Study Protocol

Title: Biomolecular Effects of Topical Curcumin in HSIL Cervical Neoplasia

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Biomolecular Effects of Topical Curcumin in HSIL Cervical Neoplasia

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(Topical Curcumin for Precancer Cervical Lesions)

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Lay Summary

Cervical cancer is the third most common cancer worldwide. The causative agent responsible for cervical cancer is the persistent infection with oncogenic Human Papillomavirus (HPV). Rates of cervical cancer and HPV infection are increased particularly in HIV-infected women due to immunosuppression and cervical cancer is an AIDS-defining diagnosis. Despite the promise of HPV vaccine in the prevention of cervical cancer, the widespread availability of this vaccine is limited due to cost and accessibility. Therefore, prevention strategies to reduce cervical cancer after HPV exposure entail treatment at the most severe premalignant state (high grade squamous intraepithelial lesion or HSIL). Unfortunately, this procedure is expensive and not widely available in resource-limited areas. There is a desperate need for an inexpensive, non-invasive alternative method to treat these premalignant cervical lesions.

Curcumin, an extract from turmeric, a popular culinary spice, has been used in traditional Indian medicine for its anti- inflammatory and anti-infectious properties. Recent studies have shown the potential effect of curcumin to reduce tumors and precancerous lesions in animal and human cancer cells. It is postulated that curcumin achieves its effect on cancer cells by modulating different cellular pathways as well as altering HPV effect on tissue cells.

The purpose of this study is to see if curcumin can reverse a cervical precancerous state by looking at people who have the condition and intervening with a study drug or placebo (an inactive drug), prior to planned therapeutic LEEP which is a treatment procedure for removing cervical cancer.

We plan to explore the effect of curcumin as a potential medical treatment in HIV uninfected and infected women with HSIL lesions of the cervix. 40 women with HSIL of the cervix will be enrolled to either insert 2000 mg capsule of curcumin in their vagina weekly or placebo. They will have regular monthly visits for the 4 months and then undergo removal of the precancerous cells as recommended standard of care.

SUMMARY

In the United States, approximately thirteen thousand new cases of cervical cancer are diagnosed annually and it is the cause of death of approximately four thousand women (1). The average age for diagnosis of cervical cancer is over 50 years, however precursors to cervical malignancy, such as cervical dysplasias and carcinomas in situ, are commonly diagnosed in a younger population (2). Also in certain high-risk populations such as HIV-infected women, the age of presentation of cervical cancer or its precursors has become increasingly younger requiring a more aggressive approach to treatment thus affecting fertility in this young population (2, 3).

There is a paramount need for a new nonsurgical treatment to stabilize or treat abnormal areas of the cervix that can lead to cancer. Current surgical treatment options carry the risk of decreasing a women's ability to have children (4, 5). Medical treatment with a natural herb, Curcumin, may allow subjects to receive treatment of cervical lesions without undergoing a surgical procedure (6-10). Curcumin is a major active component extracted from turmeric, a popular culinary spice and is also commonly used as a traditional Indian medicine in 'Ayurveda' for its anti-inflammatory and anti-infectious properties (11). Curcumin given by mouth has been shown to have anti-cancer properties (12-14) and to decrease the amount of precancerous tissue in several parts of the body including the cervix. However, the studies done did not include enough people to be significant. In addition other studies have shown that oral curcumin has poor bioavailability (15) and limited effect in target tissues. Therefore topical intravaginal curcumin has the promise of delivering this drug directly at the site of disease to ensure adequate exposure of the tissues to the drug without having systemic side effects (10). We have completed a phase 1 trial demonstrating safety of topical curcumin intravaginally up to the maximum dose of 2000 mg (appendix 1) and a recent study (10) has also demonstrated minimal side effects of administering curcumin intravaginally at 500 mg daily. This proposed study will determine the biological relevance of intravaginal curcumin on known HPV related molecular targets (E6, E7 mRNA expression, P16ink4a, P53, Rb and VEGF mRNA and protein expression, and NF- κB binding activity) within HSIL lesions of the cervix in HIV uninfected and infected women (without AIDS defining illness). Also this study will determine the tissue concentration of curcumin in the cervix of women in the curcumin treated arm and evaluate whether the expression of molecular targets is associated with its tissue concentration. This data will contribute to the development of a larger phase 2 trial examining efficacy of intravaginal curcumin for the treatment of HSIL lesions and associated changes biomarker expression.

RATIONALE

A. Curcumin is a major active component extracted from turmeric (*Curcuma longa Linn),* with anti-inflammatory and anti-infectious properties (8, 9, 11-14). Several preclinical and clinical studies have shown curcumin's ability to reduce tumors and precancerous lesions in animal and human cancer cell(8-15)

B. Curcumin modulates cytokines, growth factors, kinases, transcription factors and other enzymes (8-15). Specifically it can suppress the activation of transcription factor NF-κB and the expression and activity of VEGF and p16INK4a, biomarkers known to be elevated in cervical dysplasia (8-15). To date, there are no published data on the tissue penetration of curcumin in vivo. This will establish the level of curcumin penetration in cervical tissue as well as the cumulative effect of weekly curcumin over time. This study will establish the baseline which is crucial in designing further curcumin intervention studies. The methodology of curcumin measurement for this study is well documented in Subhashini et al (16).

C. Studies have shown that curcumin treatment alters HPV-associated molecular pathways in cancer cells and suppresses cervical cancer growth by inhibiting the transcription of the oncoproteins of HPV16 and HPV18 (designated as E6 and E7) and restoring p53 and retinoblastoma (Rb) function (8, 9, 17). This study will help us determine the most relevant duration of curcumin treatment when compared to the response of the biomarkers.

BACKGROUND AND SIGNIFICANCE

Cervical cancer is the third most common cancer worldwide with an estimated 530,000 women developing the disease in 2008 and 275,000 women dying from it during that year (18). The causative agent responsible for cervical cancer is the persistent infection with oncogenic Human Papillomavirus (HPV) subtypes and the role of HPV in the malignant transformation of cervical epithelial cells has been clearly elucidated in the literature (19-22). Recently, WHO has made the prevention of cervical cancer a priority and Bill & Melinda Gates Foundation and the Pink Ribbon Red Ribbon initiative are looking for potential low cost, innovative medical options to reduce cervical cancer worldwide especially in HIV-infected women. Rates of cervical cancer and HPV infection are increased in HIV-infected women due to immunosuppression and cervical cancer is an AIDS defining diagnosis (2, 3, 21).

