The Cervical cancer Screening and Treatment algorithms study using HPV testing in Africa (CESTA)

Multi-country generic protocol

June 2019

(v4.0)

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1. Executive Summary

The main objectives of CESTA are (1) to compare the efficacy of two cervical cancer screening algorithms: HPV test followed by visual inspection with acetic acid (VIA) and treatment (HPV + VIA + treat) and HPV test followed by immediate treatment (HPV + treat). The study will be conducted to address its objectives in women living with HIV (from now on called HIV positive women) and HIV negative women; (2) to compare the cervical intraepithelial neoplasia grade 2 or 3 (CIN2/3) cure rates of treatment with cryotherapy and thermal ablation (TA) 12 months after the treatment in HIV positive women; and 3) to model the comparative cost-effectiveness of the two screening and treatment strategies described in (1) and the 2 treatment methods described in (2).

The study will be carried out through 2 modules as the study outlines differ considerably for the Algorithms objective (Obj 1) and the cure rate objective (Obj 2). The cost-effectiveness study (Obj 3) will be carried out in both modules with the epidemiological comparisons aligned with the respective modules.

Module 1 will study the main objectives 1 and 3 among 3,000 HIV positive women in South Africa and 7,500 HIV negative women in Senegal.

HIV positive women will be recruited from HIV care clinics, also called antiretrovirals (ARV) clinics in the Durban area, South Africa. After giving informed consent, women will be screened with HPV testing and those that are HPV positive will be randomized at a 4:1 ratio into HPV + VIA + treat (Arm 1) and HPV + treat (Arm 2) in 1 of the 2 CESTA study clinics. Women in Arm 1 will receive VIA and only positive for VIA will be treated. In Arm 2, all HPV positive women will be treated. Women that are eligible for ablative treatment will be randomized into treatment with TA or cryotherapy in both arms. Others will be referred to colposcopy. After VIA in Arm 1 or before treatment in Arm 2, the nurses will collect 2-4 biopsies on all HPV positive women. The biopsies will be used as gold standard for disease detection. The first 200 women treated will be followed up intensely for 4 weeks to assess side-effects and levels of HIV shedding. Subsequently treated women will be called by telephone after 1 week and 1 month to assess side-effects and satisfaction with the procedures. Smartphone enhanced VIA (SEVIA) with patient navigation will be used in 1 of the 2 study clinics. 2-3 Pictures are taken with the SEVIA smartphone tool after application of acetic acid to the cervix. The pictures are sent in a secure way to expert reviewers who provide feed-back to the nurse in real time. The application can also be used to track adherence of the

women to the screen and treat procedures or referral and send automated reminders of appointments. Outcomes on performance of VIA and patient adherence will be compared between study clinic that uses SEVIA and the one not using SEVIA. All women who were HPV-positive or were treated will be called back after 1 year for a follow-up visit. Women will be screened with HPV testing and VIA and 2-4 colposcopy-directed biopsies will be taken from all HPV positive women. Women with remaining/recurrent CIN2+ disease will receive appropriate management.

HIV negative women will be recruited from the communities in the Dakar area. They will be screened in 1 of 4 CESTA study clinics. Procedures will be similar as in HIV positive women, except that the randomization rate will be 3:1 for Arm 1 and Arm 2 and that the women eligible for ablative treatment will be all treated with TA. SEVIA will be applied in 2 of the 4 study clinics to compare SEVIA outcomes in those clinics using it or not. Follow-up of treatment side-effects and women's satisfaction will be by telephone after 1 week and after 1 month. Similar to HIV positive women, all HPV positive women or those treated will be invited for a 1-year follow-up visit with the same procedures as described above.

The cost-effectiveness study will answer questions on the comparative value and efficiency of the two screening arms and two treatments respectively, using epidemiological data collected through the clinical study for the effectiveness portion of the analysis. Cost data collected through questions embedded in the clinical questionnaires and by more in-depth but limited interview will be supplemented by administrative data where necessary to form the cost portion of the analysis. The study will use a well-validated cervical cancer model.

