de recherche pourra avoir accès à ces informations en cas de besoin.

6. Sécurité

Rien ne sera fait pour vous nuire de quelque manière que ce soit. Tout ce qui vous sera fait sera médicalement correct et non nuisible. Vous ressentirez une légère douleur à l'endroit où l'aiguille piquera votre peau et votre veine lors de la prise de sang. En cas de blessure physique résultant des procédures de recherche, un traitement médical sera disponible et fourni gratuitement.

7. Compensation

Un montant fixe de 2000 frs sera versé pour compenser vos frais de transport de votre domicile au centre de soins.

8. Organisme de contact

Comité National d'Ethique de la Recherche pour la Santé Humaine au Cameroun (CNERSH), sis à Hygiène mobile, quartier Messa, Yaoundé. Tel: 00 237 699 87 47 65 / 00 237 699 76 74 27 E-mail : cnersh.cameroun@gmail.com

APPENDIX 2: CONSENT FORM

TITLE: PHARMACOGENETIC STUDY OF HIV PATIENTS UNDER EFAVIRENZ EXPERIENCING ADVERSE DRUG REACTIONS IN CAMEROON: DEVELOPMENT OF A DIAGNOSTIC PROCEDURE

I, Mr./Mrs./Ms._____ willingly accept to participate in this study. I have carefully read the information form and fully understood the purpose of the study, its benefits and inconveniences. I have received all answers to my questions.

I could benefit from the results of the study; all the information collected concerning me shall remain confidential. I agree to fully cooperate with the investigators to permit them carry out the study correctly by giving my blood sample. I can withdraw at any time of the study if I feel uncomfortable. I also give the authorization for the use of my blood sample in case of any further related studies if needed.

Yaoundé, the __/___/____

Participant signature ……..or thumb print

PERSONS TO CONTACT

In any case, should you need further clarification, you can contact: Prof. Wilfred Mbacham, (677579180); Prof. Barbara Atogho, (677641665)

APPENDIX 2: FORMULAIRE DE CONSENTEMENT

TITRE : Etude pharmacogénétique des patients HIV positifs sous Efavirenz ayant presenté des éffets secondaires au Cameroun : dévelopement d'une procédure de diagnostique

Je, M./Mme/Mlle _____ accepte volontiers de participer à cette étude. J'ai lu attentivement le formulaire d'information et j'ai bien compris le but de l'étude, ses avantages et ses inconvénients. J'ai reçu toutes les réponses à mes questions.

Je pourrais bénéficier des résultats de l'étude ; toutes les informations recueillies me concernant resteront confidentielles. J'accepte de coopérer pleinement avec les investigateurs pour leur permettre de réaliser correctement l'étude en donnant mon échantillon de sang. Je peux me retirer à tout moment de l'étude si je me sens mal à l'aise. Je donne également l'autorisation d'utiliser mon échantillon de sang dans le cas d'autres études ulterieures si nécessaire.

Yaoundé, le __/___/____

Signature du participant .....ou empreinte du pouce

PERSONNES À CONTACTER

Dans tous les cas, si vous avez besoin de précisions, vous pouvez contacter : Wilfred Mbacham, (677579180) ; Prof. Barbara Atogho, (677641665)

APPENDIX 3: ASSENT FORM

TITLE: PHARMACOGENETIC STUDY OF HIV PATIENTS UNDER EFAVIRENZ EXPERIENCING ADVERSE DRUG REACTIONS IN CAMEROON: DEVELOPMENT OF A DIAGNOSTIC PROCEDURE

Mr/Mrs/Miss……..has been given information about the goal, the nature of all procedure to be used in this study, then all the things that could be of concern. He/she was free to ask any question about different aspect of the study. Answers were provided in a simple manner.

Family (Guardian, parents) Declaration

I, Mr./Mrs./Ms._____ willingly accept to allow my child to be part of this study. I have carefully read the information form and fully understood the purpose of the study, its benefits and inconveniences. I have received all answers to my questions.

He/she could benefit from the results of the study; all the information collected concerning him/her shall remain confidential. I agree to fully cooperate with the investigators to permit them carry out the study correctly by giving authorization to collect my child's blood sample. The participation of my child in this study is voluntary and I can withdraw anytime without loosing eventual benefits. I can withdraw at any time of the study if I feel uncomfortable. I also give the authorization for the use of the blood sample in case of any further related studies if needed.

Yaoundé, the __/___/____

Parent/guardian signature ……..or thumb print

Participant's Code……..

PERSONS TO CONTACT

In any case, should you need further clarification, you can contact: Prof. Wilfred Mbacham, (677579180); Prof. Barbara Atogho, (677641665).

APPENDIX 3: FORMULAIRE D'ASSENTIMENT PARENTAL

TITRE : Etude pharmacogénétique des patients HIV positifs sous Efavirenz ayant presenté des éffets secondaires au Cameroun : dévelopement d'une procédure de diagnostique

Mr/Mme/Mlle_____ a reçus toutes les informations concernant le but de l'étude, la nature des procédures à utiliser et toutes autres informations utiles. Il/elle était libre de poser toutes les questions concernant différents aspects de l'étude. Les réponses les plus claires et simplifiées lui ont été données.

Declaration du parent ou tuteur

Je, M./Mme/Mlle _____ accepte volontiers de faire participer mon enfant à cette étude. J'ai lu attentivement le formulaire d'information et j'ai bien compris le but de l'étude, ses avantages et ses inconvénients. J'ai reçu toutes les réponses à mes questions.

Mon enfant pourrait bénéficier des résultats de l'étude ; toutes les informations recueillies le concernant resteront confidentielles. J'accepte de coopérer pleinement avec les investigateurs pour leur permettre de réaliser correctement l'étude en leur permettant de collecter un échantillon de sang de mon enfant. Je peux me retirer à tout moment de l'étude si je me sens mal à l'aise. Je donne également l'autorisation d'utiliser l'échantillon de sang de mon enfant dans d'autres études ultérieures si nécessaire.

Yaoundé, le __/___/____

Signature du parent ou tuteur .....ou empreinte du pouce

Code du participant…….

PERSONNES À CONTACTER

Dans tous les cas, si vous avez besoin de précisions, vous pouvez contacter : Wilfred Mbacham, (677579180) ; Prof. Barbara Atogho, (677641665)

APPENDIX 4: DATA COLLECTION SHEET

PART 1: GENERAL INFORMATION	
Study identification code:	Date of enrollment:
Sex:	Date of birth:
Telephone number:	Marital statut:
Number of children:	Educational level:
Profession:	Diagnostic date
Reason for HIV test:	Statut disclosure
PART 2: CLINICAL/BIOLOGICAL DATA	
Weight:	Concomittent drugs:
Others diseases past history:	
WHO Stage at enrollement:	Treatment adherence:
Complaints before ART initiation:	

PARAMETERS BEFORE AND DURING ART							
Date							
CD4							
Viral Load							
SGOT							
SGPT							
Creatinine							
Hemoglobine							

ART Regimen:	Initiation Date:
Complaints after ART initiation /date/ duration:	
Any change in ART regimen/new regimen:	
Date of last visit:	
Other comments:	

APPENDIX 5: RESEARCH AUTHORIZATIONS FROM THE STUDY SITE

REPUBLIQUE DU CAMEROUN
Paix - Travail – Patrie

MINISTERE DE LA SANTE PUBLIQUE

SECRETARIAT GENERAL

DIRECTION DE L'HOPITAL CENTRAL DE YAOUNDE.