Despite the HPV vaccine showing promise in the prevention of cervical cancer, the widespread availability of this vaccine in developing countries has not yet been reached due to cost and poor infrastructure (23). Therefore, prevention strategies to reduce cervical cancer after HPV exposure entail treatment at the most severe premalignant state (HSIL) (4, 21). The most common treatment for HSIL is the loop electrical excisional procedure (LEEP) (4, 5). Unfortunately, this procedure requires expensive set up, specialized training, is time-consuming and not widely available in resource-limited countries. In addition, LEEP is associated with potential significant morbidities, including preterm birth, premature rupture of membranes and cervical incompetence (5).

Another treatment modality is the cryosurgery (24, 25). However, this procedure is not recommended due to the high risk of disease recurrence in HIV-infected women who suffer disproportionately from this disease. For this reason an economical medical alternative treatment of HSIL cervical disease can have a significant global impact in countries where cervical cancer is the leading cause of death from cancer in women.

Curcumin is a major active component extracted from turmeric (*Curcuma longa Linn),* with anti-inflammatory and anti-infectious properties. Several preclinical and clinical studies have shown curcumin's ability to reduce tumors and precancerous lesions in animal and human cancer cells (7-9, 11-14). Curcumin modulates cytokines, growth factors, kinases, transcription factors and other enzymes (8, 11-15). Specifically it can suppress the activation of transcription factor NF-κB and the expression and activity of VEGF and p16INK4a, biomarkers known to be elevated in cervical dysplasia (7, 8, 11, 13-15, 26). Studies have shown that curcumin treatment alters HPV-associated molecular pathways in cancer cells and suppresses cervical cancer growth by inhibiting the transcription of the oncoproteins of HPV16 and HPV18 (designated as E6 and E7) and restoring p53 and retinoblastoma (Rb) function(7, 9, 17).

Although clinical studies have shown that curcumin is safe and well tolerated with no significant toxicity and an acceptable blood chemistry profile, its use is limited by its low systemic bioavailability (9, 10, 14, 15, 17). To circumvent this problem, we administered curcumin capsules through an intravaginal approach and tested safety, tolerability and acquired pharmacokinetic data. We found no severe adverse events or dose limiting toxicities during our phase I trial of 13 subjects treated daily for 14 days with intravaginal curcumin (appendix 1). Three subjects (1 taking 1000 mg dose, 2 taking 1500 mg dose) completed 13 of the 14 days, with the reason for non-compliance being they chose not to finish. In addition, curcumin and curcumin conjugates were not measurable in the serum, and detectable concentrations were not observed in the urine of study participants (presented at Eurogin 2011). With a low side effect profile, easy application, and low cost, this potentially therapeutic herb can be sustainable in low resource countries. We are also supported by a recent study from India conducted by Basu(10) et al 2013. Their study clearly demonstrated that intravaginal curcumin is safe and tolerable. The main reason for the

discontinuation of the drug was vulvovaginal irritation and itching. This application explores the effect of topical application of curcumin as a potential chemoprevention agent in the suppression of cervico-vaginal HPV infection and subsequent risk for cancer of the cervix. IND #103496 is in place to proceed with this study.

SPECIFIC AIMS

This application explores the effect of curcumin, a known chemotherapeutic agent as a potential medical treatment in HIV uninfected and infected women (without AIDS defining illness) with high grade squamous intraepithelial lesions (HSIL) of the cervix. We plan to address our specific aims by conducting a pilot two-arm study in which women will be randomized to 2000 mg of intravaginal curcumin versus placebo weekly for 12 weeks.

The specific hypothesis of this phase II study is that intravaginal curcumin will decrease expression of HPV oncogenic biomarkers associated with cervical cancer and this will correlate with the levels of curcumin detectable in the cervical tissue.

Specific Aim 1 (Primary Objective):

Determine the association between intravaginal curcumin on known HPV-related molecular target HPV E6/E7 mRNA expression within HSIL lesions of the cervix in HIV uninfected and infected women.

Rationale:

The HPV E6/E7 oncoproteins have significant roles in malignant transformation and are consistently expressed in malignant cervical tissue. Increasing levels of E6 and E7 expression cause genetic instability, and implies a risk for cellular changes, which results in a selective growth advantage (31, 34-37). The APTIMA® HPV Assay that we will be utilizing in our studies detects full-length HPV E6/E7 mRNA for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and correlates very well with integrated HPV, which in turn correlates with full-length HPV E6/E7 protein levels (38).

All methodologies for evaluating the expression and function of HPV E6/E7 are ongoing in the laboratory of the co-investigator (Dr. Sidell) and are described in referenced publications (27-34). The expression of HPV E6/E7 proteins will be assessed by the APTIMA* HPV Assay using liquid-based cytology specimens.

Specific Aim 2 (Secondary Objective):

To quantify levels of curcumin in the cervix of women in the curcumin treatment arm.

Rationale:

To date, there are no published data on the tissue penetration of curcumin in vivo. This will establish the level of curcumin penetration in cervical tissue as well as the cumulative effect of weekly curcumin over time. This study will establish the baseline which is crucial in designing further curcumin intervention studies. The methodology of curcumin measurement for this study is well documented in Subhashini et al (16).

Specific Aim 3 (Exploratory Aim):

To determine the association between curcumin and other known biomarkers of cervical disease and its effect on the vaginal microbiome.

