Biomarker and Safety Study of Clozapine in Patients with Benign Ethnic Neutropenia (BEN)

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Study Protocol

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Introduction:
Current monitoring guidelines for treatment with the medication clozapine have cut off values for white blood cell (WBC) counts that were based on Caucasian populations and do not take into account lower white blood cell counts and lower fluctuations seen in people of African descent. This leads to many people of African descent not receiving appropriate care with clozapine. This study will examine clozapine safety in patients of African descent with a history of a low absolute neutrophil counts (ANC), known as Benign Ethnic Neutropenia (BEN). Normal patterns of week-to-week fluctuations in ANC levels in individuals of African ancestry with BEN are not known, and no published research has examined variation in genes in African ancestry clozapine-treated patients with BEN. Conducting such research will generate genetic marker and safety data that could be used to expand access to clozapine for African American patients who otherwise are eligible to receive this superior treatment option.

We have recently been funded by NIMH to study clozapine in this population. We are working closely with the Food and Drug Administration (FDA) and have obtained an Investigational New Drug (IND) approval for the project.

This protocol outlines the details for an open label prospective study of six months of open label clozapine treatment that follows a six week period of WBC count monitoring to establish baseline values. It also contains the modified monitoring guidelines that will be used in BEN patients throughout the study. MPRC will be the coordinating site and will be the initial site to be IRB approved and running. Subsequently, we will also include Howard University and the Federal Neuropsychiatric Hospital Yaba, Lagos, Nigeria.

Background:
Schizophrenia is a chronic and disabling medical illness that starts early in life and results in loss of productivity and poor quality of life. Symptoms in 40-60% of individuals with schizophrenia do not respond to currently available medications. In this subgroup of treatment-refractory patients, clozapine is the only medication with demonstrated effectiveness in controlling symptoms, preventing multiple hospitalizations, and enabling community integration. Furthermore, clozapine is the only medication with an FDA-approved indication for suicidal ideation. The estimated savings in healthcare costs are $9,000/patient/year for patients on clozapine for two years, and $23,000/patient/year for patients treated with clozapine for over 2 years. The medication is affordable, readily available, and safe following FDA guidelines based on normative values of absolute neutrophil counts (ANCs) in European-ancestry populations.

In African ancestry populations, a deletion mutation of the Duffy Antigen Receptor for Chemokines gene (*DARC*-null genotype), which abolishes expression of DARC protein, has been positively selected for its protective role against malarial infection (*Plasmodium vivax*).

This *DARC* null mutation has been recently identified as a major determinant of benign ethnic neutropenia (BEN) in people of African descent, including African-Americans. BEN, defined as the occurrence of neutropenia based on normative values of ANCs in Caucasian populations, is not associated with increased susceptibility to infection or agranulocytosis.

In the U.S, compared with European-Americans, African-American patients are less likely to be initiated on clozapine and their clozapine treatment is twice as likely to be abruptly discontinued for ANC counts that fall below the normative values in Caucasians. BEN is the likely primary determinant of this barrier to clozapine treatment in African-American patients. However, the relationship between *DARC* genotype, ANCs, and occurrence of clinically significant fluctuations in ANC during clozapine treatment in African-ancestry schizophrenia patients is unknown. Pilot data from our group at MPRC and records from over 650 clozapine-treated sub-Saharan African patients at the Federal Neuropsychiatric Hospital Lagos, Nigeria clearly show no increased incidence of agranulocytosis in this ethnic population compared with European ancestry populations.

STUDY OBJECTIVES AND HYPOTHESES:

New knowledge is needed to reduce this healthcare disparity and care gap for African-American patients who would otherwise benefit from clozapine treatment. To generate this knowledge, the objectives of the study are:

Aim 1: To study and understand the normal pattern of white blood cell fluctuations in BEN patients. We will study 250, mostly African ancestry, patients to determine the fluctuations of within-participant WBC and ANC (mean, S.D., frequency and duration of episodes of mild, moderate or severe neutropenia), measured weekly over a period of (up to) 6 weeks

> *Hypothesis:*
> *BEN patients will have lower mean ANC and WBC than normal values reported in the literature. The normal variation in ANC and WBC will create sporadic episodes with ANC below guidelines in BEN patients.*

Aim 2: To test to see if people with BEN have a genetic risk that may put them at risk of having a naturally lower WBC count than people who are White. We will establish ANC and WBC patterns in both those with and without the *DARC* allele.

We will also genotype a panel of variants in the human leukocyte antigen (*HLA*) complex gene recently shown to be associated with agranulocytosis in Caucasian patients, to explore the prevalence of co-occurrence of these HLA variants and the *DARC* null genotype in BEN patients, which might increase risk of agranulocytosis in AA and SSA participants with BEN. We anticipate that the high risk HLA variants will be in very low frequency in the AA and SSA population

> *Hypothesis: Patients with the DARC null allele genotype will have lower ANC and WBC than people without the DARC null allele genotype.*

Aim 3: To test the safety of clozapine treatment in people with BEN. We also will study the normal fluctuations of WBCs and ANCs during 6 months of clozapine treatment. We will study the risk of potential episodes of agranulocytosis or serious infection. We will develop an upper 95% confidence interval for risk of agranulocytosis (< 500 cells/mm^3), which may be compared to the known 1% agranulocytosis risk in European ancestry populations treated with clozapine. We will also estimate the risk of serious infection in clozapine-treated BEN patients.

Hypothesis 1 (DARC null allele patients):
The 6-month risk of agranulocytosis in clozapine-treated BEN patients will not differ from reported rates in the literature. In the DARC null allele patients, normal variation in ANCs and WBCs will create sporadic episodes with ANCs below usual U.S. monitoring guidelines (<1500 cells/mm^3), but not higher rates of agranulocytosis or severe infection. These patients will have lower mean ANC and WBC than patients without the polymorphism, and not have higher rates of agranulocytosis.

Hypothesis 2: (non DARC null allele patients)
BEN patients without the polymorphism will have higher mean ANC than the group with the genetic polymorphism (DARC null alleles), and will be less likely to have episodes with ANCs below usual U.S. monitoring guidelines (<1500 cells/mm^3). We will evaluate any identifiable causes (e.g., infection) of low ANC in such participants

STUDY PARTICIPANTS:

Inclusion Criteria
Eligible and recommended for clozapine treatment (e.g. treatment resistant schizophrenia, schizoaffective disorder, bipolar disorder, other psychotic disorder, delusional disorder, hostility, other documented rationale)
Male or Female
African Ancestry (African, African-American or African-Caribbean). This population will make up the majority of the study. However, there are cases of BEN in individuals of Middle Eastern, Caucasian, and other ethnicity. We will accept these patients as they may have unknown African ancestry and genotyping will be important. Caucasian subjects will only be recruited if they have a history of an absolute neutrophil count (ANC) <2500 cells/mm^3 in past 24 months
Age: 18 to 64 years.
Documented ability to sign informed consent. This is a score of ≥10/12 on ESC.
Effective birth control if of child bearing potential

Exclusion Criteria
Pregnancy or lactation
History of myeloproliferative disorder
Uncontrolled seizure disorder
History of paralytic ileus
History of 3 or more successive ANC values below 500 cells/mm^3

Systemic Lupus Erythematosus, Multiple Sclerosis, Hashimoto's Thyroiditis, Sjogren's Syndrome, Grave's Disease*
Medical condition whose pathology or treatment would likely alter the presentation or treatment of schizophrenia or significantly increase the risks associated with the proposed protocol.
Medical condition affecting patient's ability to mount an immune response
Current symptomatic bacterial or viral infection*
Sickle cell anemia
Temperature > 38.0 º Celsius, 100.4 º Fahrenheit*
Current treated or untreated cancer*
Documented nutritional deficiencies (such as Beriberi, Pellagra, Rickets, Scurvy, Keshan Disease)*

*exclusion criteria for giving clozapine to neutropenic patients are similar to exclusion criteria for giving chemotherapy to BEN patients.

Smokers are permitted. All sites will pay careful attention to hospital admissions in smokers to ensure that the change in smoking status does not affect clozapine blood levels. Smoking is permitted at the hospital sites and blood levels should be drawn at the time of inpatient/outpatient change. This is just an extra precaution to ensure that changes in the amount and routine of smoking do not have large effects on clozapine optimization in these patients.