N° 889 L/MINSANTE/SG/DHCY/ CM

REPUBLIC OF CAMEROUN
Peace – Work - Fatherland

MYNISTRY OF PUBLIC HEALTH

SECRETARIAT GENERAL

DIRECTORATE OF THE YAOUNDE CENTRAL HOSPITAL

Yaoundé, le 14 JUIL 2015

AUTORISATION DE RECHERCHE

Je soussigné professeur Pierre Joseph FOUDA, Directeur de l'Hôpital Central de Yaoundé, marque mon accord pour la recherche de Madame NGUEFEU NKENFOU Carine, étudiante en biochimie à l'université de Yaoundé I, dans le service Hôpital de jour de l'Hôpital Central de Yaoundé.

Cette étude a pour thème : « pharmacogénétic study of HIV positive patients in cameroon : developement of a diagnostic procedure »

L'intéressée est tenue au strict respect de la règlementation en vigueur à l'Hôpital Central de Yaoundé.

En foi de quoi la présente autorisation lui est délivrée pour servir et valoir ce que de droit. /-

Pour le Directeur et par ordre
Le Conseiller Médical

Pr. Djientcheu Vincent

REPUBLIQUE DU CAMEROUN
PAIX – TRAVAIL – PATRIE

MINISTERE DE LA SANTE PUBLIQUE

SECRETARIAT GENERAL

DELEGATION REGIONALE DE L'EST

HOPITAL REGIONAL DE BERTOUA

DIRECTION

REPUBLIC OF CAMEROON
PEACE-WORK-FATHERLAND

MINISTRY OF PUBLIC HEALTH

SECRETARY GENERAL'S OFFICE

EAST REGIONAL DELEGATION

BERTOUA REGIONAL HOSPITAL

DIRECTOR'S OFFICE

N°2137/15 /AFS/MINSANTE/SG/DRE/HRB/D

AUTORISATION DE RECHERCHE

Je soussigné, Dr N.G. Nguefé Max. Médecin Gynécologue Obstétricien, Directeur de l'Hôpital Régional de Bertoua,

Marque mon accord pour la recherche de Madame **NGUEFEU NKENFOU Carine,** étudiante en biochimie à l'Université de **Yaoundé 1,** dans le service Hôpital du jour de l'Hôpital Régional de Bertoua.

Cette étude a pour thème : « pharmacogenetic study of HIV positive patients in Cameroon ; developement of a diagnostic procedure ».

L'intéressée est tenue au strict respect de la réglementation en vigueur à l'Hôpital Régional de Bertoua.

En foi de quoi, la présente autorisation est délivrée à l'intéressé pour servir et valoir ce que de droit. /.

Bertoua, le 28 JUL 2015

LE DIRECTEUR

Dr N.G. Nguefé Max. Médecin Gynécologue Obstétricien

APPENDIX 6: ETHICAL CLEARANCE

COMITE NATIONAL D'ETHIQUE DE LA RECHERCHE POUR LA SANTE HUMAINE

Arrêté N° 0977/A/MINSANTE/SESP/SG/DROS/ du 18 avril 2012 portant création, organisation et fonctionnement des comités d'éthique de la recherche pour la santé humaine au sein des structures relevant du Ministère en charge de la santé publique

N° 2014/12/-670/CE/CNERSH/SP

Yaoundé, le 1er décembre 2015

Cnethique_minsante@yahoo.fr

CLAIRANCE ETHIQUE

Le Comité National d'Ethique de la Recherche pour la Santé Humaine (CNERSH), en sa session extraordinaire du 1er décembre 2015, a examiné le projet de recherche intitulé **«Pharmacogenetic study of HIV positive patients in Cameroon : development of a diagnostic procedure»** soumis par **Mme Carine NGUEFEU NKENFOU**, Investigateur Principal, Faculté des Sciences, Université de Yaoundé1.

Le projet est d'un grand intérêt scientifique et social. Le but de cette étude est d'obtenir des données pharmacogénétiques dans une population camerounaise sur les gènes CYP2B6 et ABCB1 codant pour des protéines et molécules intervenant dans le métabolisme des antirétroviraux et proposer un algorithme pour une meilleure gestion des patients HIV positifs au Cameroun. Les risques liés au prélèvement de sang sont présentés ainsi que les mesures pour les éviter et les minimiser. La notice d'information et le formulaire de consentement éclairé, en français et en anglais, sont bien élaborés et simples à comprendre. Les mesures prises pour garantir la confidentialité des données collectées sont présentes dans le document. Les CVs des Investigateurs les décrivent comme des personnes compétentes, capables de mener à bien cette étude. Pour toutes ces raisons, le Comité National d'Ethique approuve pour une durée d'un an, la mise en œuvre de la présente version du protocole.

L'étudiante **Carine NGUEFEU NKENFOU** est responsable du respect scrupuleux du protocole approuvé et ne devrait y apporter aucun amendement aussi mineur soit-il, sans avis favorable du CNERSH. L'investigateur est appelé à collaborer pour toute descente du CNERSH pour le suivi de la mise en œuvre du protocole approuvé. Le rapport final du projet devra être soumis au CNERSH et aux autorités sanitaires du Cameroun.

La présente clairance peut être retirée en cas de non respect de la réglementation en vigueur et des recommandations susmentionnées.

En foi de quoi, la présente clairance éthique est délivrée pour servir et valoir ce que de droit.

Ampliations

- MINSANTE

LE PRESIDENT

Pr Lazare KAPTUE

N.B : cette clairance éthique ne vous dispense pas de l'autorisation administrative de recherche (AAR), exigée pour mener cette étude sur le territoire camerounais. Cette dernière vous sera délivrée par le Ministère de la Santé Publique.

APPENDIX 7: PREPARATION OF SOLUTIONS

20% Chelex-100 (50mL)

Chelex-100: 20 g

1X PBS: 100 mL

Autoclave and store at 4°C

1X Phosphate buffer saline (PBS), 200ml pH 7.2

PBS tablet: 1g

Complete to 200mL with distilled

Autoclave and store at 4°C

0.5% Saponin

Saponin: 0.5g

Complete to 100mL with 1X PBS

Autoclave and use immediately

10X TBE (1l)

Tris base: 108 g

Boric acid: 55g

0.5M EDTA 40mL

Complete to 1L with distilled water and store at room temperature

DNA Loading Buffer (50mL)

0.25% Bromophenol Blue: 0.125 g

0.25% Xylene Cyanol: 0.125g

15% Ficoll: 7.5 g

20 mM EDTA: 2 mL of 0.5M

Complete to 50 mL with distilled water

Store at room temperature

Molecular Weight Marker (200 µL)

100 bp ladder : 20 µL

1x TE : 140 µL

Loading Buffer: 40 µL

Store at -20°C

Tris EDTA Buffer (TE), 100mL pH 8

1M Tris : 1mL

0.5M EDTA : 200uL

Complete to 100mL with distilled water

Autoclave and store at room temperature

Ethidium Bromide (10mL)

Ethidium bromide: 0.15g

Distilled water: 10mL

Store at room temperature

2% Agarose gel (100mL)