Rationale:

To determine the most relevant duration for curcumin treatment, as well as identify additional biomarkers that are regulated with the pharmacological activity of curcumin, we will measure mRNAand/or protein expression levels of p16INK4a, p53, Rb, VEGF and NF-κB in cervical biopsy specimens. A combination of immuno-histochemical methods and other molecular assays (RT-PCR, ELISA, TransAM NF-κB DNA binding ELISA) to determine mRNA levels and protein levels on transcription factor binding activity will also be done.

p16INK4a is an indirect marker of cell cycle dysregulation and has been shown to be expressed in cervical dysplasias and carcinomas associated with high risk HPV infections(30, 33, 34). As such p16INK4a has emerged as a valuable surrogate marker for high-risk HPV infection and shows increased expression with worsening grades of CIN(30, 33). High HPV E6/E7 oncogene expression has been shown to be consistent with high expression of p16INK4a in the clinical assessment of CIN grade (29, 30, 33, 34). This knowledge has led to the use of p16INK4a immunohistochemistry in many laboratories as a useful diagnostic tool (29).

p53 and Rb are important cell cycle regulator proteins in cervical carcinogenesis which are suppressed in most cervical cancer cells(35, 37) Increased levels of these proteins have been linked to regression of cervical cancer lesions(35, 37). In in vitro studies utilizing HPV+ cervical cancer cell lines, curcumin was shown to enhance protein expression of p53 and up regulate the activity of Rb through alteration of its phosphorylation profile (6-8)

VEGF is known to play an important role in the development of cervical dysplasia and CIN progression, and VEGF expression has been shown to correlate with severity of cervical cancer precursor (CIN) lesions and invasive disease(28, 32). The laboratory has extensive experience in the analysis of VEGF production including its transcriptional and translational regulation by steroid hormones in reproductive tissue.

NF-κB upregulation is related to the grade of CIN although the significance of NF-κB activation per se to CIN lesion development and its prognostic value in cervical cancer have not been well defined (27, 33). The known molecular link of reduced NK-κB function to curcumin treatment in conjunction with the other biomarkers studied may shed light on this issue (12, 14, 15). Since most downstream (e.g. anticancer) effects of curcumin are known to be mediated through suppression of NK-κB activity, the analysis of NF-κB binding activity will provide a direct molecular benchmark for assessing curcumin treatment responses independent from its therapeutic effects.

RESEARCH DESIGN AND METHODS

We are proposing a prospective, randomized, two-arm, open label pilot study to demonstrate the in vivo effects previously seen in preclinical studies using cervical cancer cell lines16-20. Study participants will be recruited from the Grady Cancer Center of Excellence and the Infectious Disease Program at Grady's Ponce Clinic. A convenience sample of 40 HIV uninfected and infected participants with biopsy-proven HSIL will be randomized to one of two arms (2000 mg of curcumin powder in capsules or placebo inserted intravaginally weekly at bedtime for 12 weeks, (excluding the time when they are on their menses) as demonstrated in the schema.

Cervical cytology, vaginal swabs, colposcopies and biopsies will be performed every 4 weeks during each study visit for the study duration for the primary objectives outlined in specific aims 1-3. These time points were selected to acquire cervical samples prior to treatment, during treatment and 4 weeks after treatment completion (LEEP specimen). Study drug and placebo will be administered during weeks 1-12 (12 weeks of treatment) and no treatment will be administered during weeks 13-16 (washout period). Cervical biopsies will be performed in areas with a colposcopic suggestion of HSIL disease and four additional cervical punch biopsies (one specimen from each of four quadrants) of the cervix will be done for downstream biomarker analysis. The laboratory, pathology and data analysis staff will be blinded to treatment arm. The clinicians performing the colposcopies nor the participants will not be blinded secondary to the evident color of curcumin.

Study Population

HIV uninfected and infected women will be recruited for this study. We will focus only on HIV infected women without an AIDS defining disease, given that the risk of progression from high grade lesions to invasive carcinoma is faster in HIV infected subjects who are significantly immune compromised.

We are targeting HIV infected subjects because in low resources countries this is the population that can benefit the most from a medical therapy for pre-invasive cervical dysplasia until the women can have the opportunity to obtain care for definitive treatment. All over the world in low resource countries women are dying from cervical cancer because they do not have trained clinicians to treat the disease in the preinvasive state. Curcumin can be a cheap herbal chemopreventive alternative until definite treatment can be performed. It may also give these women options if they desire to have treatment to delay treatment until childbearing is complete. Also it has been proven by data from the WIHS (women interagency HIV study) that well followed women who are HIV infected have the same rates of invasive cervical cancer as their HIV uninfected counterpart. Therefore women regardless of their HIV status will be enrolled in the study.

Higher rates are only seen in women with poor compliance. Even though we are opening recruit to the greater Atlanta area, our focus of recruitment for our HIV population will be women from the WIHS study at Emory where there is a high degree of compliance for study visits. This is a drug to be used mainly worldwide not in high income industrialized countries like the United States.

The maximum dose found to be safe in Phase I of my study and will be used for this Phase II study is 2000 mg. The overall focus is to conduct the study in the patient population most likely to present with disease, need a form of medical treatment due to lack of trained doctors or providers to treat them at the pre-invasive stage and give alternatives to women who want to defer treatment for childbearing.

Study Intervention

Description of the Study Drug

The investigational product/active study drug for this study, curcumin (Curcumin C3 Complex), a constituent of the spice turmeric, is considered to be a low-toxicity, dietary-derived agent with chemopreventive and therapeutic benefits. The Curcumin C3 Complex® capsules will be prepared by Sabinsa Corporation. All Curcumin C3 Complex® capsules will be directly shipped to Emory University Investigational Drug Service under the supervision of Philip Powers, PharmD or Kay Woodson, PharmD who will distribute the drug. The manufacturer of curcumin is Sabinsa Corporation 750 So. Innovation Circle Payson, UT 84651.

Study Drug Administration

All study subjects will be instructed to insert 2000 mg total curcumin capsules at bedtime every week. The study subject will place four 500 mg Curcumin C3 Complex® capsules into the opening of the vaginal applicator and insert the capsules using the applicator while lying on their back with their knees bent (Figure 1). The subject will be instructed to gently inserted the capsules into the vagina as far as it will go comfortably (Figure 1), similar to a tampon insertion or other common intravaginal drugs (fungal treatments). All required capsules should be inserted at one time. After successful insertion of the capsules, the subject will remove the vaginal applicator for disposal. The study subject who is randomized to the gelatin capsules will insert the placebo capsules in the same manner as the study drug. Dr. Flowers holds the FDA IND (no.103,496) for the intravaginal use of curcumin for human clinical trials.

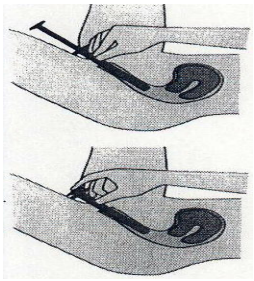


Figure 1. Insertion of study drug

Dosing regimen

2000 mg total (4 capsules) of curcumin powder in capsules or placebo inserted intra-vaginally weekly at bedtime for 12 weeks, (excluding the time when they are on their menses).