Module 2 will study the main objectives 2 and 3 among 920 HIV positive women with CIN2/3 in South Africa. An estimated 143 women will have CIN 2/3 on biopsy from Module 1 and can be directed to participate in Module 2. A remaining 777 women with CIN2/3 will need to be recruited through 8-9 ARV clinics. Since 2018, women are screened routinely with liquid based cytology (LBC) in South Africa. We will invite women with LBC results of low-grade squamous intraepithelial lesions or worse (LSIL+) to participate in the CESTA Module 2 study in 1 of the 2 CESTA study clinics dedicated to Module 2. After giving informed consent, 2-4 cervical biopsies will be taken by the study nurses. Women with biopsy results <CIN2 will be referred back to the ARV clinic for routine cervical cancer screening. Women with CIN2/3 and that are eligible for ablative treatment will be randomized into TA and cryotherapy. Others will be referred to

colposcopy for appropriate management. Similar to Module 1, women will be called by telephone after 1 week and 1 month to assess side-effects and satisfaction with the treatment.
All women who were CIN2/3 and were randomized to ablative treatment or referred to colposcopy will be invited for a 1 year and 2 year follow-up visit. Similar as in Module 1, Women will be screened with HPV testing and VIA and 2-4 colposcopy-directed biopsies will be taken from all HPV positive women. Women with remaining/recurrent CIN2+ disease will receive appropriate management.
The cost-effectiveness study will assess the comparative value and efficiency of the two treatment methods.

2. Background

2.1. Clinical need

Cervical cancer remains a serious public health problem, with more than 500,000 new cases and 300,000 deaths occurring every year, particularly in developing countries where 87% of cases occur. Although rates of cervical cancer vary considerably, cervical cancer ranks first or second in all individual sub-Saharan countries, while it is the leading cause of cancer deaths at 56,444 cases per year [1] in the subcontinent. Cytology based cervical cancer screening programs have successfully reduced cervical cancer incidence in developed countries but, with few exceptions, not in low- and middle income countries (LMIC). Programs using cervical cytology, which detects cellular changes indicating cervical intraepithelial neoplasia or cancer, are very complex to implement properly and the method has limited sensitivity and low reproducibility, which imposes the need for repeating tests frequently. This results in high cost and logistic complications which hamper the implementation and success of cytology-based screening programs in LMIC. Alternative screening methods have been developed, the two most studied being testing for human papillomavirus (HPV) and visual inspection with acetic acid (VIA). VIA can be implemented at low cost and allows for an immediate result to be given to the patient. This can be followed by a treatment at the same visit. However, VIA is operator dependent, lacks reliable means of quality assurance control, has a low positive predictive value and results in overtreatment of large numbers of women [2].
It is now clear that a group of 13 human papillomavirus (HPV) types are the causal agents of cervical cancer and that HPV 16 and 18 are responsible for about 70% of all cervical cancer.

HPV is a very common infection usually acquired shortly after initiation of sexual activity, but most infections are cleared by the immune system within 2 years after acquisition and only a few persist and progress to cancer.

The recently introduced HPV vaccine has the potential to prevent 70% of cervical cancers, and a newer version has the promise to offer even more protection against cervical cancer. Vaccination needs to take place before the beginning of sexual activity, the target age recommended by the WHO for vaccination being 9-14 years old [3]. The protective effect on cervical cancer incidence, however, will only be noted several decades later, as the peak of cervical cancer incidence is in women in their mid-forties. Even in the most optimistic scenario where vaccination coverage in the target age group would reach 100% within the 10 coming years in all countries of the world, based on Globocan data we estimate that nearly 28 million women will still be affected by cervical cancer in the coming 40 years [1].

Therefore cervical cancer screening programs remain a high priority for most countries but especially for developing countries, where most cervical cancer cases occur.

In 2014 WHO updated its evidence-based recommendations for comprehensive cervical cancer control including vaccination, screening, treatment and palliative care [WHO 2014]. An important gap in the evidence, emphasized by the international expert panel, was the lack of clinical data directly comparing screening by HPV testing with or without VIA triage testing in HIV negative as well as HIV positive women. Notably data on the benefits, side-effects and cost-effectiveness of the screen and treat algorithms using these tests were lacking [4]. This lack of evidence results in recommendations on the use of algorithms without indications on what would be the most cost-effective approach in which conditions.

Therefore, the 2 screen and treat algorithms of interest that need to be carefully compared and evaluated in order for WHO to provide strong recommendations to low- and middle-income countries (LMIC) are: (1) Screening by HPV test followed by VIA for triage of HPV positive women and; (2) Screening by HPV test alone; each followed by ablative treatment of screen-positives. We will also compare two ablative treatment methods for cervical precancer: cryotherapy and the novel thermal ablation. WHO has recently recommended that TA or cryotherapy can be used to treat eligible cervical legions, however these recommendation were based on weak evidence in HIV negative women and non-existent evidence for HIV positive women.