STUDY DESIGN AND METHODS:

- This is a single treatment group, open-label, prospective study. This study will take place at three sites; the Maryland Psychiatric Research Center (MPRC), University of Maryland School of Medicine, Howard University, and the Federal Neuropsychiatric Hospital Yaba, Lagos, Nigeria (FNPHY). The study will begin at the MPRC site and the other sites will come on board only when the studies and consents are fully approved at each site. We will notify the UMB IRB when each site is fully functioning.

- MPRC will be the lead site for regulatory compliance, coordination, design, leadership, data storage and analysis. The Nigerian site and Howard University both are well established research sites with ongoing NIH research and an enriched environment for clinical research. Patients will be enrolled for 9 months duration from a cohort of 250 African ancestry (African-American or Nigerian) subjects who have had evidence of neutropenia (ANC<2500 cells/mm^3) in the past 24 months and are otherwise clozapine eligible.

- We will enroll a target of approximately 80 at MPRC, approximately 130 in Nigeria and approximately 40 at Howard University with a range of 70-90 at MPRC if needed, a range of 130-150 if needed in Nigeria and 40-50 at Howard if needed.

- All participants will be recommended to undergo 6 weeks of weekly monitoring for ANC and WBC fluctuations and clinical evaluations. This may be fewer if the PI or treatment team feel waiting to begin clozapine would not be in the best interest of the patient. At least one week of observation is needed. This could also go longer if a patient is enrolled and clozapine start is delayed for a short period. After this period, subjects still meeting eligibility criteria will be treated for 6 months with open label clozapine with weekly WBC/ANC

monitoring. We will minimize the burden of assessments to focus on safety and retention and in order to simulate treatment as usual in a naturalistic design.

- Assessments: A full schedule of events is included below. At baseline, all participants will have a standard medical workup including a physical examination, Body Mass Index (BMI) measurement, and EKG (In USA only). Blood will be collected for complete blood count (CBC), Chemistry 14 panel with fasting blood glucose, lipid panel, HIV, thyroid panel, and liver function tests. Additional blood smear analyses for malaria parasites will be performed at FNPHY. A description of the time points for all of the assessments and laboratories are included in the schedule of events.

SCHEDULE OF EVENTS	Visit 1 Screening	Visit 2-5 (Pre-clozapine)	Visit 6 (Week prior to Clozapine start	Visit 7-30
Medical and Psychological evaluation	X			
Demographic information	X			
Inclusion/Exclusion criteria documentation	X			
SCID and DDF form	X			
Smoking status, Fagerstrom Test for Nicotine Dependence (can be done at visit 1 or 2-5)		X		
Blood draw for genotyping (one for storage and one for named genes)	X			
Cytokines (US only)		X		X (2 times)Visit 18 and 30)
Documentation of previous ANCs	X			
Consent and patient monitoring form	X			
HIV testing	X			
Concomitant medication list		X (weekly)	X	X (weekly)
ANC signoff each week		X (weekly)	X	X (weekly)
Blood smear for malarial parasites (Nigerian site)	X	X (every 2 weeks)	X	X (every 2 weeks)
Education/reminder on reporting infection	X	X	X	X (weekly)
Medical record review	X			

CBC with differential	X	X (weekly)	X*	X (visit 7-10 and 30)*
WBC/ANC finger stick**			X	X (weekly) until week 4 than optional thereafter
Chemistry 14 including lipids, liver enzymes, thyroid function, FBG, electrolytes	X		X	X (monthly) Visits 10,14,18,22,26,30)
Vital Signs (heart rate, BP, temperature, and respiratory rate)	X	X (weekly)	X	X (weekly) (Twice during week 7 and then weekly)
BMI assessment	X		X	X (monthly) Visits 10,14,18,22,26,30)
EKG (USA only)	X		X	
BPRS, CGI, CDS (when possible 2 x pre clozapine start)		X	X	X (monthly) Visits 10,14,18,22,26,30)
Pregnancy test (Females)	X		X	X (monthly) Visits 10,14,18,22,26,30)
Side Effect Checklist	X		X (2 x this week)	X (weekly)
Questionnaire on clozapine blood draw preferences			X (or earlier)	X (week 2 and 4)
SAS, BAS, AIMS	X		X	X (monthly) Visits 10,14,18,22,26,30)
Clozapine Serum Level (venous and capillary) ***				X (2x for first month, monthly thereafter)
Retrospective Chart review				X

*this will change according to participant and monitoring parameters if ANC <1200 cells/mm^3

** WBC/ANC capillary lab results (3 part differential) will be drawn each week and will superscede full CBC (5 part differential) after 4 weeks in some instances. CBC can also be drawn when PI or physician request.Weekly monitoring of either venous or capillary will be required weekly throughout the study.

*** capillary blood draw for clozapine level is optional at the MPRC site and performed three times, once at baseline, once at approximately week 4 and once at approximately week 8. This is a prick of the finger with maximum of 3 ml for each stick. This is separate from fingerstick for ANCs that are only a few drops of blood

LABORATORY MONITORING:

White Blood Cell (WBC) and Absolute Neutrophil Counts (ANC):

- All WBC with 3 to 5 part differential (by capillary and/or venous, respectively) will be obtained at the same time at each drawing when possible (approximately 9am to 12 pm) to avoid the confound of morning pseudoneutropenia and will be performed weekly for 6 weeks prior to clozapine and 6 months after clozapine. In participants who discontinue clozapine during the trial, weekly WBC/ANC measurements will be performed for an additional 4 weeks. The WBC and ANC at the two US sites will be performed by Lab Corp, which is fully licensed by the State of Maryland. The Laboratories Unit of the Maryland Department of Health and Mental Hygiene licenses laboratories and is responsible for ensuring federal certification of all laboratories located in Maryland, including federal certification in the Clinical Laboratory Improvement Amendments of 1988 Program, required for all clinical laboratory testing sites used in analysis of samples from US patients. WBC and ANC counts are calculated by automated cell counter by Lab Corp or by using The Athelas One lab using the Automated Haematology Analyzer .
- WBC and ANC at the Nigeria site will be performed in the well-equipped FNPHY Medical Laboratory using the Beckman Coulter (3 to 5 part differential) for automated counts with use of a microscope for manual WBC backup, under the direct oversight of Mrs. Adeola Adebayo, Head Medical Lab Technician. This laboratory routinely performs CBCs, is registered by the Medical Laboratory Sciences Council of Nigeria, the body for registration of diagnostic labs in Nigeria, and will abide by all requirements that are indicated when analyzing samples from Nigerian patients.
- WBC and ANC cutoff and reference criteria used for safety monitoring for clozapine are standardized in this protocol, so using different laboratories for these measurements are not of concern.
- If morning neutropenia occurs the afternoon (12-4pm) draw time is acceptable. The draw time will be documented.

Clozapine Blood Levels:

Venous blood samples will be drawn to measure clozapine levels once during the titration phase (Day 14), or more often if needed during titration for clinical purposes, monthly during the 6-month clozapine treatment phase, and at the conclusion of the study. Levels will be obtained prior to the administration of the morning dose of clozapine. Samples will be analyzed using High Performance Liquid Chromatography (HPLC). Clozapine levels (clozapine, norclozapine) are sent out to LabCorp at the MPRC and Howard University site. At the MPRC these may also be sent to Saladax Biomedical, Inc, Bethlehem PA for immunoassay. HPLC equipment is budgeted for the Nigerian site so that all laboratory tests are done in the same lab to ensure standardization and QC. The purpose of the blood levels are 1) to ensure clozapine is being taken and 2) examine relationship of clozapine levels to ANC levels. Lab values that are considered to be abnormal may be repeated to verify accuracy. A corresponding capillary draw will occur at baseline, approximately week 4 and 8 to confirm it matches venous blood levels. This is optional and only offered at MPRC site with capillary tubes.

Other Laboratory and Metabolic Monitoring:

A chemistry panel (liver enzymes, BUN, creatinine, electrolytes, thyroid), and metabolic profile (lipids, fasting blood glucose) will be drawn at baseline and repeated monthly. All laboratory values outside normal ranges will be discussed and addressed with the medically accountable physician and clinical team at each site. A determination of

appropriate diagnostic procedures and/or follow up treatment will be made by the medically accountable physician. Lab values that are considered to be abnormal may be repeated to verify accuracy. We also will be collecting existing lab values if possible and will request only with patient permission for previous records. Cytokines labs will be drawn prior to the start of clozapine, and at the middle and endpoint of the clozapine trial.