Agarose: 2g

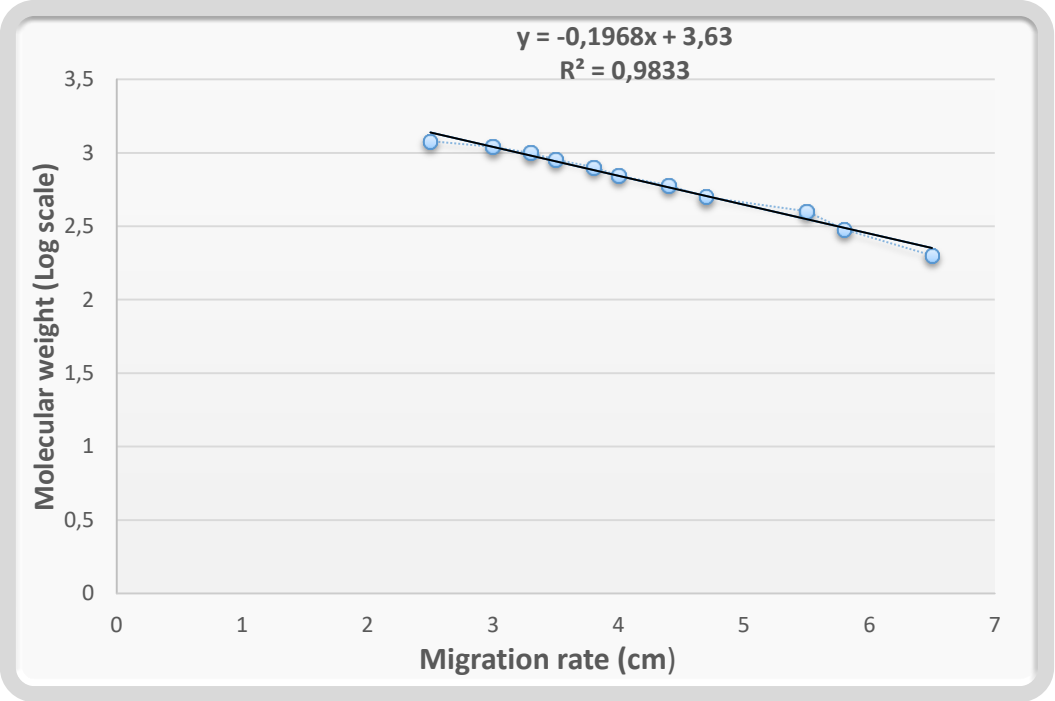
1X TE: 100mL

Boil for 4 minutes on bursen burner and allow to cool to 60ºC, add

2.5µL 1.5% ethidium bromide and cast in electrophoresis tank.

Allow to solidify for 45 minutes before use in electrophoresis.

APPENDIX 8: STANDARD CURVE FOR THE DETERMINATION OF MOLECULAR WEIGHT OF PCR PRODUCTS



APPENDIX 9: THE MANUAL OF STANDARD OPERATING PROCEDURES

At the end of this research work, a manual of procedures was produced. This manual is structured in chapters and standard operating procedures (SOPs), which provide amendments to the challenges faced on the field. In the manual of procedures, there are 5 parts and each consists of a set of SOPs which entail a series of tasks and procedures to follow and there by facilitating the reader to carry out his own pharmacogenetics study successfully. All the SOPs were prepared by Carine Nguefeu Nkenfou Tchinda, proofread by Prof. Atogho Barbara Tiedeu and Dr. Jean Paul Chedjou and validated by Pr. Wilfred Fon Mbacham.

PART 1:

Preparatory phase

Before initiating a research study, it is important that a foundation is laid down. To avoid some challenges which sometimes lead to drastic changes in the course of a study, measures have to be taken to identify all the resources needed (human, financial, material, time, etc). In order not to omit any step in this phase and ensure a safe kick-off of a study, a checklist has to be written so as to guide the researcher. The best way of validating a checklist is by validating a set of questions. Some of such questions include: Where should the study be conducted? How to present a research protocol? Where will the funding come from? How long will the study take? Which regulatory bodies should issue ethical clearance for the study? How should the questionnaire be designed? In this part, a total of 4 Standard Operating Procedures (SOPs) were elaborated, which help in securing a proper planification of the study before launching. These SOPs are entitled:

- SOP001 - Drafting of protocol

- SOP002 - Filling for Ethical Clearance application form and submissionof the protocol

- SOP003 - Contacting study sites

-SOP004 - Sites preparation

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Standard Operating Procedure N^{0}001

Drafting of protocol

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Proofread by: Prof Babara Atogho Tiedeu and Dr Jean Paul Chedjou
Validated by: Prof Wilfred Mbacham Date of effect: 24/03/2021

1. **Objective:** To present a well detailed document stating why and how the study will be conducted and which can be submitted to request for an ethical clearance.
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Computer, power supply, relevant literature, Microsoft Word software, endnote software, Biostatistician, Internet connection
4. **Place of Work:** Office desk
5. **Procedure:**

- A good and precise title should be proposed. From the title, the reader should be able to have a brief idea on what the study will entail.
- The background information should be written from existing literature, justifying the existence of an issue/problem/lack which has to be addressed. A rationale should follow the background information, justifying the need for the study to be conducted.
- Research hypothesis should be written and the main and specific objectives enumerated to make the feasibility of the study visible.
- A study methodology should be well designed, and should include the following: study endpoints, study site; study duration (duration of subject enrolment and study period); selection of subjects; inclusion and exclusion criteria; genotyping methods.
- The statistical methods used for data analyses should be described and the level of significance should be stated.
- Quality Control/Quality Assurance of the study should be highlighted, stating regular audits, source data verification, GCP, GLP, SOPs
- The risk/benefit assessment should be enumerated with approval forms by the national ethics committee and the. Measures should be emphasised on participant informed consent and patient/data protection and confidentiality.

- The recording and handling of data should be done using data collection sheets, and they should be properly handled and kept.
- The source of funding of the project should be stated. Any compensation for the study participants should also be highlighted.
- A detailed chronogram of activities should be written, Including: all the tasks as derived from the objectives. The chronogram should be spread according to weeks (most preferably) and should begin from the development of the protocol to the submission of the final study report.
- References for every scientific backing should be listed in the bibliography. The referencing style will depend on the funder/sponsor (Vancouver, Chicago, APA).
- Appendices should include: Informed consent and patient information sheet, letter of approval from the study sites (if available), ethical clearance from the national ethics committee.

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Standard Operating Procedure N^{0}002

Filling for the Ethical Clearance application form and submission of the protocol

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1. **Objective:** To submit to the National Ethics Committee to obtain ethical clearance
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Computer, power supply, application form, Microsoft Word software
4. **Place of Work:** Office desk
5. **Procedure:**

- The form is to be completed and signed by the principal investigator (PI);
- In filling the form, the title of the project has to be written as well as the names and qualifications of the PI. In a maximum of 300 words, the outline of the proposed project should be given. Sufficient detail of the protocol must be presented to allow the Committee to make an informed decision without reference to other documents;
- In a maximum of 300 words, the intended value of the project should be stated. If this project or a similar one has been done before, clarify the reason for repeating;
- In a maximum of 300 words, specify , with scientific justification for sample size, age of participants, gender, source and enrolment method;
- State the likely duration of the project, and where it will take place;
- Specify the procedures, including interviews, involving human participants with brief details of actual methods;
- State the potential discomfort, distress or hazards that research participants may be exposed to (these may be physical, biological and/or psychological); State which precautions are anticipated to alleviate these risks. Include information on hazardous substances that will be used or produced, and how to handle them;
- Risk assessment procedures including travel safety are important.