Each of the aims will be evaluated in different modules: Mod 1, a randomised controlled trial (RCT) to compare the efficacy of the two screen-and-treat algorithms, and Module 2, another

RCT to compare the 12 and 24 months CIN2+ cure rates of each ablative treatment. The first module will be carried out among HIV positive and HIV negative women in South Africa and Senegal, respectively. The comparison will be based on HPV positive women with histological high grade squamous intraepithelial lesions or worse (HSIL+) adequately treated, those left untreated and those who were treated and did not have confirmed disease (overtreatment) randomized to each algorithm.

The second module will be carried out in HIV positive women with histologically diagnosed CIN2/3 at the time of randomization. The third aim on cost-effectiveness will be evaluated within each module to provide a complete picture on cervical cancer screening strategies in the context of the cervical cancer elimination initiative.

2.2. HPV and cervical cancer in HIV-positive women

Most healthy women clear HPV infection on their own, while clearance in women with human immunodeficiency virus (HIV) is more limited, and women with HIV are more likely to experience progressive cervical changes [5]. Studies suggest that HIV-infected women were respectively 1.8, 2.1 and 2.7 times more likely to display high, intermediate and low risk HPV infections in the cervix than HIV uninfected women [5, 6]. Thus, HIV-positive women have higher rates of HPV persistence and progression to invasive cancer when compared to HIV-negative women and in sub-Saharan Africa, HIV-infected women are 6 times more likely than HIV uninfected women to develop cervical cancer [7].

The effect of antiretroviral therapy (ART) on cervical cancer precursor lesions (CIN2+) was evaluated in a recent meta-analysis. It was found that women living with HIV on ART have a lower prevalence of both high-risk HPV and CIN2+ lesions, compared to HIV positive women not on ART. Furthermore, a reduction in the incidence and progression of CIN2+ lesions was seen in women on ART, and an increase in regression of CIN2+ lesions. Notably, these effects remained after adjusting for immune restoration indicators such as CD4 cell count and duration of ART use [8].

HIV positive women typically have more remaining/recurrent disease after treatment of pre-cancerous lesions. These will be discussed in the sections of the respective treatments.

2.3. HPV testing in cervical screening

Practically every invasive cancer and the vast majority of CIN 2/3 express high-risk (hr) HPV [9]. Currently, there are several laboratory tests for detection of cervical HPV infection which have high sensitivity and are highly reproducible. These tests are recommended by WHO and considered to replace cervical cytology for primary screening. More and more countries are introducing HPV tests in their programme. They are highly sensitive to detect cervical cancer precursors (sensitivity typically >90%), highly reproducible, and have the capacity to detect more disease at an earlier stage [10]. In addition, HPV testing has a high negative predictive value that allows extension of the screening interval, with consequent savings that can compensate the possibly higher cost of the test compared to cytology or VIA. An additional advantage of HPV testing is the possibility to use vaginal specimens that are self-sampled by the woman. It has already been shown that this approach can increase the coverage of a screening program [11]. Recently, several HPV tests have been developed that are technically easier to implement and provide results more rapidly, compared to the conventional HPV tests. Two of those tests, the *care*HPV test (Qiagen) and HPV GeneXpert test (Cepheid) were recently prequalified by the WHO in-vitro diagnostics group, which would make procurement easier for middle and low income countries.

Screening with HPV has the problem that transient HPV infections are very common, particularly among young women, where the majority of infections will regress spontaneously. Even among women over 30 years of age, HPV infection still tends to regress and only in a fraction of cases with persistent infection, it can lead to true cancer precursors and cervical cancer. Thus, one of the main issues to resolve is how to triage HPV positive results, i.e. to identify, for further evaluation and treatment, those HPV-positive women who are most likely to have or to develop in the near future significant disease that requires treatment. Direct treatment of HPV-positive women results in overtreatment, as the positive predictive value of HPV testing is around 15%. In general, a degree of overtreatment with cryotherapy is accepted because the treatment is safe with no adverse effects, well tolerated and serious side effects such as cervical stenosis are extremely rare [12]. In addition, ablation treatment of the cervix may have a prophylactic effect on future infections with HPV. Recent work has also shown that the junction between the ecto- and endocervix harbours embryonic reserve cells that have the potential to transform into neoplastic cells when infected with oncogenic HPV, and that their destruction may be protective of future cervical neoplastic developments [13]. In high-income

countries (HIC), triage of HPV positive women is usually done by cytology. In LMIC, where it is challenging to implement good quality cytology, triaging by visual inspection with acetic acid (VIA) is recommended, as well as no triage approach [4]. One of the major concerns is that the addition of VIA which has a known limited sensitivity may jeopardize the overall performance of both tests combined.