INFECTION MONITORING:

- Body temperature will be taken at each visit. Since clozapine treatment can raise the basal body temperature by up to 1 degree Fahrenheit, a slight increase in body temperature from pre-treatment baseline would not necessarily be indicative of infection. Fever is generally defined as an increase in body temperature to over 38.0 º Celsius using a tympanic thermometer or to over 37.5 º Celsius using an axillary thermometer. Fever is usually accompanied by sickness behavior, which may consist of lethargy, anorexia, sleepiness, hyperalgesia, and inability to concentrate. The medically accountable physician will obtain a history, conduct a review of systems, and perform a physical examination on any participant that develops a fever during the study. Because infection in neutropenic patients can proceed rapidly, a close initial evaluation is necessary and the medically accountable physician will promptly evaluate the seriousness and origin of the infection. As part of the fever workup, laboratory and radiological tests will be performed as deemed necessary.
- At each visit, participants will be asked about symptoms such as sore throat, mouth ulcers, flu-like symptoms, chills, rigors, arthralgia, or any other symptoms of infection. Participants will be instructed to contact the study physician immediately, if they develop any of these symptoms.
- A determination on study discontinuation, antiviral, antibiotic, antifungal, or, in Nigeria, antimalarial treatment will be made with the physician and infectious disease consultant as needed. The same criteria apply to all three sites. We will also pay special attention to clozapine levels and order a blood level during an infection as clozapine blood levels can rise.

SYMPTOM MONITORING:

- Psychiatric symptoms will be monitored at 2 times pretreatment to establish a baseline (approximately 6 weeks prior to clozapine start, and 1 week prior to clozapine start), and every four weeks thereafter during the six months of clozapine treatment.
- Decisions regarding response to clozapine and continuation of clozapine treatment will occur in collaboration with the treatment team.
- The following guidelines will be generally observed and recommended based on current literature: Eight weeks is often needed for complete response and few patients demonstrate additional response after this time period. Study discontinuation from clozapine will be recommended if significant clinical worsening occurs. The treating physician will help make the decision.
    - Either the treating psychiatrist, medically accountable physician, or the PI may determine that a patient is experiencing clinical worsening. Clinical worsening may be defined as the following: the subject is judged to be entering an exacerbation of his/her illness by the treating clinician; an increase of 3 or more points from baseline on a BPRS positive symptom item; an increase of 3 or more points from baseline on BPRS somatic concern, conceptual disorganization or hostility; or an increase of 2 or more points from baseline on the CGI global severity rating or in

the case the CGI is at a ceiling for beginning, clinical judgment will be made. Transient rating changes do occur so the symptom increases must be accompanied by the clinician's feelings that they are entering exacerbation.

- Clozapine discontinuation may take place in the case of a significant side effect, or if there is no adequate symptom response to clozapine and discontinuation is thought to be a better option. Response is defined as 20% improvement and a final score of ≤ 35 in total BPRS score or CGI ≤ 3. These scores generally result in a response rate of approximately 68% with another 25% demonstrating partial response. Partial response is defined as < 20% improvement on total BPRS with two clinicians agreeing that there is notable improvement not evident on BPRS. We anticipate that 7% of patients will not achieve any response and may be discontinued at 8 weeks. Since achieving response after 8 weeks is not likely and the risks may be higher, symptoms will be monitored monthly to determine whether clozapine should be continued.
- Once a participant is discontinued from clozapine, for whatever reason, they may remain in the study for continued monitoring. When any participant is discontinued from the study, completion ratings and assessments (including laboratory tests and rating scales) will be performed if possible. If a patient is prematurely terminated from the study, the investigators will work with the clinical team to ensure the patient will continue clozapine or resume other antipsychotic treatments for their symptoms. More information on discontinuation is later in the protocol in the withdrawal criteria section.

Brief Psychiatric Rating Scale (BPRS): Total symptoms will be measured using the total score on the 18 item version. Psychotic symptoms will be calculated using the conceptual disorganization, hallucinations, suspiciousness and unusual thought (delusion) items.
Clinical Global Impression Scale (CGI): The CGI severity of illness item will be used to assess global changes in symptomatology.
Calgary Depression Rating Scale (CDS): This 9-item scale is designed specifically to assess depressive symptoms in patients with schizophrenia. The CDS has been shown to be reliable and have good construct validity. The CDS total score will be used to measure depressive symptoms and assess suicidal ideation and actions. Suicidality may be decreased with clozapine, however we will monitor the suicide item for changes during the study.
Retrospective chart review will be conducted to compliment and confirms the data collected by in person interviews already completed

SIDE EFFECT MONITORING

All side effect scales will be performed monthly with exception of Side Effect Checklist, which will be done at each visit.
Simpson-Angus Extrapyramidal Symptom Rating Scale (SAS): The modified 11-item version of the SAS will be used to assess EPS.
Abnormal Involuntary Movement Scale (AIMS): This is a 12-item scale, with 7 items designed to assess abnormal facial, oral, extremity, and trunk movements; 3 global judgment items; and 2 current dental status items.
Barnes Akathisia Scale (BAS): This is a 4-item scale designed to assess objective and subjective components of akathisia.

Side Effect Checklist (SEC): This scale is designed to assess vital signs and directly inquire about 25 commonly occurring antipsychotic side effects. Specifically, it covers side effects seen with clozapine, side effects that relate to infection (i.e., sore throat, gum soreness, cold, pneumonia) and other common side effects. We will provide anticonstipation medications and other side effect medications as needed.

GENOTYPING INFORMATION:

- Single-Nucleotide Polymorphism (SNP) genotyping will be performed on gDNA isolated from blood or saliva with QIAamp DNA Maxi Kit (Qiagen) using TaqMan polymerase chain reaction (PCR) technology.
- We will use a validated commercially available 5'->3' exonuclease assay; Assay IDs; (C__15769614_10) and (C__2493442_10) for determining the genotype at the Duffy variant (rs2814778) and missense mutation (rs12075) of *DARC* gene.
- We will use the context sequence of genomic regions for the SNPs extracted from the GWAS study of clozapine-induced agranulocytosis to design forward and reverse primers and design customized assays for genotyping the SNPs that met genome wide significance for association with clozapine-induced agranulocytosis in the CIAG study. Customized genotyping assays will be used to genotype SNPs, rs41549217 and rs13073300, for *HLA-B* and *HHLA2* genes, respectively, and SNPs rs143211074 and rs28362679 of *BTNL2* gene. All PCR will be performed in Dr. Wonodi's Biogenetics Lab, on standard 96-well reaction plates, as previously described. For each PCR reaction, 2 ng of genomic DNA will be used in a 5-µl reaction mixture (1.3 mM MgCl2, 200 µM dNTP mix, 0.25 µM of each primer, 5% DMSO, 1.5 U Taq polymerase). The PCR program includes: denaturation of DNA (5 min at 94°C), 30 cycles of 30s at 94°C, 30s at 62°C, and 30s at 72°C. This will be followed by a final extension of 10 min at 72°C.
- All PCR reactions will be performed using the Biorad Multiplatform Thermocycler, which has 4 blocks of 384 wells (Bio-Rad Laboratories Inc., Hercules, CA, USA). PCR results will be read by reporter dye fluorescence (Vic/Fam) and allelic discrimination analysis with 7900 Sequence Detection Systems (SDS) software v2.3 (Applied Biosystems) to assign genotype groups to the SNPs. The TaqMan genotyping assay is quick and accurate and has worked well for our research team on this project.
- The DNA will be shipped from Nigeria to the MPRC using either Oragene DNA kits or Whatman FTA Elute Micro Cards. DNA is relatively stable and currently DNA is being shipped on other collaborative grants without problems.

CLOZAPINE DOSING:

- Following consent and prior to initiation of clozapine, eligible patients will have weekly WBC/ANC. During this period, patients will be registered with the clozapine manufacturer and other medications will be appropriately titrated. Patients may be inpatients or outpatients.
- Clozapine will be recommended to be prescribed twice daily (other regimens permitted). The dose will be titrated within a range of 200-900 mg daily. We *do permit* individualized dosing, as occurrence of neutropenia and agranulocytosis is not clozapine dose-related. See recommended titration schedule below. If a patient has not received clozapine for two days or more he/she will need to have their clozapine initial dose be reinstated.