- Specify how confidentiality will be maintained with respect to the data collected. When small size population is involved, indicate how possible identification of individuals will be avoided;
- State the manner in which consent will be obtained and supply copies of the information sheet and consent form. Written consent is normally required wherever possible. Where not possible, a detailed explanation of the reasons should be given and a record of those agreeing kept.
- If the study involves blood samples and/or any other tissue collection, list samples which will be taken. Confirm insurance that any staff involved in the research has been trained;
- If payments are made to participants these should usually not be for more than travelling expenses and/or loss of earnings and must not represent an inducement to take part;
- Give details of the research sponsor if any.

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Standard Operating Procedure N^{0}003

Contacting study sites

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1. **Objective:** To ensure that the study sites are well informed on the modalities of the study before initiation
2. **Scope:** Applicable to all Pharmacogenetic studies
3. **Requirements:** Computer, internet modem, telephone, communication credit
4. **Place of Work:** Office
5. **Procedure:**

- While requesting research authorizations from the selected study sites, also collect the contacts (email and telephone numbers).
- Send emails to all study sites and follow up with phone calls and explain in detail how the study will be conducted. Inform them when the study will start.

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Standard Operating Procedure N^{0}004

Sites preparation

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1. **Objective:** To ensure that the study sites are well equipped with human and material resources before study initiation
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** research team, transport costs (fuel money, bus, train or air tickets), lodging and feeding, camera
4. **Place of Work:** Study sites
5. **Procedure:**

❖ Prepare a trip to all study sites
❖ On arrival at each site ensure the following:
  - The study site is highly attended by the target population of the study
  - The infrastructure is adequate enough to carry out the study
  - There are enough qualified personnel (medical doctors, nurses, pharmacists, laboratory technicians) actively present in each site
  - The equipment in the laboratories are functional
  - There is sustainable power supply in the sites (electricity and water)
❖ Take pictures where needed
❖ When all this has been evaluated and noted, a medical doctor, nurse and laboratory technician should be designated as the support staff to carry out the study at site.

PART 2:

CLINICAL PROCEDURES

This phase is performed ideally at the study site, and starts with screening for eligibility of potential study participants. The activities in this phase are vital for the success of the study and would therefore need rigorous supervision throughout the study. In this part, 4 SOPs were developed. These SOPs guide the reader to systematically recruit participants in the study. They are entitled:

- SOP5 - Screening for eligibility
- SOP6 - Obtaining informed consent
- SOP7 - Enrolment of participants into study and blood samples collection
- SOP8 - Filling of data collection sheets

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Standard Operating Procedure N^{0}005

Screening for eligibility

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Validated by: Prof Wilfred Mbacham: Date of effect: 24/03/2021

1. **Objective:** To harmonise the methodology for selecting eligible patients for the study
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Prospective study participants medical files, papers, pen
4. **Place of Work:** HIV treatment centre/unit
5. **Procedure:**
    - Screening of patients medical records filled by physicians
    - Identification of patients with or without ADRs associated to Efavirenz
    - Establishment of a repertoire with patients' telephone numbers for further calls

❖ **Inclusion Criteria**

- Willing to participate in the study with signed informed consent
- > 18 years
- Under ART
- No concomitant drugs
- Being followed up

❖ **Exclusion Criteria**

- Withdrawal from the study

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Standard Operating Procedure N^{0}006

Obtaining Informed Consent

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1. **Objective:** To include interested participants by having them sign a document to prove they agree with all the terms of the study
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Informed consent form, study participants
4. **Place of Work:** HIV treatment centre
5. **Procedure:**

❖ Explain the objectives and the procedures for the study to the participant.

❖ Allow them to read the Information Sheet and Consent Form while explaining in either English, French, their local language or pidgin-English.

❖ Answer to all questions asked by the participant

❖ Then formally ask the patient consent to participate in the study.

❖ Patients who can read and write and consent to enter the study should sign the Consent Form.

❖ Those who cannot read should have the Consent Form read out by the recruiting team member with a witness to sign for them. The participant can also provide a finger print.

❖ Participants must be made aware that if they do not enter the study they will not be disadvantaged in any way, and will receive standard treatment from the hospital as required.

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Standard Operating Procedure N^{0}007

Enrolment of participants into study and blood samples collection

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1. **Objective:** To harmonise the procedure for enrolling patients into the study and blood sample collection
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Study participants, 5 mL Purple top (Ethylene diamine tetra-acetic acid (EDTA)) blood tubes, 10 mL Syringe or Vacutainer system with butterfly needle attachment, Cotton Swab, Alcohol Swab, Tourniquet, Plastic gloves
4. **Place of Work:** HIV treatment unit
5. **Procedure:**

❖ Ensure all necessary material is ready at hand in a tray.

❖ Identify a good-sized vein

❖ Apply a tourniquet proximal to the site of venipuncture to ensure engorgement of vein with blood.

❖ Prepare a 10 mL syringe or prepare the vacutainer with a butterfly needle. The type of device used to collect blood is dependent upon operator's preference.

❖ Clean the site of venipuncture with an alcohol swab.

❖ Insert needle into vein looking for blood flashback in the level of the syringe.

❖ Gently withdraw approximately 5mL of blood into the syringe or alternatively place purple EDTA tubes into the Vacutainer to allow self-filling of blood.

❖ Once enough blood has been withdrawn, undo the tourniquet with the needle still in place.

❖ Take cotton swab and place over site of needle insertion (Venipuncture) and gently remove the needle.

❖ Apply direct pressure with a dry cotton over the puncture site to stop any bleeding. This should be last for 2mins, after which the swab should be removed to ensure bleeding has stopped.

- Transfer blood from syringe into purple EDTA tubes, either by directly puncturing the top of the EDTA tube in the center or remove the tube top and gently inject blood into the empty tube then recap. If a vacutainer device is used, the above would not be necessary as tube would self-fill. Slowly invert samples 8 to 10 times to ensure the mixing of the sample with the anti-coagulant liquid inside the tube.
- Carefully label the tubes with patient ID study number and date blood sample was taken, aligned in order they are readily recognized as this will help with processing in the laboratory.

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Standard Operating Procedure N^{0}008

Filling of data collection forms

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1. **Objective:** To collect sociodemographic, clinical and biological data of each participant for eventual analysis
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Data collection form, blue or black ballpoint pen, study participants
4. **Place of Work:** HIV treatment centre
5. **Procedure:**

- Only the investigators and or research team members are authorised to make entries on the data collection sheets. Entries should be made with a black or blue ink ballpoint pen to ensure legibility.
- Corrections should be made by drawing a single line through the original entry, entering the new value.
- Follow each line of each page by filling all required information needed, starting with the code of the study participant.
- Then fill the patient's sociodemographic, clinical, past medical history and biological data obtained from his medical report and from answers given to specific questions.
- Completed forms will be dated and signed by the investigator or/and the authorised study personnel.