Treatment Plan

There is no concern about progression of cervical intraepithelial lesion (CIN) 3 disease or severe dysplasia within the time course of this study (4-5 months) based on the natural history of CIN 3 (severe cervical dysplasia). Approximately 30% will regress and 50-70% will progress to cancer however the rate of progression to cancer takes 5-10 years to occur. Though the data of the natural history of cervical dysplasia was in HIV uninfected women; data from the Women Interagency HIV study has also reported that in a well followed cohort of HIV infected women, rates of invasive cervical cancer was the same in HIV infected women as in HIV uninfected women. We expect the same outcomes in both cohorts of women.

In addition time, course to cancer is not significantly faster to require treatment to occur sooner than their HIV negative counterpart. Presently, HIV positive young women with histologic HSIL disease (CIN 2,3) are now offered in clinical practice active monitoring with colposcopies every 6 months instead of treatment of cervical dysplasia to avoid the negative pregnancy outcomes from treatment. This change in practice is due to the number of HIV positive women desiring fertility and pregnancy and the effectiveness of HAART in pregnancy.

Enrolled subjects will be asked to agree to the use of reliable birth control: Combined oral contraceptive pill (OCP), Long Lasting Reversible Contraceptive (LARCP), Bi-Lateral Tubal Ligation (BLT) and Depo-Provera Shot/Birth control shot and they will be given a partner notification letter advising them to use a condom when having vaginal sex with their partner and the risks associated with lack of use of a condom.

Clinical care practice guidelines indicate colposcopy every 6 months. We will be following these subjects every month with colposcopy for research purposes of this study, which is more than sufficient to clinically monitor and capture any women at risk for progression of disease. Subjects will be informed that the alternative to this study or if disease progression occurs, is referral for continued clinical care with their provider where they will this they will discuss the treatment (Leep with continued active monitoring-colposcopy with biospies) with their provider at the Grady Cancer Center of Excellence treatment Clinic or at the Emory Clinic.

Randomization Method

The treatment assignments will be generated with the use of a pseudo-random-number generator with randomly permutated blocks that will be used to ensure balance between the number of subjects assigned to each treatment (1st: 2000 mg of curcumin for 12 weeks versus placebo capsules for 12 weeks). Before the study starts, the Pharmacist at Ponce Clinic (IDP) or Grady CRN will be sent a batch of sealed, sequenced, opaque envelopes containing the treatment assignment. The envelopes will have a unique ID consisting of a sequence number determining the treatment arm. Once informed consent is obtained on a subject, the Study Coordinator/P.I. will set up an appointment for the screening visit at the Ponce clinic (IDP) or Grady CRN to confirm clinical eligibility. Once the participant is cleared to enter the study, they will be given a unique ID associated to the batch of envelopes in the care of the Grady CRN or Ponce Clinic (IDP) Pharmacist.

Subject Recruitment:

All subjects will be recruited from Grady Cancer Center of Excellence at Grady Memorial Hospital (GMH) and the Grady Infectious Disease clinic at Ponce (IDP) per the inclusion and the exclusion criteria below.

<u>Inclusion Criteria:</u>

1. Age 21 years and older
2. HIV uninfected and infected women (without AIDS defining illness)
3. HSIL cytology with no invasive features identified on colposcopy or the baseline biopsy
4. Compliant on combined anti-retrovirals (cART) if HIV infected
5. On continuous antiretrovials with CD4 count >200 cells/ml with sustained undetectable viral load for at least 3 months. (HIV positive women)
6. Patient on reliable (OCP, LARC, BTL or Depo Provera) birth control: Combined oral contraceptive pill (OCP). Long Lasting Reversible Contraceptive (LARCP), BiLateral Tubal Ligation (BLT) and DepoProvera Shot/Birth control shot.
7. Patient willing to conform to the study requirements
8. No risk factors for microinvasive disease (no colposcopic features of microinvasion, adequate colposcopy and negative endocervical curettage)

Exclusion Criteria:

1. Age over 21 years of age
2. Invasive features on colposcopy and the biopsy specimen
3. Not compliant with anti-retroviral therapy (cART) (HIV infected participants)
4. CD4 count <200 cells/ml and detectable viral load within the least 3 months (HIV infected participants
5. Lactating and pregnant women
6. Patient with irregular cycles (more than once a month).
7. Patient not on reliable birth control.
8. Previous hysterectomy and/or prior treatment for cervical precancer condition

Study visits

Subjects that are eligible and volunteer to take part, they will be asked to do the following:

A. Sign the combined ICF/HIPAA Authorization Form and a medical release form.
    a. Update their contact information.
B. Respond to Questionnaires
C. Undergo a thorough medical evaluation at their screening visit. This will include
    i. Review of their medical records
    ii. Medical history obtained by study personnel
    iii. Physical examination by study personnel.
    iv. Have urine take for a pregnancy test at each visit.
    v. Receive a pap smear
    vi. Vaginal sampling.

D. Use a reliable form of birth control and keep a pill diary for the duration of the study.
E. Place the four capsules, (curcumin or placebo), into the vagina at bedtime during the course of the study.
F. Undergo cervical sample collection, colposcopy, and cervical biopsy during study visits (every 4 weeks).
G. Give their partner sign a notification letter that informs them of the study and the precautions to be taken during vaginal sex while their partner is in the study.
H. Undergo LEEP procedure.
I. Respond to phone contact twice a week
J. Optional: If the consent, attend a 2-3 hour focus group (maximum of 20 individuals will be selected for) at the end of the study which will be conducted by Dr. Paula Frew. This Focus Group session aims to discuss the acceptability of the product as a treatment for pre-invasive disease using the.

Costs for this study are covered by the study and this includes the associated procedures which are covered by effort by the PI and others listed on the study.

Partners of subjects enrolled in this study

As part of the study, enrolled subjects will be given a partner notification letter informing them on the study, the purpose of this study, route of administration of the capsules, that oral sex is not permitted, and the importance of using condoms for vaginal sex whilst their partner is on the study. The notification letter will also indicate that, male partners may see a temporary discoloration and irritation on their male genetilia if a condom is not used.

Assessment of Compliance

Compliance with weekly intravaginal curcumin/placebo capsules for the duration of the study is measured by the number of nights of reported use as tabulated in the study pill diary.