Recommended Clozapine Titration Schedule (3 week)

This is only a recommended titration schedule. This can be modified if necessary.

Day 1:	12.5 mg BID (1/2 of the 25 mg tablet)	
Day 2:	25 mg AM, 0mg HS	
Day 3:	25 mg AM, 25 mg HS	Extra visit, vitals, side effect and safety check
Day 4:	25 mg AM, 50 mg HS	
Day 5:	50 mg AM, 50 mg HS	
Day 6:	50 mg AM, 75mg HS	
Day 7:	50mg AM, 100 mg HS	Visit, vitals, side effect and safety, WBC
Day 8:	50mg AM, 100mg HS	
Day 9:	100 mg AM, 100 mg HS	
Day 10:	100 mg AM, 100 mg HS	
Day 11:	50 mg AM, 200 mg HS	
Day 12:	50 mg AM, 200 mg HS	
Day 13:	100 mg AM, 200 mg HS	
Day 14:	100 mg AM, 200 mg HS	clozapine blood level will be drawn, visit, vitals, side effect and safety, WBC
Day 15:	125 mg AM, 200 mg HS	
Day 16:	125 mg AM, 200 mg HS	
Day 17:	150 mg AM, 200 mg HS	
Day 18:	150 mg AM, 200 mg HS	
Day 19:	175 mg AM, 200 mg HS	
Day 20:	175 mg AM, 200 mg HS	
Day 21:	200 mg AM, 200 mg HS	Visit, vitals, side effect and safety, WBC

The white blood cell count level is not related to the dose of the medication. The dose throughout the study will not be altered based on white blood cell counts, but if the white blood cell counts fall lower than the established monitoring parameters for each patient the patient may be discontinued from treatment.

MONITORING GUIDELINES:

Participants enrolled in the study at all 3 sites will receive the modified guidelines (table below) in addition to their informed consent and the investigator and patient will sign the consent acknowledging the modified guidelines. More stringent monitoring may take place if ANC levels fluctuate and modifications from this guideline may occur on a case by case basis with the consultation of a physician, hematologist and PI. During the study subjects will be very closely monitored as per below. Subjects will be educated concerning signs and symptoms of infection and the need to immediately report these throughout the study.

The Food and Drug Administration (FDA) has approved the modified monitoring procedures under an IND:

Absolute Neutrophil Count (ANC) Value	**Modified Monitoring Plan Recommendation for Patients with History of or Current Benign Ethnic Neutropenia (BEN).** **Changes can be made with consultation from hematology. Always consider other medications that may be contributing to ANC suppression.**
Clozapine Initiation	1. All patients initiating clozapine in the study will have had ANC <2500/mm^3 in the past 24 months and those enrolled will not have a history of ANC<500/mm^3. 2. Patients with two baseline ANC levels ≥1000/mm^3 will be initiated with weekly blood draws. *3.* Patients with baseline ANC levels between 500-999/mm^3 may be initiated *a.* After hematology consult and *b.* With specific oversight plan approved by hematology and study team. *c.* If initiated, patients will have ANC drawn twice weekly for first month of treatment prior to be considered for weekly monitoring. If ANC levels fall below 500/mm^3, treatment will be interrupted as per protocol below. d. *Justification for deviation from Risk Evaluation Management System (REMS) recommendations: We recognize that these are more lenient criteria than REMS, however, patients are enrolled in clinical trial with increased monitoring and we will be seeking a hematology consult and oversight prior to initiation and to make a plan for increased monitoring once treatment initiated.*
ANC ≥1000/mm^3	Continue weekly ANC monitoring.
ANC <1000/mm^3 to ≥500/mm^3	1. Continue clozapine treatment 2. Immediate redraw ANC to ensure second ANC value 3. Must contact hematologist at site or consultant hematologist. Provide hematologist with WBC/ANC history and review of medications. **Sophie Lanzkron MD, MHS** **Associate Professor of Medicine and Oncology** **Johns Hopkins School of Medicine** **410-502-8642** **slanzkr@jhmi.edu.** 4. If fever is present, will likely discontinue 5. Twice weekly ANC monitoring until ANC ≥1000/mm^3 or at least patient's known baseline* *a. Justification for deviation from the REMS recommendations: We recognize that these are more lenient criteria than REMS, however, we will be undertaking a hematology consult and closely monitoring patients as per our clinical trial protocol* 6. Once ANC ≥1000/mm^3 or at least patient's known baseline, check ANC weekly
ANC <500/mm^3	1. Redraw immediately 2. Interrupt therapy for suspected clozapine-induced neutropenia 3. If fever, discontinue clozapine

4. Monitor ANC daily until ≥500/mm^3, then twice weekly until ANC reaches patient's established baseline.
5. Must contact hematologist at site or consultant hematologist ASAP for plan of action similar to steps below:
    a. Discontinue treatment is likely and most probable but will notify and consult hematologyand make decision prior to discontinuation for <500/mm^3 and no fever.
    b. Possible Plan of Action with Consultation from Hematology and FDA:
        i. Daily ANC until ANC >500/mm^3
        ii. Twice weekly CBC until ANC >1000/mm^3
        iii. Complete full history looking for signs and symptoms of infection.
        iv. Subjects will continue with daily monitoring for fever.
        v. If temp increases to > 38.5°C, the subject will be directed to the Emergency Department or arranged for admission. If subject remains < 500 /mm^3, they may be admitted and covered with antibiotics for neutropenic fever according to institutional standards. Work-up may include blood cultures, urine cultures and chest x-ray as well as work-up for localizing symptoms if they exist.
        vi. Once ANC increases to >500/mm^3, antibiotics may be discontinued if no source of infection is identified.
        vii. For subjects with recurrent, persistent fever after 48 hours of antibiotics, infectious disease consultation may be requested
        viii. Only with compelling clinical circumstances should you consider starting granulocyte colony stimulating factors. In this case either filgrastim daily at 480 mcg subcutaneously (SQ) daily until ANC is >1000 /mm^3 or a single dose of pegfilgrastim 6 mg SQ. The study nursing staff will arrange for seeing the patient for injections. This will be determined with hematology consult ONLY. These subjects will have daily contact by phone with a research nurse. Growth factor use can be associated with fever so use only if directed by hematology.
        ix. For all subjects whose ANC does not increase above 500 /mm^3 20 days after last dose of clozapine or hematology recommendation, there may be consideration of bone marrow aspirate and biopsy.
6. If a participant falls below 500/mm^3 he/she will likely be discontinued from the study. In this case, the research team will contact the clinical teams to check on the status of the patient and the outcomes of the patient event will be recorded, however the participant will not continue to with study visits and assessments. Only if a participant is seen by the ER, admitted for infection symptoms or receives filgrastim or pegfilgrstim this will be documented as a serious adverse event (SAE) and information sent to all regulatory bodies.
7. If participant is rechallenged with clozapine, resume treatment as a new patient monitoring level once ANC ≥1000/mm^3 or at least patient's known baseline.

Pharmacy and General Overall Guidelines	1. All US participants will be registered the clozapine REMS program prior to initiation of clozapine. 2. US participants will be registered as BEN patients and, if necessary, the REMS program will be apprised of modified guidelines. 3. Clozapine will be dispensed as a seven day supply, so long as the ANC is at least 1000 to coincide with recommended blood draws. For patients whose ANC is > 500/mm^3 and < 1000/mm^3, medications may be dispensed as a three or four day supply based on appointment dates or as indicated below*. 4. If clozapine needs to be discontinued, participants will be contacted by phone or in person and advised to stop medication and come in for a follow-up visit. 5. A centralized research designee (clinical pharmacist) will coordinate with the dispensing pharmacy (if different than centralized) and physician involved with participant to ensure PDA is available where needed (outpatients) or that eligibility criteria is provided (inpatients). The centralized research designee clinical pharmacist shall act as designee for pre-identified prescribers working in the study. Per these guidelines, after physician review and approval, these specialized designees shall enroll patients and enter ANC levels for the study. *a. Justification for this deviation from REMS guide: All treatment decisions will be made by the prescribing psychiatrist after review of ANC and clinical criteria. Our specially trained clinical pharmacist is part of the clinical research team with advanced expertise in treating BEN patients with clozapine so highly qualified to accurately enter this information into the REMS system.*
Other Information	*daily, weekly or twice weekly blood draw and dispensing (particularly in the range of <500-<1000 /mm^3) will be determined based on additional recommendations from the FDA, REMS implementation or based on individual patient parameters. Patients that remain between 500-1000/mm^3 for longer than 4 weeks may be recommended for weekly clozapine blood draws and dispensing. Also going from twice weekly to weekly monitoring should occur after 4 values are back above 1000 cells/mm3 Emergency supply of two doses is given to all patients.