PART 3

LABORATORY PROCEDURES

This phase is done simultaneously during the clinical procedures. Depending on the time allocated in the entire study, the molecular analysis could be done during the clinical procedure or immediately after. This phase helps to prepare collected samples needed for laboratory analysis. The 8 SOPs in this part show how these samples should be treated, what biomarker should be analysed and how this analysis should be done in the laboratory. They are entitled:

- Patient sample registration and identification
- Realization of Dried Blood Spots on filter paper
- Human DNA extraction
- Human DNA amplification
- Agarose gel electrophoresis of amplified DNA products
- Restriction Fragment Length Polymorphism
- Determination of molecular weight of PCR and digestion products
- Waste management

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Standard Operating Procedure N^{0}009

Patient sample registration and identification

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1. **Objective:** To be used for patient registration and assignment of patient IDs at the laboratory.
2. **Scope:** Applicable to all pharmacogenetic studies.
3. **Requirements:** Laboratory log book, black or blue ballpoint pen, enrolment log book.
4. **Place of Work:** Molecular Biology Laboratory
5. **Procedure:**

- Assign a unique laboratory ID (study ID) for the participant's sample as indicated in the participant's information sheet. This ID will be used as the enrolment ID and for all tests performed subsequent to enrolment. **The patient's name should never be used anywhere in the registration or enrolment of the participant.**
- Enter the details in the enrolment log.
- Ensure the following details are captured in the laboratory log:
  - Enrolment ID
  - Lab. ID No.
  - Date of reception

NB:

Label the specimen or sample containers or tube appropriately for traceability.

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Standard Operating Procedure N^{0}010

SOP 010- Realisation of Dried Blood Spots on filter paper

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1. **Objective:**To transfer fresh blood sample to a filter paper in order to obtain dried blood spots for the molecular analysis of the DNA
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** gloves, cotton, HB pencil, 70% ethyl alcohol, sterile filter paper, envelopes, pipets and pipets tips, blood sample in EDTA tubes
4. **Place of Work:** Molecular Biology Laboratory
5. **Procedure:**

- Wear a pair of clean non-sterile gloves.
- Prepare sterile filter paper
- Pipet 7µL of whole blood in EDTA tube and put gently on the receiving ends of the filter paper.
- Label the closed end of each filter paper with an HB pencil. Filter papers should be labelled bearing the following: patient ID (001, 002) and date of collection (DD/MM/YYY).
- The filter paper should be labelled for one patient first before proceeding with the next patient.
- Allow them to dry at room temperature
- Store the filter papers in separate and labelled envelopes as described in SOP 017.

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Standard Operating Procedure N^{0}011

Human DNA extraction

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1. **Objective:** How to extract Human DNA from dried blood spot on filter paper
2. **Scope:** Applicable to human DNA extraction at LAPHEB
3. **Requirements:** Sterile forceps, a pair of scissors (sterile), a bunsen burner, 1.5mL microfuge tubes, heat block or boiling water bath set at 100˚C, 4˚C refrigerator, micro centrifuge, dried blood spots on filter paper, 1xPBS (sterilised by autoclaving), 1xPBS/0.5% Saponin (sterilised by autoclaving), 20% w/v Chelex-100 solution in 1xPBS (autoclaved), double distilled water (DNase-free water), disposable paper tissue, distilled water, fine tip permanent marker, lab notebook. PBS = Phosphate Buffer Saline
4. **Place of Work:** Molecular Biology Laboratory
5. **Procedure:**

- First, select and register the number of samples (**n**) to be extracted in the lab notebook with their respective code IDs.
- Wear clean gloves throughout the procedure. As the blood potentially contains infectious material, work in a biohazard containment cabinet if available. Any form of liquid waste should be discarded into a 10% bleach solution to inactivate infectious material. Clean working space and all equipment with 10% bleach and alcohol. Flame scissors and rinse with distilled water, then dry with a clean paper tissue.
- For each sample, label 4 Eppendorf tubes with a fine tip permanent marker with the same code ID as in the lab notebook. BE CAREFUL NOT TO CONTAMINATE THE TUBES WHEN REMOVING THEM FROM THE PACKET. USE FORCEPS IF NECESSARY.
- Remove the DBS filter paper from the plastic bag using forceps. Excise the area of filter paper with the blood spot using a scissors and get the piece into sterile 1.5mL Eppendorf tubes. DO NOT ALLOW THE SCISSORS OR FORCEPS TO COME INTO CONTACT WITH THE BLOOD SPOT. Remember in between each sample to flame the scissors and rinse with distilled water, then dry with a clean paper tissue.

- Add 1mL of 0.5% Saponin in 1XPBS (sterile) to each tube. Invert the tubes 5-10 times and store in a 4˚C refrigerator overnight.
- After overnight incubation, discard the brown solution from each Eppendorf tube into a beaker containing 10% bleach, without removing the filter paper. Refill the tubes with 1mL PBS and invert them again. Place the tubes in the 4˚C refrigerator again for 15-30 minutes.
- While the tubes are in the refrigerator, add 50µL of 20% Chelex solution (mix well before use) into 150µL of DNase-free water in a second set of **n** number of 1.5mL Eppendorf tubes. Heat the filled tubes to 100˚C in a heat block.
- After 15-30 minutes, take the tubes containing the filter paper (from the refrigerator), then remove and discard the PBS into the 10% bleach container still without removing the filter paper from the tubes. Using forceps, remove the filter paper and place into the respective tubes containing the hot Chelex. Clean the forceps after each sample by flaming as before. Vortex vigorously for 30 seconds and replace in the heat block at 100˚C for 10 minutes. Repeat the vortex procedure twice for each tube, always remembering to incubate in the 100˚C heat block for 10 minutes after each vortex.
- After 3 rounds of vortexing, place the tubes in a micro centrifuge. Spin the tubes at 10,000 rpm for 2 minutes. Remove 100µL of the supernatant into a third set of **n** number of tubes. Spin this new set of tubes (100µL) at 10,000 rpm for 2 minutes still. Transfer 50µL of the supernatant into a final (fourth) set of tubes. Do not transfer any of the Chelex matrix.
- Store the DNA solution at -20˚C (short term) or at -70˚C (long term).

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Standard Operating Procedure N^{0}012

Human DNA amplification

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1. **Objective:** To describe the procedure to amplify DNA isolated from DBS by PCR
2. **Scope:** pharmacogenetic analyses
3. **Requirements:** Thermocycler (Biometra), -20˚C deep freezer, -32˚C deep freezer, 4˚C refrigerator, laminar hood, electronic balance (Sartorius), pH meter (Hanna), Gilson micropipettes (p10, p20, p50, p100, p200, p1000), magnetic stirrer, micro centrifuge, electrophoresis apparatus (Fisher Biotech), autoclave or bunsen burner, paper tissue, disposable gloves, alcohol, cotton wool, 10% bleach, graduated cylinders, 250mL and 500mL bottles, ice pile, pipette tips, PCR tubes (0.2mL, 0.5mL), tube racks, 50mL conical centrifuge tubes, fine tip permanent marker, plastic bowl, laboratory notebooks, 10XTBE, multipurpose agarose, DNA molecular weight marker, gel loading dye, ethidium bromide, *Taq* DNA polymerase, primers, dNTPs, restriction enzymes, DNase-free water, 10x thermopol buffer.
4. **Place of Work:** Molecular Biology Laboratory
5. **Procedure:**

Amplification of DNA for the detection of CYP 2B6 516G>T and CYP 2B6 983T>C polymorphism

- First, select and record the number of samples to be amplified in the lab notebook. Include positive and negative controls for each primer in every run.
- Label PCR tubes with the fine tip permanent marker for the primary amplification reactions. BE CAREFUL NOT TO CONTAMINATE THE TUBES WHEN REMOVING THEM FROM THE PACKET. USE FORCEPS IF NECESSARY.
- Remember to include controls: One will need a positive control and a negative control (with no DNA template added).