Endpoints

Subjects will be monitored during the trial for disease progression, adverse events, tolerability and compliance. All study participants will undergo definitive excisional treatment (LEEP) at week 16 of the study period after a 4 weeks washout period. In addition to histology the LEEP specimens will also be tested for biomarkers and curcumin levels.

Samples:

At each visit, cervical biopsies will be divided and sent (1) to pathology for p16 staining to determine the level of dysplasia and grading/documentation of disease and (2) storage for use at a later date for research purposes only. We have designated pathologists, collaborating on this study to ensure standardization of evaluating and grading tissue and other proposed biomarkers.

SCHEDULE OF EVENTS

	Event				
	Screening Visit	**Baseline Visit**	**Study Visit Wk 4, 8**	**Study Visit 12**	**Study Visi Wk 16**
Informed Consent	❖				
Urine pregnancy test	❖	❖	❖	❖	❖
HIV test if status is unknown*	❖				
Full physical exam	❖				
Colposcopy with Pap	❖	❖	❖	❖	❖
Pelvic exam		❖	❖	❖	❖
Vaginal sampling with colposcopy		❖	❖	❖	❖
Targeted cervical biopsies		❖	❖	❖	❖
30 day supply of medication (curcumin/placebo)		❖	❖		
Pill diary review			❖	❖	❖
Partner notification letter (distribution)	❖				
Leep Procedure					❖

All subjects will be asked to complete an acceptability questionnaire at the end of the study.
Focus group: 20 subjects will be invited to attend a focus group to discuss the acceptability of the medication. *HIV test will not be performed if there is a negative test documented in the medical records 3 months prior to screening visit.

SCHEDULE OF EVENTS

Screening Visit

Screening evaluations will determine eligibility and will occur prior to the participants' enrollment into the study. At the screening visit subjects will have:

- A urine test to see if they are pregnant.
- HIV status will be confirmed based on medical records or testing during the screening visit
- They will be asked to agree to the use of reliable birth control: Combined oral contraceptive pill (OCP), Long Lasting Reversible Contraceptive (LARCP), Bi-Lateral Tubal Ligation (BLT) and Depo-Provera Shot/Birth control shot.
- They will be given a partner notification letter about the study.
- A full physical exam will be done.
- A colposcopy with Pap smear to check for the presence of human papilloma virus; vaginal sampling for infection and microbiome to get a baseline state of the vagina.

Study Visits

Baseline and all other study visits will occur after the screening visit.

Baseline:

- Urine test to see if they are pregnant.
- A pelvic exam will be done.
- Vaginal sampling and colposcopy with targeted cervical biopsies will be done.
- They will be given a pill diary and a 4-week supply of the study medication (curcumin or placebo).
- Instruction on insertion of the intravaginal capsules will be given and they will be reminded of potential side effects of the medication.
- They will be asked to return for another study visit 4 weeks later.

Study visits wk 4 and wk 8:

- Urine test to see if they are pregnant.
- A pelvic exam.
- Vaginal sampling and colposcopy with targeted cervical biopsies.
- Their pill diary will be reviewed (aspects of time, frequency and date of distribution of the medication)
- They will be given another 4-week supply of the study medication (curcumin or placebo).
- They will receive a phone call twice a week to address any issues.
- They will be asked to return for another study visit 4 weeks later.

Study visits wk 12:

- Urine test to see if they are pregnant.
- A pelvic exam.
- Vaginal sampling and colposcopy with targeted cervical biopsies.

- Their pill diary will be reviewed (aspects of time, frequency and date of distribution of the medication)
- They will receive a phone call twice a week to address any issues.
- They will be asked to return for another study visit 4 weeks later.

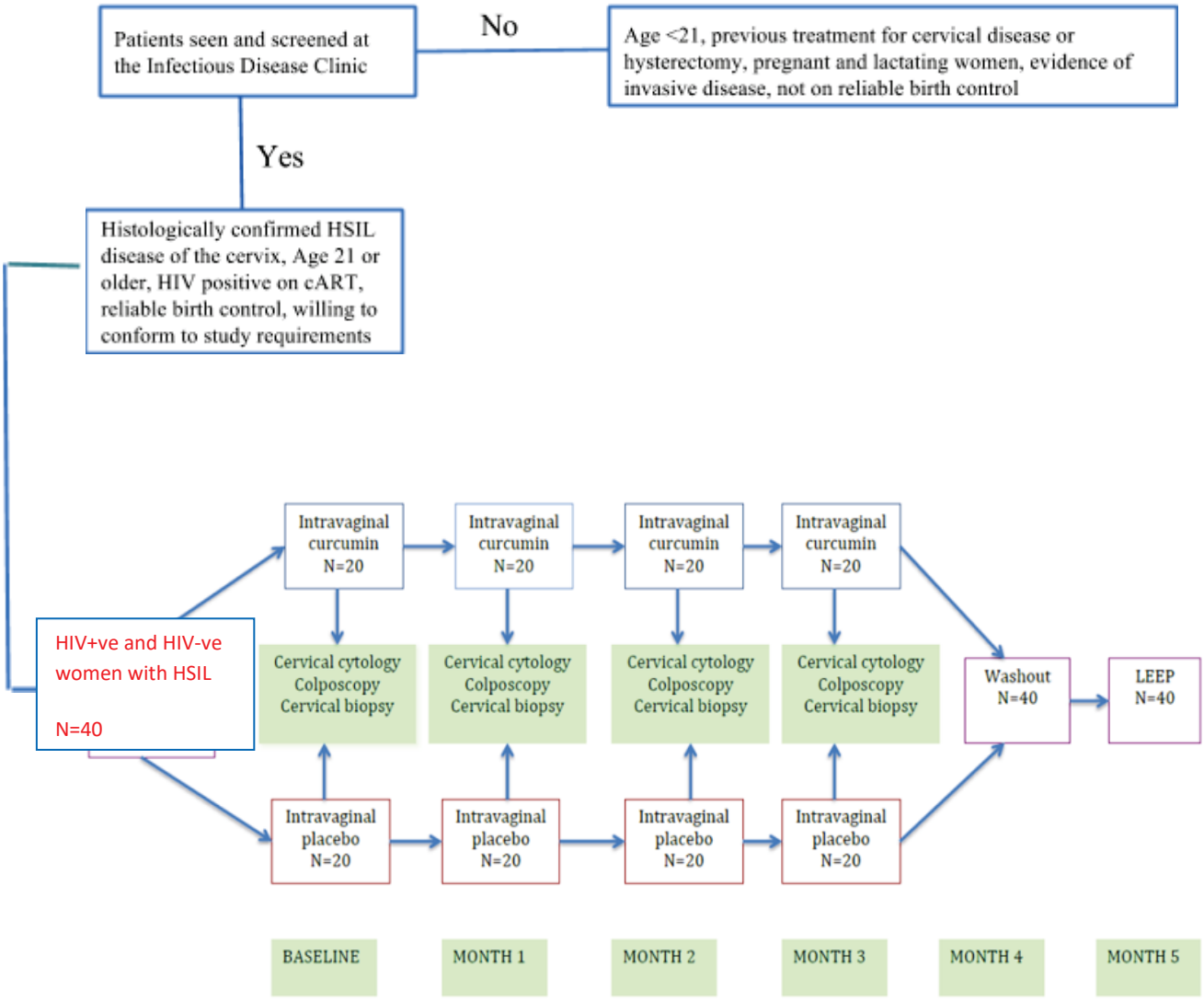
Study visit wk 16:

- Same as above for wk4-wk12, but no further dispensing of a 4-week supply of the study medication (curcumin or placebo) will occur.

Focus Group:

- If subjects consent, a maximum of 20 individuals will be asked to attend a 2-3 hour focus group at the end of the study (after week 16) to discuss acceptability of the product as a treatment for pre-invasive disease.

STUDY SCHEMA



Subjects will be contacted twice a week to ensure adherence
Subjects (maximum of 20 individuals) who consented to participate in the options section, Focus Group Session, will be invited to attend a 2-3hour focus group at the end of the study discussing acceptability of the product as a treatment for pre-invasive disease.

CLINICAL AND LABORATORY EVALUATIONS

1 Clinical Evaluations

1.1 LEEP:

Loop electrical excision procedure of the cervix (LEEP) refers to an outpatient procedure that uses a fine wire loop with low-voltage electrical current to remove, a cone-shaped biopsy of cervical tissue. The procedure starts much like a regular pelvic exam. Subjects will need to lie down on an examining table and put their feet in the stirrups. A speculum is then inserted into the vagina to hold the vaginal walls open the physician can view the inside of the vaginal walls and the cervix. A dilute vinegar solution is applied to the cervix to make the abnormal cells visible and a colposcope is used to visualize the cervix. The cervix is numbed with local anesthesia. An electrically charged loop made of thin wire is inserted through the speculum and up to the cervix. As the loop is passed across the cervix, it cuts away a thin layer of surface tissue, removing the abnormal cells. This tissue is then sent to the lab to be tested for abnormal cells. In some instances, a medicated paste is applied to the area to prevent bleeding. . Subjects may feel some vaginal or pelvic cramping. The tissue specimen(s) will then be sent to the laboratory for microscopic examination by a pathologist.

1.2 Pap with Cervical swabs:

A speculum will be inserted into the vagina to visualize the cervix. A sample of cells from the outer opening of the cervix is collected with a plastic cytobroom/endocervical brush which is rotated in the central opening of the cervix. The cells are placed on in liquid media (SurePath) and ThinPrep (HPV) and taken to the laboratory to be checked for abnormalities /cervical disease and HPV.

1.3 Follow up:

Subjects will be contacted within 48 hours after the start of the medication to inquire on any issues affecting your adherence to the study medication after the baseline visit.
Subjects will receive a call twice a week from week 4 through week 16 to inquire on any issues affecting your adherence to the study medication.
All women will be contacted by telephone to discuss all cytology and histology results and Cytology and histology results will be available for all study participants PCP if a medical release waiver is signed by the subject. We will be following these subjects every month with colposcopy which is more than sufficient to capture any women at risk for progression and they will be referred to treatment is progression of disease occurs.

2 <u>Laboratory Evaluations</u>

2.1 Pap and HPV testing will be performed by Grady pathology lab.

2.2 The APTIMA® HPV Assay (HPV viral load quantitative/ Detection of HPV mRNA Levels)

The Aptima HPV assay offers a unique screening approach by targeting high-risk human papillomavirus (HPV) mRNA from the E6/E7 oncogenes. The Aptima HPV assay offers the same excellent sensitivity and improved specificity as compared to DNA-based tests. The expression and function of HPV E6/E7 a will be conducted in the lab of Dr Sidell.

STATISTICAL ANALYSIS:

Aim1: To determine the association between intravaginal curcumin on known HPV-related molecular target HPV E6/E7 mRNA expression within HSIL lesions of the cervix in HIV uninfected and infected women. Biomarkers are known to be differentially regulated in cervical disease. Current literature shows that HPV E6/E7 mRNA, NF-κB, p16INK4a and VEGF levels are up-regulated, whereas p53 levels are down-regulated in cervical disease. Biomarker outcome, defined as the level of mRNA and/or protein in the samples collected, will be summarized with standard descriptive statistics and represented graphically with displays such as box plots by treatment group. For this study the biomarkers whose levels will be quantified are as follows:

- Primary endpoints: Human papillomavirus *E6/E7* (HPV E6/E7)
- Secondary endpoints: NF-κB, p16INK4a, Rb, p53 and VEGF.

HPV E6/E7 mRNA testing has been demonstrated to be a useful tool in the diagnosis of cervical cancer prevention and will be the primary end-point for this aim. The primary objective for this study is to look at the association between curcumin and cervical disease. In vitro work shows that curcumin treatment inhibits the transcription of HPV E6/E7, thus restoring the expression of proteins such as p53 and that over expression of HPV E6/E7 is a good indicator of risk and progression to cancer. (Maher DM, 2011 Jan;50(1)). Collectively the vitro and our in vivo findings may show that curcumin should be considered an effective chemopreventive and therapeutic agent for cervical cancer prevention and treatment.

Aim2: To quantify levels of curcumin in the cervix of women in the curcumin treatment arm. The mean tissue concentration of curcumin in the cervix at each time point (baseline, 1,2,3,4, and 5 months) will be calculated. This secondary objective quantifies the pharmacologically active levels of curcumin recovered from cervical tissue, for information to be gathered on the levels of curcumin that is absorbed into the cervical tissue and thus eliciting a direct pharmacological effect.