A patient version to give out at time of consent is included at the end of the protocol.

The FDA has given us advice to try less conservative monitoring guidelines after we initially treat participants in the study and gain more experience. The following will be considered for a modification but only used once amended to the IRB:

1. ANC 1000-1499 /mm^3 :monitor weekly for 6 months and then every two weeks for 6 months, then monthly
2. ANC 500-999 /mm^3 :hematology consult, continue clozapine, monitor 3x weekly until ≥ 1000/mm^3 then weekly for weeks then back to baseline monitoring
3. ANC <500/mm^3 :stop clozapine, hematology consult, monitor daily until ≥ 500 /mm^3, then 3x weekly until back to baseline, do not rechallenge unless benefit outweighs risk.

In the US Dr. Sophie Lanzkron from Johns Hopkins University will be the hematologist and consultant hematologist to Nigeria. Dr. Akinbami, the Nigerian consultant hematologist from Lagos University Teaching Hospital (LUTH) will be on the protocol in Nigeria. In the case of

severe Neutropenia, one strategy to increase ANC values is to start lithium treatment, however we will not recommend this strategy.

Concomitant Medications:
Subjects may remain on adjunctive anticholinergic, antidepressant, most mood stabilizers and/or antianxiety agents. Subjects on such medications are included to increase the generalizability of study results and to relate the study more closely to treatment issues addressed in usual clinical practice. Any medication that may lower WBCs such as carbamazepine will not be permitted to be initiated but treatment for 3 months and consultation with hemaogolosit with treatments that may lower ANC.

Subject Recruitment: Subjects will be recruited from three sites. The MPRC will be coordinating the study. Dr. Ikwunga Wonodi, the primary coinvestigator affiliated with MPRC, along with Dr.Lawal from FNPHY, will oversee study procedures and recruitment from the Nigerian site and Dr. Evaristas will be the primary coinvestigator for the Howard University Site. All sites will have approval by an Institutional Review Board (IRB), however UMB will be the central IRB of record. We plan to enroll at least 250 AA subjects with BEN or past possible BEN (past neutropenia) from the three sites, with approximately 130 subjects from Nigeria (easy to recruit with pure *DARC -/-* population), 120 subjects from the MPRC and 40 subjects from Howard University over a 5 year period. This will be approximately 30 per year from Nigeria, 16 per year from MPRC and 8 per year at Howard University, all fewer than the amount of clozapine starts annually at each site.

A partial HIPPA waiver will be obtained to permit the identification of potential participants through chart review. In addition, participants will be referred by the person's treatment team for consideration of study participation. A chart review will be completed for all potential participants to reduce the likelihood that they will be found ineligible after participating in more extensive assessments. The study recruiter will verify with the primary clinician that a potential participant is sufficiently stabilized to consider participation and has capacity to provide consent. This is done prior to the study recruiter approaching a potential participant. The study recruiter will be introduced to the person and provide a brief overview of the project.

Participants may also be recruited from internet based advertising (HP-00061828) that will refer interested participants to the MPRC web site as well as working with clozapine prescribers from the state of Maryland identified through collaboration with the Behavioral Health Administration and Medicaid.

We will also collect data on WBC, ANC and demographic information on people previously treated in Maryland on a waiver system. Their data is available in clinical charts at Spring Grove Hospital and will only collect with approval from the hospital and the Spring Grove Research Committee. These patients will not be eligible to reenroll in this study but the data will be available to add to the study as it will help decrease the final sample size needed to enroll in the study. We will link this data from another study approval where a waiver of informed consent has been granted to study antipsychotic use (HP-00040084).

PRIMARY OUTCOME VARIABLES:

The primary outcomes are to measure ANC levels, side effects and patient safety.

STATISTICAL ANALYSES:

Analysis of Effect of clozapine on WBC and ANC levels: We will characterize variation of WBC and ANC levels in participants by *DARC* null genotype. We anticipate 212+ AA participants with BEN who are homozygous for the *DARC* null allele who will be the focus of this analysis, with other participants with BEN to be examined in a separate analysis. We will compute within-patient means and standard deviations of weekly WBC and ANC by three months intervals, starting with the three-month pre-treatment phase, and continuing for the six -months of follow-up on clozapine treatment, and observe the frequency of ANC values observed that exceed specific levels of concern (e.g. <500, <700, <1000 cells/mm^3). We will estimate the change from baseline in overall average WBC and ANC during each three month interval after initiation of clozapine, and assess whether these change with time using a mixed model for repeated measures ANCOVA, controlling for baseline. Frequency of other events of concern (serious infection, need for treatment for very low ANC) will also be reported. We will also perform analyses of effects of mean participant pre-treatment ANC, clozapine dose and clozapine blood level on mean ANC during clozapine treatment and ANC<500 cells /mm^3 during clozapine treatment, using linear and logistic regression. To date all published work and US guidelines are based on non-BEN samples and not specific to schizophrenia patients.

For the study when we report the results we will evaluate a subgroup of people with a history of ANC < 1500 cells/mm^3

Risk of Agranulocytosis - Analysis and Power: The analysis of this aim will resemble that for an equivalence trial comparing effectiveness of two treatments, only instead we will be comparing safety outcomes between two populations (patients with and without BEN), using an extremely large historic control group rather than newly recruited controls. We will compare estimated risk of agranulocytosis in our study to a large historic cohort study estimating risk of agranulocytosis in non-BEN patients followed according to current FDA guidelines during clozapine treatment. In a report on 11,555 clozapine-treated patients followed for agranulocytosis. There is an incidence rate of 0.9% at one year (upper 95% CI 0.99), with over 80% of cases observed within the first three months, from which we conclude that our planned six-month follow-up on clozapine is adequate to cover the period of highest risk. Some participants will discontinue clozapine prior to conclusion of the six-month treatment phase. In the CATIE trial, 15% of participants assigned to clozapine had discontinued treatment by six months. To adjust for missing data due to dropout, we will use the Kaplan-Meier method for censored survival data to calculate a point estimate of the proportion of cases of agranulocytosis by six months. However, we anticipate few if any cases of agranulocytosis in our cohort, so that usual methods of calculating a CI for the percent without an event at six months which rely on large sample estimators may not work well. Accordingly, we will also use all clozapine-treated cases, ignoring variation in follow-up on clozapine, to calculate a binomial approximation for an upper 95% confidence limit for the risk, r, of agranulocytosis during the first six-months on by solving the equation $0.95= 1 - \Sigma_j\, n!/(j!(n-j)!)\, r^j(1-r)^{n-j}$, where r is probability (risk) of agranulocytosis, n is the total number of participants, k is the number of cases of agranulocytosis, and the summation runs from j=0 to k. If no cases are observed, this reduces to $0.05 =(1-r)^n$; we will use similar a similar formula to calculate the lower confidence limit (LCL); if the LCL does not include the risk level in the historic control cohort, we would reject strict equivalence of risk. With n=212 *DARC* null homozygous participants, zero cases observed would give an upper limit on the risk of 1.4%, approximately 1.4 x the estimated risk of agranulocytosis in non-BEN patients reported in a U.S. cohort of 11,555 patients treated with clozapine. Thus, we will not be able to demonstrate complete equivalence to the risk of

clozapine in non-BEN patients, but can limit the excess risk to a level potentially acceptable to justify gaining further clinical experience with use in BEN patients.