- Remove stored DNA samples from deep freezer and thaw. Spin briefly in a micro centrifuge (10,000 rpm for 1 second) to pellet any Chelex matrix and place immediately on ice.
- Prepare a PCR premix (Nuclease free water-NFW, 10xThermopol buffer, dNTPs, primers, Taq DNA polymerase), WORKING ON ICE AT ALL TIMES. For each sample, one will need 22µL of premix (17.75µL NFW, 2.5µL of 10xThermopol buffer, 0.5µL of 10mM dNTPs, 0.5µL of primers forward and reverse respectively, 0.25µL of Taq DNA polymerase), with a little extra (say 2 sample volumes extra or 44µL extra) to allow for pipetting errors.
- Aliquot 22µL of the premix into each of the labelled PCR tubes, recapping each tube as you go. Don't forget the negative control.
- Add 3µL of the appropriate DNA template (sample, positive control) or water (negative control) to each tube, and keeping tubes capped at all times when not in use. The final volume in each tube should be 25µL.
- Place the tubes into the PCR machine and run the appropriate pre-entered programme.
- Load 10µL of the PCR products into wells of a 2% agarose gel (SOP 013) and run at xxxvolts, cccamp; for xxxminutes. Don't forget to load alongside into another well the DNA molecular
- weight marker (DNA MWM)
- Check for approximate band sizes against DNA MWM provided.

Some causes of common problems encountered during amplification of DNA by PCR

Problem	**Possible cause(s)**	**Suggested actions**
All reactions are negative, including positive control	Component (s) missing, wrong thermocycler programme used and contaminated reagents	Repeat PCR with new set of positive control and enzyme mix
No amplicon with positive control while some sample present amplified products (positive)	Control DNA degraded or not added	Repeat PCR with new set of positive control
Smears	Excess product, false amplification	Reduce amount of primers or modify annealing conditions
Non-specific bands	Wrong primer set, wrong amplification profile	Troubleshoot, readjust quantity of reagent as well as amplification conditions
Test bands remain in wells but MWM migrated and uniformly distributed	Contamination of samples, inhibitors in samples	Troubleshoot

LAPHEB

Standard Operating Procedure $N^0$013

Agarose gel electrophoresis of amplified DNA products

Date of issue of this version: 24/03/2021
Prepared by: Carine Nguefeu Nkenfou Tchinda
Version No: 1
Proofread by: Prof Babara Atogho Tiedeu and Dr Jean Paul Chedjou
Validated by: Prof Wilfred Mbacham Date of effect: 24/03/2021

1. **Objective:** To describe the procedure of a 2% agarose gel electrophoresis of PCR products
2. **Scope:** Applicable to all agarose gel electrophoresis of PCR products at LAPHEB
3. **Requirements:** Electrophoresis tank and tray, gel combs, 250mL Duran bottles with lids, power supply system, tripod, bunsen burner, P20 pipette, multipurpose agarose, 1xTBE, ethidium bromide, PCR products, DNA molecular weight marker, 10x loading buffer, Parafilm, sterilised pipette tips, paper tissue, 10% bleach, 70% alcohol, , lab notebook, digital camera, computer
4. **Place of Work:** Molecular Biology Laboratory
5. **Procedure:**

- Clean working space with paper tissue soaked with distilled water. Clean combs and casting tray with distilled water and **NOT** with alcohol and dry with disposable tissue paper.
- Place the tray in a gel-forming clamp. Place a well comb in position, making sure that about 1 millimetre of space exists between the bottom of the comb teeth and the surface of the tray. This helps to prevent leaks of the sample out of the well. Balance the position of the tray with a pie.
- Prepare a 2% agarose gel. Weigh out and gently pour 1g agarose directly into a Duran bottle containing 50mL of 1xTBE buffer (larger gels will require proportionately larger amounts of agarose and TBE). Make sure the agarose doesn't touch the walls of the bottle when pouring. Cover the bottle with a lid. Place bottle on tripod bunsen burner and allow gel to cook for complete dissolution (when the solution starts to boil then the gel is dissolved). The bottle will be very hot so remember to handle with paper towels. Allow the bottle to cool at room temperature to about 55˚C (hot but can hold with hand). Add 2.5µL ethidium bromide, swirl till its colour disappears and then pour the liquid gel into

electrophoresis tray with well comb in place. Allow to cool completely for at least 20-30 minutes. This allows for the gel to solidify.

- Prepare the DNA molecular weight marker as follows: 20µL of 100bp or 50bp ladder + 140µL of 1xTBE + 40µL loading buffer.
- Remove the tape from the gel tray and place in the electrophoresis tank with the sample wells towards the negative (**black**) electrode. Fill the mini gel tank with about 100mL of 1xTBE buffer to cover (barely) the gel surface. Gently remove the comb, making sure the well lips are covered completely with 1xTBE.
- Load wells with 10µL PCR sample (after mixing with 2.5µL loading buffer on a Parafilm) using a P20 micropipette. Place the pipette tip barely at the top of the well. BE CAREFUL NOT TO PUNCTURE THE AGAROSE AT THE BOTTOM OF THE WELL. Dispense the sample into the well slowly. Be sure to record the order of sample loading. Load 10µL of DNA marker on first and last lanes.
- Hook up electrodes (**red to red and black to black**) and electrophorese samples toward the positive electrode (**from black to red**) at 50 volts (maximum 100 volts). Allow marker dye to travel one-half to two-thirds the distance to the end of the gel.
- Carefully remove gel from tank, place and observe under UV trans-illuminator, record results in the lab notebook, take photographs and upload into a computer.
- Score the bands as not visible, weakly visible, medium intensity or strong relative to the DNA size marker.
- Decontaminate waste in 10% bleach and 70% alcohol before disposal.

LAPHEB

Standard Operating Procedure N^{0}014

Restriction Fragment Length Polymorphism

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1. **Objective:** To describe the procedure to digest amplified DNA products with restriction enzymes and how to determine the genotype from fragment pattern generated
2. **Scope:** Used to determine the presence of mutations in the CYP 2B6 gene
3. **Requirements:** Thermocycler (Biometra), *BsmA* I and *Bsr* I enzymes, Nuclease-free water, NE Buffers 2 and 3, Bovine Serum Albumin, PCR products, Gilson micropipettes (p10, p20, p50, p100), disposable gloves, alcohol, cotton wool, 10% bleach, PCR tube racks, PCR tubes (0.2mL),ice pile, pipette tips, laminar hood, micro centrifuge, laboratory notebook, fine tip permanent marker
4. **Place of Work:** Molecular Biology Laboratory
5. **Procedure:**

- First, select and record the number of samples to be tested in the lab notebook. Include positive and negative controls.
- Label PCR tubes with the fine tip permanent marker for digestion. BE CAREFUL NOT TO CONTAMINATE THE TUBES WHEN REMOVING THEM FROM THE PACKET. USE FORCEPS IF NECESSARY.
- Remember to include controls: you will need a positive control and a negative control (with no DNA added).
- Collect stored PCR products from deep freezer and thaw at room temperatureand place immediately on ice.
- Prepare a premix for all restriction enzymes to be used, WORKING ON ICE AT ALL TIMES, with a little extra (for 2 sample volumes extra or 28µL) to allow for pipetting errors.
- To identify the 516 G>T mutation in the CYP 2B6 gene, the premix for each sample will be as thus: 8µL of NFW; 3µl of Buffer; 1µL *Bsr* I enzyme.