Aim 3: To determine the association between curcumin and other known biomarkers of cervical disease. This exploratory aim seeks to use repeated-measures analyses, utilizing a linear mixed model for the curcumin group to assess the association between and each biomarker (NF-κB, p16INK4a, Rb, p53 and VEGF) and the concentration of curcumin at each time point (baseline, 1, 2, 3, 4, and 5 months).

The primary analysis will be conducted with a two sample two sided t-test to compare the each of the primary endpoints and secondary endpoints, between the two arms of the study, those on treatment (curcumin capsules) and those on the placebo, respectively. If normality cannot be assumed a Wilcoxon rank-sum test will be used instead. Chi-square test or Fisher's exact test will be used to compare categorical characteristics by treatment group.

In the secondary analyses, for the biomarker measurements, analyses of repeated measures methods are needed to properly account for the correlation between multiple observations from the same subject. Mixed-effects linear models will be employed to analyze such data while adjusting for covariates. Inference from such analyses will be limited by the small sample size of the study. However these data will provide important estimates of within-and between-subject variance that will be helpful for planning future studies. Repeated-measures analyses using mixed linear models will be performed for each biomarker outcome variable (E6/E7, p16INK4a, p53, Rb NF-κB, and VEGF). Each outcome will be analyzed with a means model with SAS Proc Mixed (version 9) providing separate estimates of the means by treatment group (curcumin and placebo) and time on study (baseline, 1,2,3,4, and 5 months). An unstructured variance-covariance form among the repeated measurements will be assumed for each outcome and estimates of the standard errors of parameters will be used to perform statistical tests and

construct 95% confidence intervals. The model-based means are unbiased with unbalanced and missing data, so long as the missing data are non- informative (missing at random). A p-value ≤ 0.05 will be considered statistically significant for the main effects (treatment and time on study) and interaction term (treatment group by time on study) from the repeated measures analysis for each outcome. If there is a significant interaction effect then separate comparisons between the two treatment groups will be performed at each time point (baseline and 1,2,3,4, and 5 months). Results from these models will focus on the magnitude of the differences for each outcome. Data will be summarized using adjusted means plus 95% confidence intervals and observed differences plus 95% confidence intervals.

Additionally these biomarkers will also be quantified using immunohistochemistry methods. Immunostaining of p16 (NK4a) will be done and defined as moderate to strong diffuse or focal staining. This characterization of the staining pattern and intensity will allow one to evaluate the histological sample/biposy as having a negative, focal/patchy, or diffuse staining pattern. Generally, 100% of the low-grade and high-grade SIL's are positive for p16 staining and a strong diffuse staining with p16 suggests a high association with high-risk HPV associated lesions.

Acceptability Questionnaires: Descriptive statistics on subject and partner responses from the acceptability questionnaires will be reported.

Power and sample size calculation: The expression of E6/7 is the primary endpoint of the study and will be used in the power and sample size calculation with a two sample two sided t-test. In this study, we will enroll 20 patients in each of the two groups. Therefore, the group sample sizes of 20 in Curcumin group and 20 in placebo group will achieve at least 80% power at the significant level of 0.05 to detect a difference of a ratio of 1.536 or higher in the expression of E6/ between the two groups, assuming the coefficent of variation is 0.5.

SAFETY EVALUATION:

Adverse Events (AEs) and Serious Adverse Events (SAEs)

This investigational agent is studied under IND # 103496 and monitoring will be performed per previously agreed upon FDA guidance.

There may be some minor irritation from the intravaginal curcumin to the vaginal mucosa and staining of underwear. Other side effects which can happen include the upset stomach, diarrhea, burning sensation in the vagina, dizziness, insomnia, rash, pruritus generalized, and fever. The literature shows that over 13 subjects in United States and 151 subjects in India (10) have been studied with curcumin without any serious adverse effects.

Our phase I study has shown that curcumin is safe in dosage up to 2000mg.

Any patient intervention related adverse effects, an unanticipated problem, or reportable protocol deviation/ non-compliance will be reported to the Emory IRB office according to IRB P & P and to the FDA according to regulations.

Any serious adverse effects will be communicated by the principal investigator to the Emory IRB using the standard adverse event reporting forms.

SAEs will be defined per FDA standards and include those that result in death, are life threatening, result in a disability, cause for hospitalization, or to a congenital abnormality or birth effect. SAEs will be reported to the DSMC, IRB and FDA and manufacturer as required.

CLINCAL OUTCOMES AND MEASURES:

The table below gives the descriptions to be used to document findings during colposcopy.

Colposcopic findings				**Presence**	
	Grade 1 (Mild) *Definitions provided*	**Grade 2 (Moderate)** *Definitions provided*	**Grade 3 (Severe)** *Definitions provided*	**Present**	**Absent**
Peeling	If both the epithelium and the blood vessels are intact	If either the epithelium is superficially disrupted or the blood vessels are disrupted	If they involve disruption of the epithelium; an area of the epithelium with bleeding should be considered deeply disrupted		
Ulcer					
Abrasion					
Ecchymosis					
Petechiae					
Erythema					
Edema					
Grossly white lesion					
Sloughing					
Laceration					

Colposcopic findings (present or absent) will be summarized at each visit. Findings will be counted once, attribute to the time point/visit at which they were first detected, and described on the basis of their characteristics when they were first detected. The same criteria outlined in the WHO Update Colposcopy Manual to assess toxicity and mucosal damage to the cervix, vagina and perineum will be carefully followed.

DATA AND SAFETY MONITORING PLAN (DSMP):

The primary risk to this study relates to potential complications with vaginal sampling, vaginal mucosa irritation and colposcopy with targeted cervical biopsies/LEEP. Dr Flowers will monitor subject safety and respond to occurrences if AEs/SAEs in a timely manner.

The Data and Safety Monitoring Committee (DSMC) of the Winship Cancer Institute will provide oversight for the conduct of this study. The DSMC functions independently within Winship Cancer Institute to conduct internal monitoring functions to ensure that research being conducted by Winship Cancer Institute Investigators produces high quality scientific data in a manner consistent with good clinical practice (GCP) and appropriate regulations that govern clinical research. Depending on the risk level of the protocol, the DSMC review may occur every 6 months or annually.