Safety Monitoring: If the risk of agranulocytosis in participants with BEN is much higher than the risk in non-BEN subjects treated with usual monitoring guidelines in the US, then the potential for fatal harm may be unacceptably high. While our best risk estimate will not be obtained until the end of the study, the appearance of any agranulocytosis cases will set a lower limit on the final point estimate of the level of risk. For example, if two agranulocytosis cases are observed in the first 50 participants, the current point estimate for risk of agranulocytosis after the 2nd case would be 2/50 = 4%, and the final risk estimate if the study continued to the full sample size of 250 with no dropouts, the risk cannot be less than 2/250 = 0.8%. We will notify the DSMB monitoring the study whenever a case of agranulocytosis occurs, along with data on fluctuations in WBC/ANC preceding the episode, the current estimate of risk, the upper and lower bound on the ultimate risk estimate if the study continued with no further cases. In practice, we assume some dropouts will occur. Assuming six month follow-up on a final n of 212 participants (15% dropout), after withdrawals for causes other than agranulocytosis, we calculated exact two-sided confidence limits on the risk (probability) of agranulocytosis using the Clopper-Pearson formula, for numbers of cases of agranulocytosis ranging from 1 to 10. The results are shown in Table 1 below

Table 1: Point estimate of agranulocytosis risk (p) and 95% confidence limits for agranulocytosis risk by number of cases observed out of a total of 212 participants.

Cases	p	95% Confidence Interval lcl	ucl
1	0.0047	0.0001	0.0260
2	0.0094	0.0011	0.0037
3	0.0142	0.0029	0.0408
4	0.0189	0.0052	0.0476
5	0.0236	0.0077	0.0542
6	0.0283	0.0105	0.0606
7	0.033	0.0134	0.0668
8	0.0377	0.0164	0.0730
9	0.0425	0.0196	0.0791
10	0.0472	0.0229	0.0850

lcl = low confidence limit; ucl=upper confidence limit

The DSMB will review data on the accumulated cases to recommend whether to terminate the study, modify the ANC monitoring guidelines or take other action, if these estimates suggest that, based on observed cases to date and expected final sample size, the anticipated lower bound on risk of agranulocytosis at end of study will be unacceptably high.

An exploratory study of fluctuations in WBC and ANC in clozapine-treated African descent participants meeting ANC criteria for BEN who are not *DARC* null homozygotes will use statistical methods similar above. In addition, we will review patient characteristics and medical history to determine any identifiable causes of episodes of benign neutropenia in this group, to further describe this subgroup of potentially heterogeneous origins. While

underpowered to obtain definitive results, this substudy will provide valuable initial data to characterize this subgroup and the potential risks and benefits of clozapine.

An exploratory study of HLA alleles associated with agranulocytosis will provide descriptive data on the percentages (plus or minus 95% CI) of these *HLA* alleles (*HLA-B* and *HHLA2)* in participants with BEN by *DARC* null genotype to provide valuable preliminary data on to whether routine screening for these *HLA* risk alleles could enhance safety of clozapine treatment in AA patients with BEN.

RISKS AND DISCOMFORTS:

i. Risks related to study medication (Therapeutic Risk). This study involves the treatment with clozapine.

Other than neutropenia (see below), the main risks to clozapine treatment are as follows: salivary hypersecretion, somnolence, weight increase, dizziness, constipation, nausea, vomiting, dyspepsia, tachycardia, hypotension, type II diabetes, hyperlipidemia, sweating, and dry mouth. Serious but rare side effects include: seizures, myocarditis, sudden death, tardive dyskinesia, neuroleptic malignant syndrome, pulmonary embolism, hepatitis, fever, intestinal blockage, respiratory and cardiac arrest. We will be closely monitoring weekly for side effects at all sites and will have standardized operating procedures across sites for close monitoring standards. Standard routine medical care will be given for all side effect occurrences. The only treatments not permitted are those that may lead to a risk of neutropenia. No benzodiazepines should be administered in the first 3 days of dose titration of clozapine.

ii. Risk of neutropenia or infection (Side Effect Risk)

The major risks of the study are the risk for infection and neutropenia. Based on all existing data, genetic testing, use in chemotherapy trials and our pilot test cases, we don't expect subject ANC levels to drop below 700 cells/mm^3, however, a number of patients may fluctuate below 1000 cells/mm^3 throughout the study. We are paying careful attention to the risk of infection and neutropenia. As listed in the methods we have a careful plan for ANC and infection monitoring. In rare instances a bone marrow biopsy or aspiration may be needed.

iii. Risk related to participating in research interviews (Research Risk)

The risk from research interviews is minimal and relatively uncommon. During assessments participants may be uncomfortable discussing their smoking habits or mental health history and treatments. Participants may become frustrated and tense when they encounter difficulty when completing these measures. Careful planning and observation of the participant's response to these sessions will allow the testing to be completed with a minimum of discomfort. Participants will take breaks when necessary to help alleviate any discomfort. All interviewers are trained to recognize signs of distress or anxiety. The participant will be reminded that they can refuse to answer any question that makes them uncomfortable and may take breaks whenever they are needed. There is a slight risk of breach of confidentiality. All data will be coded with an ID number that is unique. All data including information from chart reviews, therapist reports and laboratory results will be labeled by ID only. Only members

of the study team will have access to the link between the ID and participant's name. Data containing names and personal information will never be included in published materials.

iv. Other risks.

Blood draws may be associated with tenderness or bruising. A cream can be used to reduce the pain or discomfort from inserting the needle. The cream itself may cause the skin to become pale, red, or swollen. Inserting the needle may cause dizziness or fainting. Infection is rare, because sterile methods are used. The total amount of blood taken during the study is about half of the blood taken in one blood donation. This amount of blood is usually replaced by the body without any problems. A rash could appear at the site of the EKG pads.

CONFIDENTIALITY AND PROTECTIONS AGAINST RISK:

Protections against Therapeutic and Research Risk

We will make every attempt to minimize all study-related risks. Women of childbearing age will agree to the use of medically approved birth control, which includes condoms, oral contraceptives, diaphragms and intrauterine device during the study. Pregnant and lactating females will be excluded. We will test for pregnancy prior to clozapine start and then monthly during treatment. We will be carefully monitoring for side effects and psychiatric symptoms with around the clock psychiatric and medical coverage. A physician and coordinator will be reachable 24 hours daily.

Confidentiality:

Careful procedures will be used to protect the anonymity of the participants and the confidentiality of the data. Names will only appear on consent forms and on a master list that links them with study ID numbers (different from medical record numbers). This list will be stored in locked files in a separate location from the data. At the conclusion of the project, the list will be destroyed, unless continuation is planned and approved by the Institutional Review Board. All data (whether on forms or electronic data files) will be collected, analyzed, and reported according to the study ID number and will contain no names or other identifiers. Raw paper-based data will be stored in locked files. Electronic data files reside on desktop computers and are password protected. Sources of identifiable research materials obtained from participants include laboratory (blood) specimens, research records and ratings and computerized data. Laboratory specimens and ratings will be obtained for research purposes; however close monitoring of laboratory measures will ensure excellent monitoring for clinical treatment. This will allow clinical treatment for new onset lipid or glucose abnormalities, should they occur. Any existing data and records will be used if possible.

The Database and Biostatistics Core at the MPRC will prepare laptops to be used for the study at each site. To protect participant confidentiality, data stored on this system will be kept in password protected and encrypted files and the study laptop will be stored in a locked cabinet in the FNPHY Biological Psychiatry Research Suite and a locked research suite at Howard University. Only members of the FNPHY and HU research teams have access to the research suite and cabinets. The MPRC IT/database administration staff created a secure File Transfer Protocol (FTP) link for the transfer of encrypted data from FNPHY and HU to the MPRC server. This will be installed on the FNPHY and HU study laptops. At frequent, regular intervals, the encrypted database will be backed up to the MPRC secure server overnight at

Howard University and Nigeria when there is less Internet traffic. Additionally, as determined by the volume of data being collected, duplicate files of this encrypted database will be copied onto DVD and shipped by courier service (e.g., FedEx) to the MPRC site or brought back in person to update the combined study database and the mirror of the database on the back-up laptop. We also have a secure fax line with direct electronic data dumping for some assessments that are not easily data entered. MPRC staff will run regular queries on the files from both sites to identify records with potential data entry or other problems. This dual method of transmitting data between sites was chosen because of uncertainties about the regular availability of high-speed internet-based modes of data transmission (e.g., FTP) at the Nigerian site. This method has worked well as part of the existing NIMH-funded study between MPRC and FNPHY.

Informed Consent.