- To identify 983 T>C mutation in the CYP 2B6 gene, the premix will be as follows: 8µL of NFW; 3µL of Buffer; 1µL *BsmA* I enzyme.
- Aliquot 12µL of the *BsmA* I and *Bsr* I premix respectively into each of the labelled tubes, recapping each tube as you go. The final tube should be for the negative control.

- Add 8µL of the appropriate DNA (sample, positive control) or water (negative control) to each tube, and keeping tubes capped at all times when not in use. The final volume in each tube should be 20µL.
- Place the tubes into the PCR machine and run the appropriate programme.
- For *BsmA* I digestion, set the PCR machine at 55˚C for 20 hours.
- For *Bsr* I digestion, set the PCR machine at 65˚C for 20 hours.
- Load 10µL of the digestion products and run on a 2% agarose gel (SOP 13).
- Interpret the band pattern obtained (genotype or allele reading)

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Standard Operating Procedure N^{0}015

Determination of molecular weight of PCR and digestion products

Date of issue of this version: 24/03/2021
Prepared by: Carine Nguefeu Nkenfou Tchinda
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1. **Objective:** To determine the molecular weight of amplified or digested DNA fragment
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Computer, ruler, pictures of electrophoregram, Microsoft Excel, pen, notebook, calculator
4. **Place of Work:** Office
5. **Procedure:**

- Open a Microsoft Excel document
- Open a picture of electrophoregram.
- Using a ruler, measure the distance of migration in centimetres of each band of the molecular weight markers. Use the level of the wells of the gel as a baseline for all measurements.
- Calculating the log10 of each of the molecular weight markers, plot a curve of the distance of migration (ordinate), against log10 of molecular weight marker (abscissa) (see graph below).
- Extrapolate graphically, the molecular weight of each DNA fragment by applying the standard formula **y = mx+c**, where y=log10 of molecular weight; m=gradient (constant); x=distance of migration of gene; and c=y intercept (constant).

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Standard Operating Procedure N^{0}016

Waste management

Date of issue of this version: 24/03/2021
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1. **Objective:** Describe the procedures adopted in the handling, packaging and safe disposal of biological waste to protect individuals and the environment from exposure to biological hazards.
2. **Scope:** Applicable to all pharmacogenetic studies

Definition: Biological waste is any waste that is potentially bio-hazardous, infectious or pathological. The categories of biological waste generated in this lab are as follows:

- **Human Blood and Blood Products:** All human waste blood, serum, plasma, and other blood products or components are considered infectious wastes. Items that are saturated, dipped, or contain human blood, serum, plasma, other blood components, are also considered infectious, E.g. Cotton
- **Contaminated Sharps:** All discarded sharp materials (sharps), which have been used in patient care/treatment, present with the double hazard of inflicting injury and inducing disease. All needles, syringes, and cover slips are considered sharps.
- **Chemicals:** All chemical liquids generated in the lab have the potential to cause burns, and expose to infections.

3. **Requirements:** Non-sterile gloves, 10% hypochlorite solution, Incinerator, Sharps container (puncture proof), Bio hazard bags, Bins, Carton, Labels, Kerosene, and Logbook.
4. **Place of Work:** Laboratory
5. **Procedure:**

- **Handling and disposal of biological waste**

  - Biological waste should be decontaminated before disposal, either by autoclaving or bleaching.

- Segregate waste at source; discard disposable plastic ware and reusable glassware into separate bins, containing 10% hypochlorite. Leave them overnight in the 10% hypochlorite solution.
- The following day, drain the 10% hypochlorite from the discard bins into the sink.
- Reusable glassware is subjected to washing procedure.
- Collect the vials/tubes/syringes containing blood, serum or plasma or cotton/gauze soaked or soiled in blood, into a biohazard bag, placed in a small carton.
- Place the carton in a second, durable bag (secondary container); seal or tie each bag
- When the biological waste container is 3/4 full, seal and transport the waste to the waste depository of the laboratory.
- Transport the bag containing biomedical wastes from laboratories into the incinerator, located in the hospital and burn the waste, using kerosene.

❖ **Handling and disposal of sharps**

- Since sharps can easily cut or puncture skin or plastic bags, they need to be disposed into puncture proof containers**.**
- Sharps should be segregated at source by discarding into a sharps container containing 10% hypochlorite. The container should be puncture and leak-proof plastic boxes with a narrow entry.
- Leave them overnight in 10% hypochlorite.
- The following day, drain the 10% hypochlorite from the sharps container into the sink.
- Empty the contents carefully into a biohazard bag, and then into a carton.
- Wash and re use the sharps container
- Label the carton as “Sharps”
- Once ¾ full, place the carton in a durable bag (secondary container); seal the bag and dispose in an incinerator as described above.

PART 4

MANAGEMENT OF STUDY MATERIAL AND TERMINATION OF STUDY

The proper management of study material is a major challenge in studies. Samples collected will have to be properly stored and transported for analysis. Adequate and conducive means of transport should be put in place for each sample type to be transported. Another major challenge is the management of left over material. All these are needed when drafting the report. In this part, 3 SOPs have been developed to show how these samples and study material will be stored, and how they will be transported for laboratory analysis. They are entitled:

- Storing of filter paper containing Dried Blood Spots
- Management of data collection sheets
- Validation and transportation of study left-over material

LAPHEB

Standard Operating Procedure N^{0}017

Storing of filter paper containing Dried Blood Spots

Date of issue of this version: 24/03/2021
Prepared by: Carine Nguefeu Nkenfou Tchinda
Version No: 1
Proofread by: Prof Babara Atogho Tiedeu and Dr Jean Paul Chedjou
Validated by: Prof Wilfred Mbacham Date of effect: 24/03/2021

1. **Objective:** To ensure all DBS filter paper are properly conserved
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Silica gels, A5 envelopes, transparent zip lock bags, sterile gloves
4. **Place of Work:** Laboratory
5. **Procedure:**

- Wear a pair of sterile gloves. Ensure all filter paper are appropriately labelled.
- By holding the free and empty end of the filter paper, gently place each filter paper in a separate A5 envelope, remembering always **NOT** to touch the blood containing end.
- Add one silica gel in each of the envelopes with a filter paper.
- Label the envelope with its corresponding DBS filter paper code
- Assemble the labelled envelopes and place in a transparent zip lock bag. Ensure the zip lock bag contains 5 to 7 silica gels in it.

Store the zip lock bags at room temperature in a dry draw.

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Standard Operating Procedure N^{0}018

Management of data collection sheets

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1. **Objective:** To ensure the confidentiality of personal information of study participants and maintain the integrity of the data collection sheets-DCSs.
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** DCSs, study coordinator, book files/carton boxes, cupboard
4. **Place of Work:** Archive room
5. **Procedure:**

- ❖ Ensure that all forms are properly filled and up to date.
- ❖ Classify the forms in a chronological manner as per the study codes.
- ❖ Insert the filled forms in book files or carton boxes to prevent any possible damage from natural hazards (water, dust, insects, rodents etc)
- ❖ Label the files/boxes appropriately-study title, starting date, number of forms
- ❖ Place these files/boxes in a fire-proof cupboard and lock with a key.