For this study: deemed Moderate Risk, initial study monitoring will occur within 1 year from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. Subsequent monitoring will occur in routine intervals per the Winship Data and Safety Monitoring Plan (DSMP).

The DSMC will review pertinent aspects of the study to assess subject safety, compliance with the protocol, data collection, and risk-benefit ratio. Specifically, the Winship Cancer Institute Internal Monitors assigned to the DSMC may verify informed consent, eligibility, data entry, accuracy and availability of source documents, AEs/SAEs, and essential regulatory documents.

Following the monitoring review, monitors will provide a preliminary report of monitoring findings to the PI and other pertinent individuals involved in the conduct of the study. The PI is required to address and

respond to all the deficiencies noted in the preliminary report. Prior to the completion of the final summary report, monitors will discuss the preliminary report responses with the PI and other team members (when appropriate). A final monitoring summary report will then be prepared by the monitor.
Final DSMC review will include the final monitoring summary report with corresponding PI response, submitted CAPA (when applicable), PI Summary statement, and available aggregate toxicity and safety data. The DSMC will render a recommendation and rating based on the overall trial conduct. The PI is responsible for ensuring that instances of egregious data insufficiencies are reported to the IRB. Continuing Review submissions will include the DSMC recommendation letter. Should any revisions be made to the protocol-specific monitoring plan after initial DSMC approval, the PI will be responsible for notifying the DSMC of such changes. The Committee reserves the right to conduct additional audits if necessary.

Adverse events observed or reported by subjects will be monitored by the physicians from this study and will also be reviewed by professional colleagues within infectious disease, GynOb and pathology. Subjects will be given the contact phone numbers to call if they experience any problems (increased discharge, vulvo-vaginal irritation). They will be followed routinely, every 4 weeks by the physicians using colposcopy, biopsy and cytology to identify any high risk features or progression of the disease.

Stopping/Pausing Rules

Symptom or AE	**Toxicity Grade**	**Duration**	**Criteria**	**Action required**
Colposcopic findings	• Grade II or greater • Grade I not resolved in 2 weeks	• N/A	One participant report the product-related symptoms at a specified grade	Prompt notification of DSMB, IRB, NIH, FDA and CTRC. All product application is help until further notice, follow up and resolution

This study does involve intervention with a product and the protocol does require the collection of sensitive and confidential information, biological samples (genital tract), physical examination, colposcopy and pharmacological intervention (curcumin). As with any study that includes these procedures there may be side effects from the study procedures that are not known at this time. However, for those common risks and discomforts expected in this study they are outlined in the consent forms, and also listed below with the methods to minimize any associated risk:

1: Physical exams

Participants may feel some minor physical discomfort. This should be minor and will go away quickly. All physical tests and examinations will be performed by a trained and experienced health care practitioner.

2: Biologic Sample collection (genital tract sample collection) and colposcopy with biopsy:

For genital tract sample collections, participants may feel some minor physical discomforts and experience spotting or bleeding from cervical biopsy collections.

3: As this study involves pharmacological intervention subjects will be administered a pregnancy test will be administered by an experienced health care practitioner before enrollment and at each study visit during the course of the study.

4. This study requires intravaginal placement the capsules (curcumin or placebo). Pill diaries will be given and monitored at each visit. Subjects will also be contacted twice a week to ensure adherence and be given the ability to report any adverse events/side effects like vaginal discharge and vulvo-vaginal irritation.

5. Subjects will be informed of potential risks and sign a written informed consent for participation. Subjects will be asked to agree to the use of reliable birth control: Combined oral contraceptive pill (OCP), Long Lasting Reversible Contraceptive (LARCP), Bi-Lateral Tubal Ligation (BLT) and Depo-Provera Shot/Birth control shot.

5b. Partner notification letters will indicate the need for condom use and the risk of discoloring and/or irritation if a condom is not used when having vaginal sex with an enrolled female participant.

6. Patient confidentiality will be protected by identifying study subjects and samples by code number. Study records will be kept on password protected computers or in a locked office. Only the study coordinators, Nurse Practitioners, Interviewers, and PIs will have access to de-identified information. Collaborators and staff outside Emory listed on the study will not have access to HIPAA protected information.

7. Careful training of study staff on ethical research practices, informed consent, and confidentiality. The Investigative team will meet on a bi-weekly basis during subject enrollment and monthly to review AEs and any SAEs, discuss recruitment, drop-outs/withdrawals and any protocol problems.

8. Summaries of adverse events (Grades 3 or 4) and other targeted AEs will be reviewed regularly by study investigators. The standard Emory IRB and FDA reporting guidelines for adverse events will be followed.

Written IND safety reports will be submitted to the FDA for serious, unexpected suspected adverse reactions within 15 calendar days of learning of its occurrence. If the event is fatal or is deemed to be life threatening, the report will be made within 7 calendar days. The IND sponsor will also make an assessment of whether the event constitutes an unanticipated problem posing risks to subjects or others (UP). This assessment will be provided to the Emory University IRB, which, in turn will make a final determination. If the Emory IRB determines an event is a UP it will notify the appropriate regulatory agencies and institutional officials.

Standard procedures will be followed to ensure the safety and security of subject information. Throughout this process, patient confidentiality will be maintained via de-identification of patient information. Electronic information will be stored only on password protected media, and any required paper documentation will be kept in a locked and secured location.

CLINICALTRIALS.GOV REGISTRATION

We will ensure to submit the required clinical trial information prior to enrollment of the first participant for this study.

HUMAN SUBJECTS

I. 1 Responsibilities of the Investigator

In implementing this protocol, the investigators will adhere to the basic principles of "Good Clinical Practice" as outlined in CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," CFR 21, part 50 and CFR 21, part 56 and Section 4 of ICH Harmonized Tripartite Guideline for GCP.

II. IRB Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the Emory University IRB. A signed and dated, Emory IRB-approved consent form will be obtained from the subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A signed copy of the consent form will be given to the subject, parent, or legal guardian, and this will be documented in the subject's record**.**

III. 8.3 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by coded number to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB or the OHRP.

IV. 8.4 Study Discontinuation

A study participant may elect to discontinue participation in the study at any time. The study may be discontinued at any time by the IRB, the OHRP, or other government agencies as part of their duties to ensure that research subjects are protected.

BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72.

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