Research staff members are trained to recognize symptoms of severe mental illness and cognitive impairment that could undermine an individual's ability to provide informed consent. Interested people will be provided study information and an informed consent form that contains all pertinent details of participation and includes the following: a brief explanation of the purpose of the research and a brief explanation of the requirements of the participant, including: a) willingness to be treated with clozapine, b) completing a series of interviews about one's symptoms, c) completing assessment tasks, and d) being available for follow-up assessments.

The consent form will include an explanation of the risks and benefits of participation; assurances of confidentiality; and an explanation that participation is entirely voluntary, the decision to participate will in no way influence or restrict the participants access to clinical services and care at participating sites, and the participant is free to withdraw at any time with no negative consequences. As some potential participants will have poor reading skills, the consent form will be read aloud to all participants in tandem with their own silent reading of the document. The individual securing consent will review any points about which the participant is unclear, and the participant will be invited to ask questions as needed.

After reading the consent, and before obtaining a signature, a brief questionnaire is administered to verify that the participant is competent to provide consent and has demonstrated comprehension of the consent document. This questionnaire is attached to the informed consent form and is completed immediately after explaining the informed consent form and before obtaining the participant's signature on the form. If the participant does not understand the consent form, the recruiter will try to explain points of confusion, and administer the questionnaire again. Those failing to answer the questions adequately will not be recruited into the study. The recruiter will also make a clinical judgment and not recruit participants who appear unable to grasp key aspects of the procedure. This approach, which requires a proactive demonstration on the part of the participant that they understand what is being requested, has been used by investigators at all three sites. Included participants must also be judged competent to consent by the Evaluation to Sign Consent (ESC) questionnaire, and provide voluntary informed consent. Per University of Maryland School of Medicine IRB regulations, a copy of the signed consent form is given to the participant, a copy is placed in the person's medical record, and the original is kept in the laboratory.

Informed consent forms will include consent to share de-identified subject data with a clinical trial data repository maintained by the National Institute of Mental Health, using procedures

outlined in NIH NOT-MH-14-015 (http://grants1.nih.gov/grants/guide/notice-files/NOT-MH-14-015.html). This depository will allow other researchers access to data from this clinical trial, with the assurance that the participant's individual name or other identifying information will not be included in the data shared with other researchers.

WBC and infection monitoring:

All subjects in the study will be evaluated weekly during clozapine treatment for WBC and ANC. The team will closely examine any risk of severe neutropenia or infection. Twice weekly monitoring will be instituted in most cases when ANC drops below 1200 cells/mm^3. The full procedure for WBC monitoring is in the protocol. In addition to monitoring by the PI and somatic physician, a expert hematology consultants are available to the research teams as well as the hematology team from the FDA at their request. Weekly temperatures will be taken and signs and symptoms of infection will be closely monitored.

We will notify the Data Safety Monitoring Board (DSMB) monitoring the study whenever a case of agranulocytosis occurs. The DSMB will review data on the accumulated cases to recommend whether to terminate the study, modify the ANC monitoring guidelines or take other action. If cases of agranulocytosis occur, the DSMB will review, based on the number of observed cases and the projected total sample size, whether the final estimated risk of agranulocytosis from this study in BEN subjects treated with clozapine using the guidelines described in this protocol is likely to be too high to warrant modification of clozapine treatment guidelines for BEN subjects.

Monitoring of adequate psychiatric symptom response:

Clozapine discontinuation will take place in the case of a significant side effect, or if there is no adequate symptom response to clozapine and discontinuation is thought to be a better option. Response is defined as 20% improvement in total BPRS Score and a final score of ≤ 35 or CGI ≤ 3. These generally result in a response rate of approximately 68% with another 25% demonstrating partial response. Partial response is defined as < 20% on total BPRS with two clinicians agreeing that there is notable improvement not evident on BPRS. We anticipate that 7% of patients will not achieve any response and may be discontinued at 8 weeks due to incomplete response. Since response after 8 weeks is not likely and the risks may be higher, symptoms will be monitored monthly to determine whether clozapine should be continued.

Once a participant is discontinued from the study for whatever reason, completion ratings will be performed if possible. These include laboratory tests and clinical rating scales. If a patient is prematurely terminated from the study, the investigators will work with the clinical team to ensure the patient will resume other antipsychotic treatments for their symptoms.

Suicidality:

Clozapine is the only antipsychotic with documented efficacy in reducing suicidal ideation and intent. Suicidality may be increased in participants with treatment resistant symptoms. Based on these considerations, we will use the Calgary Depression Rating Scale (CDS) to monitor for presence of significant suicidality during screening and active treatment with clozapine. If any participant has a score on the suicidal item greater than 0 they will be evaluated. If moderate suicidal ideation or intent is observed during screening, the participant will be offered the opportunity to try immediate clozapine treatment, and a “suicide prevention plan” will be implemented to provide appropriate additional behavioral interventions. This may

also include dosage adjustment or increase. If serious suicidal ideation or intent is observed during clozapine treatment, the “suicide prevention plan” will be implemented immediately and if patients do not respond to a dose increase or other treatments, they will be discontinued from study. The “suicide prevention plan” consists of immediate psychiatric evaluation, implementation of a suicide prevention plan, provision of 24 hour access to a physician, referral to emergency services as necessary and continued evaluation using the CDS.

Protection from research interview and data-gathering-related risks.

In previous studies with the target population, we have developed procedures for conducting interviews in a manner that is sensitive to the needs of the participants and the emotional nature of the interviews, while maintaining high scientific standards. Several of the strategies that we have successfully used to minimize the potential distress for participants include: informing participants before the interviews about the topics that will be covered in the interview; reminding participants that they may choose not to answer certain questions, or to terminate the interview at any time; and taking breaks during the interview when the participant feels tense or distressed. Interviewers will be trained in the recognition of, and appropriate response to (including the involvement of other professionals), specific kinds of participant negative responses.

Certification on Protection of Human Subjects in Research:

All research staff participating in this study at all three sites are required to undergo the online Collaborative Institutional Training Initiative (CITI) Course on Human Subjects Research. Their certificates of completion are uploaded (as research staff) in the University of Maryland Baltimore IRB-approved study protocol in CICERO (Collaborative Institutional Comprehensive Evaluation of Research Online) – the University of Maryland School of Medicine's Research Evaluation Portal and are compliant with the Health Insurance Portability and Accountability Act (HIPAA).

Federal Certificate of Confidentiality:

We will be obtaining a Federal Certificate of Confidentiality in the US. This is not required for Nigeria.

Collaborative Protocol and Consent Form Approval:

The University of Maryland Baltimore IRB-approved study protocol and consent form will be reviewed by the Ethics Committee at the Federal Neuropsychiatric Hospital Yaba, Lagos and the Howard University IRB. Any significant changes requested by the Ethics Committee or Howard University IRB would be re-presented to the UMB IRB. At the Nigerian site, these documents would bear the seal of the Federal Neuropsychiatric Hospital Yaba and will be downloaded from CICERO in Lagos for use in consenting study participants in Lagos. Howard University will also have their consent forms. Furthermore, the UMB IRB-approved consent forms will be translated into two indigenous Nigerian languages by a team of linguistic experts and back-translated to English by an independent team of linguistic experts. Thus, consent forms at FNPHY will be available in English, Yoruba, and Igbo at the Nigerian site.

Treatment:

We have developed a slow and standardized dosing titration to protect against side effects and promote tolerability. This titration is consistent with that approved by the FDA and is included in the methods section.

Medication Adherence:

Patients receiving >75% of their assigned medication will be considered adherent. Outpatient adherence will be monitored through weekly pill counts and participant interviews. Medications will be dispensed on a weekly basis and will only be dispensed after adherence is assessed and all other assessments are completed. Inpatient adherence will be monitored through inpatient medication records. If a patient is observed to have an adherence problem, this will be discussed with the patient and a plan formulated to bring the patient back into adherence. These monitoring procedures have been used in other MPRC studies and have resulted in high levels of adherence (~90%). Non-adherence (not receiving >25% assigned study medication) will be reason for termination from the study. Clozapine discontinuation for more than two consecutive days will need re-titration. If this happens more than once the participant will be discontinued from the study but may continue to take clozapine as per the local clinical team recommendations.

Development of New Onset Diabetes and Hyperlipidemia:

If a participant develops new onset diabetes and/or hyperlipidemia during the study, a consult will be called by the treating physician, and the participant will receive treatment according to the current guidelines.