Ensure the room where the cupboard is found is always locked when unattended.

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Standard Operating Procedure N^{0}019

Validation and transportation of study left-over material

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1. **Objective:** To validate all study and left-over material and transport for analysis
2. **Scope:** Applicable to all Pharmacogenetic study
3. **Requirements:** study checklist, human resources, transport costs (fuel money, bus, train or air tickets), lodging and feeding of study team
4. **Place of Work:** recruitment site, Traveling agency
5. **Procedure:**

- use the checklist to validate item by item, all the assembled study material by each study site
- Counter validate by a second person for accuracy.
- Seal the material in their respective boxes and prepare for transportation (car, bus or air).

PART 5

DATA MANAGEMENT

Data collection during recruitment is a pivotal step in every study. Data collected during each study has to be entered in a statistical software and analysed. This analysis is essential in affirming or rejecting the research hypothesis. This part entails 2 SOPs which are data entry and validation, and data analysis.

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Standard Operating Procedure N^{0}020

SOP 020-Data entry and validation

Date of issue of this version: 24/03/2021
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1. **Objective:** To enter all relevant data from the data collection forms or sheets into a database
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Computer, power supply, internet modem, Microsoft Access/Excel, DCSs
4. **Place of Work:** Office
5. **Procedure:**

- Using Microsoft Access or Excel, create a database of all relevant information from the DCS (sociodemographic, clinical and biological data)
- Enter information from each DCS into the database in a chronological manner.
- Save guard the database by giving it a name such as "DATA PHARMACOGENETIC STUDY"
- Store the database in a folder.

LAPHEB

Standard Operating Procedure N^{0}021

Data analysis

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1. **Objective:** To analyse entered data, using statistical methods
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Computer, Microsoft Access/Excel, SPSS/Epi Info software
4. **Place of Work:** Office
5. **Procedure:**

- Open the database stored in Microsoft Access or Excel. Transport the database into an SPSS/Epi Info software. Set statistical significance at **0.05** for all tests.
- For frequencies of the CYP2B6 516G>T and 983T>C genotypes and phenotypes in the study population use descriptive statistics
- For the association between the genotype/phenotype of the CYP2B6 516G>T and 983T>C and adverse drug reactions associated to EFV in the study population use Chi Square test.
- Odds ratio (OR) for likelihood of developing adverse drug reactions with 95% confidence intervals.

Store all results in a Microsoft Word document.

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Standard Operating Procedure N^{0}022

Cost estimation for the genotyping of CYP2B6 516G>T and 983T>C SNPs of 100 samples

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1. **Objective:** To estimate the cost for the genotyping of CYP2B6 516G>T and 983T>C SNPs of 100 samples
2. **Scope:** Applicable to all pharmacogenetic studies
3. **Requirements:** Computer, Internet connection, calculator
4. **Place of Work:** Office
5. **Procedure:**

- ❖ Write down all the different steps the samples will go trough
- ❖ Write down all the requirements for different steps and estimate needed quantities
- ❖ Use the internet to search all the prices for each reagent
- ❖ Use a calculator to have the cost for each step then make addition to have the total price

Step 1: Blood samples collection

Requirements	**Availability**	**Unit cost (FCFA)**	**N0 of units**	**Total (FCFA)**
5mL EDTA tubes	100 tubes/ box	10.000	1	10.000
5mL syringes	100 syringes/ box	7.000	1	7.000
Cotton	350g	500	2	1.000
Alcohol	1L	3.000	2	6.000
Gloves	50 pairs/ box	4.000	2	8.000
Small size envelops	50/ box	1.000	2	2.000
Bleach	1L	1.000	1	1.000
Whatman (903)Filter paper	100 cards/ box	59.000	1	59.000
Communication credit	200 FCFA/ patient	200	100 patients	20.000
Total	**114.000 FCFA**			

Step 2: DNA extraction

Requirements	Availability	Unit cost (Euro)	N0 of units	Total (Euro)	Total (FCFA)
PBS	100 tablets pack	97	1	97	63.050
Saponin	50g pack	80	1	80	52.000
20% Chelex	25g pack	73	1	73	47.450
Nuclease free water	500mL bottle	77	1	77	50.050
Gloves	50 pairs/ box	6	4	24	15.600
1.5mL Eppendorf microfuge tubes	500 tubes pack	34	1	68	44.000
100-1000µL pipette tips	1000 tips pack	20	1	20	13.000
10-100µL pipette tips	960 tips pack	14.85	1	14.85	9.650
0.1-10µL pipette tips	960 tips pack	96.50	1	96.50	62.725
Total	**357.425 FCFA**				

Step 3: DNA amplification

Requirements	Availability	Unit cost (Euro)	N0 of units	Total (Euro)	Total (FCFA)
PCR tubes	1000 tubes/box	70	1	70	45.500
Taq polymerase	1000 reactions (5U/ µL)	387	1	387	251.550
dATP	250µL vial (100 mM)	116	1	116	75.400
dCTP	250µL vial (100 mM)	116	1	116	75.400
dGTP	250µL vial (100 mM)	116	1	116	75.400
dTTP	250µL vial (100 mM)	116	1	116	75.400
983T>C primers	2 mM vial	26	1	26	16.900
516G>T Primers	2 mM vial	20	1	20	13.000
Termopol buffer	6mL vial (10X)	22	1	22	14.300
Total	**642.850 FCFA**				

Step 4: Digestion with enzymes

Requirements	Availability	Unit cost (Euro)	N0 of units	Total (Euro)	Total (FCFA)
BSmA I	1.000Units	70	1	70	45.500
BSr I	1.000Units	71	1	71	46.150
Total	**91.650 FCFA**				

Step 5: Gel electrophoresis

Requirements	Availability	Unit cost (Euro)	N0 of units	Total (Euro)	Total (FCFA)
Agarose gel	25g pack	242	1	242	157.300
Ethidium bromide	1g pack	83	1	83	53.950
DNA MWM	50µg pack	274	1	274	178.100
Loading buffer	5ml vial	20	1	20	13.000
Parafilm	1ea (each assy)	46	1	46	29.900
Total	**432.250 FCFA**				

THE TOTAL COST FOR ALL THE STEPS = 1.638.175 FCFA

APPENDIX 10: PUBLICATIONS FROM THESIS

1. **Carine Nguefeu Nkenfou**, Barbara Atogho Tiedeu, Celine Nguefeu Nkenfou, Akindeh M. Nji, Jean Paul Chedjou, Calvino Tah Fomboh, Charles Kouanfack, Wilfred Fon Mbacham. Adverse drug reactions associated to CYP 2B6 polymorphisms in HIV/AIDS-treated patients in Yaoundé, Cameroon. *The Application of Clinical Genetics 12; 261-268 (2019)*

2. **Carine Nguefeu Nkenfou-Tchinda**, Barbara Atogho Tiedeu, Celine Nguefeu Nkenfou, Akindeh M. Nji, Aristid Ekollo Mbange, Jean Paul Chedjou, Calvino Tah Fomboh, Charles Kouanfack, Wilfred Fon Mbacham. ‘’Adverse Drug Reactions and associated factors among adult HIV-positive patients taking art at the Yaoundé Central Hospital, Cameroon’’. *Journal of Applied Pharmaceutical Science 10(11); 67-73(2020)*