Withdrawal Criteria:

We have criteria for discontinuation of clozapine and the ability to continue regular monitoring. Participants can stop study drug and continue in the monitoring portion of the study.

A. Clozapine Discontinuation Criteria (clinician and PI opinion, following are potential causes of discontinuation).

Symptom Worsening:

Either the treating psychiatrist, medically accountable physician, or the PI may determine that a patient is experiencing clinical worsening. Clinical worsening may be defined as the following: the subject is judged to be entering an exacerbation of his/her illness by the treating clinician; an increase of 3 or more points from baseline on a BPRS positive symptom item; an increase of 3 or more points from baseline on BPRS somatic concern, conceptual disorganization or hostility; or an increase of 2 or more points from baseline on the CGI global severity rating or in the case the CGI is at a ceiling for beginning, clinical judgment will be made. Transient rating changes do occur so the symptom increases must be accompanied by the clinician's feelings that they are entering exacerbation.

No symptom Improvement

Clozapine discontinuation may take place in the case of no adequate symptom response to clozapine and discontinuation is thought to be a better option. Response is defined as 20% improvement and a final score of ≤ 35 in total BPRS score or CGI ≤ 3. These scores generally result in a response rate of approximately 68% with another 25% demonstrating partial response. We anticipate that 7% of patients may not achieve any response (partial or full) and may be discontinued.

Low White Blood Cell Count

If a participant drops below 500 cells/mm^3 (with stat redraw for confirmation) than clozapine will be discontinued. Participants may be discontinued due to a rapid falling white blood cell count if the hematologist or PI has reason to be concerned at a higher level.

Significant Side Effects.

The clinical and research team may discontinue clozapine if the participant is having significant side effects or side effects that are not in the best interest of the patient to continue for safety reasons. This will be determined by the research study team along with clinical team.

Medication Nonadherence

Clozapine discontinuation for more than two consecutive days will need re-titration. If this happens more than once as nonadherence the participant may be discontinued from clozapine

Non-adherent to Study Procedures

If the participant is nonadherent to white blood cell monitoring, clozapine will be discontinued.

Once a participant is discontinued from clozapine, for whatever reason, they may remain in the study for continued monitoring.

B. Discontinuation from Study and all Monitoring:

- All attempts, as long as participant agrees, to follow-up monitoring when clozapine is stopped will occur
- Discontinuation from the study will occur if the participant is not adherent to study procedures and blood draws or if the participant no longer wants to be enrolled or the physician determines blood draws and/or study participation are not in the best interest of the patient
- If a patient is discontinued from open label clozapine for reasons described above, they will continue as usual for the follow-up lab work, symptom ratings and clozapine blood levels
- If the patient is recommended for clozapine discontinuation or study termination the PI or medically accountable physician will meet with the participant to describe why they are recommended for clozapine discontinuation or study termination.
- If the participant is discontinuing clozapine the research and clinical team will work together to ensure a plan for followed medication treatment in order to help with the appropriate titration of a new medication. This will be done clinically with no research protocol for cross titration and other medication treatment.
- If the participant is discontinuing clozapine but continuing in the study for monitoring no additional expectations are required
- When any participant is terminated from the study altogether, the study team will schedule with the participant as soon as possible to complete ratings and assessments (including laboratory tests and rating scales). This may result in a midweek visit. The participant will be compensated for any study visit time.
- If a participant is discontinued from clozapine they will continue, if they agree, to continue the follow-up monitoring for the entire 6 month treatment period.
- If a participant if nonadherent with blood work during clozapine they will be terminated from the study and no further data collection will occur past the final assessments.

BENEFITS:

By participating in this study and receiving a closely observed treatment trial with clozapine, the patient may have improvement in variety of symptom domains. In addition, we may gain understanding of the hematologic fluctuations with clozapine to help guide future treatment with this medication. Patients treated with clozapine in this study likely have been denied clozapine as a treatment in the past due to their lower ANC. This provides a unique opportunity to have an otherwise denied clozapine trial.

There are several other potential benefits to participating in the study. Patients in the study will receive many elements of treatment commonly received by patients with psychotic disorders but with a heavier emphasis on continuity of clinical care and close clinical monitoring for signs of exacerbation. By virtue of their participation in numerous assessments and evaluations necessary in a combined clinical/research endeavor, they will receive more attention than is ordinarily given as part of standard clinical care. Their progress will be frequently and closely monitored from a variety of clinically relevant standpoints. In addition, patients will gain specific information concerning their neurological status and observations of treatment response which might guide their future pharmacologic treatment. Moreover, patients and their families should receive considerable education about the patient's illness. Such knowledge can provide significant benefits by increasing the capacity of the patient and his/her family to monitor and seek early clinical attention for future manifestations of illness.

Importance of the Knowledge to be Gained

The importance of the knowledge gained far outweighs any risks that may occur in this study. Patients with BEN who were never before able to have a trial of clozapine may for the first time receive treatment. By treating these patients prospectively with a pretreatment comparison we will be able to test and measure the fluctuations of ANC in BEN treated patients and will understand the feasibility and science of incorporating genetic testing. Lastly, our data may help shape and change actual treatment guidelines and prescribing practices.

DATA AND SAFETY MONITORING PLAN

A Data Safety Monitoring Board (DSMB) is already established for monitoring studies at the MPRC and will be used as the central DSMB for this study. The DSMB is comprised of three psychiatrists (2 involved in treatment and research, 1 who is a clinical practitioner), a pharmacist and a statistician. The psychiatrists will be experts in the clinical treatment of people with schizophrenia. The DSMB will be charged with the following responsibilities: 1) to establish a regular meeting schedule; 2) to review the protocol; 3) to review the consent form; 4) to monitor the occurrence of side effects/adverse events, and serious adverse events throughout the course of the study; 5) to review with investigators, the study data management system; and 6) to establish stop rules for the study as a whole. This study is scheduled for initial review by the DSMB on 4-8-15. The PI will plan to have this study reviewed by the DSMB quarterly if the DSMB determines that necessary. As a rule, the DSMB determines the frequency of review throughout a study based on risk, safety concerns and recruitment. All serious adverse events (SAEs), including new cases of agranulocytosis, will be reported to the DSMB, PIs, and the University of Maryland, School of Medicine IRB. The PIs will receive all SAE reports within 24 hours of their occurrence. If as a result of data monitoring or interim analysis, the DSMB determines that the study poses an unreasonable or unnecessary risk to study participants, the DSMB and the PI will determine what possible

protocol modifications are required to minimize the future occurrence of such events. Unexpected adverse events will be reported in accord with NIH and U.S Federal requirements. Non-serious and expected adverse events will be reported annually to the IRBs. The PI is invited to the DSMB meetings and is asked to give a review of the past quarter with a particular emphasis on safety, side effects and enrollment numbers. The data would remain blinded unless risks to participants justify unblinding. To safeguard confidentiality, data are presented in aggregate or are identified only by an ID number

The DSMB has reviewed this protocol initially and has approved.

ALTERNATIVES TO PARTICIPATION:

The participant may choose not to participate in this study. If he/she chooses not participate in this study, their treatment at any facility or clinic including MPRC and Spring Grove Hospital will not be affected. Their continued care and treatment will not change if they choose not to take part in this study.

PARTICIPANT COMPENSATION:

During the first 6 weeks participants will have approximately 8 (or fewer) visits for screening and blood draws. During the last 6 months participants will have approximately 24 visits. Participants will be compensated $20 per visit for their time for a total of approximately $640 for participation in the study if they complete approximately 8 months. If any portion is completed participants will be paid only for the visits they completed. Participants will be paid by cash or check monthly and will also provided transportation to and from visits as needed.

PATIENT MONITORING

Absolute Neutrophil Count (ANC) Value	**Modified Monitoring Plan Recommendation. Modifications may be made for each patient with hematology consult. Must be given to patient at time of consent.** **Patient Name _____** **Date received _____**
ANC ≥1000/mm^3	Continue weekly WBC monitoring.
ANC <1000/mm^3 to ≥500/mm^3	1. No Interruption of therapy 2. Redraw WBCto ensure second ANC value within range 3. Twice weekly WBC monitoring 4. Return to once weekly WBC monitoring when > 1000 /mm^3

WBC=white blood count, ANC=absolute neutrophil count