2.4. VIA testing in cervical screening

VIA is a technique in which cervical neoplastic lesions are visually diagnosed after application of 3-5% acetic acid without using any magnification device. The examination can be performed by any trained health worker, mostly nurses. The training in VIA is still not standardized and especially supervision after initial training is very difficult to organize. Therefore the technique remains subjective as it is based on the interpretation of what is seen by the health care provider after application of the acetic acid on the cervix. As a result, the reported test characteristics of VIA vary greatly,. One recent multi-country study in Nicaragua, Uganda and two sites in India, reported sensitivity between 22% in one site in India and 74% in Uganda with specificity of 95% and 67%, respectively, and associated positivity rate of 6% and 34%, respectively [14]. One advantage of the technique is that it allows a single-visit approach in which women are screened and treated with ablative treatment (cryotherapy or thermal ablation) at the same clinic visit.

In case of a screen and treat algorithm that does not involve VIA (e.g. HPV testing), a variant of VIA is applied before ablative treatment, in order to assess that the woman does not have any condition that would make her ineligible for ablative treatment, such as a lesion or squamo-columnar junction (SCJ) that reaches endocervically, an apparent lesion that covers >75% of the ectocervix, and suspicion of invasive cancer. All other cases are amenable for treatment, even without visible lesion.

2.5. Treatment of pre-cancerous lesions

Recent WHO guidelines recommend ablation by cryotherapy or thermal ablation, and excision by large loop excision of the transformation zone procedure (LLETZ, also called LEEP) as different forms of out-patient surgical treatment of CIN2/3.

2.5.1. Cryotherapy

Cryotherapy consists of freezing the lesion and underlying tissue to a temperature of at least -20° Celsius. The treated tissue necrotises and is replaced by new healthy epithelium. A reusable probe is applied to the cervix and the freezing is obtained through decompression of either carbon dioxide (CO2) or nitrous oxide (N2O) gas. The main side-effect is a watery vaginal discharge for up to 2-3 weeks, which is generally well tolerated. The equipment is simple, no electricity is needed and the technique is relatively easy to perform. Cryotherapy can be provided by a variety of healthcare personnel including general doctors, nurses, midwives and auxiliary health workers who can be trained in short sessions. For those reasons, cryotherapy is very suited for LMIC. In addition, it can also be used in a single visit screen-and-treat approach in combination with a screening test that provides immediate results, such as VIA. One potential impediment of the technique is the inadequate availability or elevated cost of the freezing gas. Lesions that are not eligible for cryotherapy are those that reach >2mm in the endocervical canal; are larger than the cryoprobe; cover more than 75% of the ectocervix; and should be treated with LLETZ. Lesions that are suspicious of invasive cancer should be biopsied and treated according to diagnostic work up.

A meta-analysis showed that cryotherapy is 92% and 85% effective to eliminate CIN2 and CIN3, respectively (cure : normal cytology at follow-up) [15].

In a recent meta-analysis of studies reporting cryotherapy and LLETZ in HIV positive and HIV negative women, Debeaudrap P. *et al.* observed that residual or recurrent high-grade lesions were detected in 21.6% of women treated with cryotherapy, compared to 12.6% after LLETZ among HIV-positive women treated for CIN2+/HSIL+ [16]. These patients were twice as likely to suffer from treatment failure compared to HIV-uninfected women. The authors mentioned that there were no studies reporting on cure rates of thermal ablation in HIV positive women fitting the inclusion criteria of the meta-analysis. Greene S. *et al.* recently reported a RCT among 400 HIV-infected women with cervical intraepithelial neoplasia CIN 2/3 disease that were randomized to receive cryotherapy or LLETZ. At 12-months, more women treated with cryotherapy experienced recurrent HSIL than those who underwent LEEP (27% vs 18%; P=0.031). At 24 months, HSIL increased in both arms and remained significantly higher in the cryotherapy arm (37% vs 26%; P=0.018). Overall, the rate of recurrence of HSIL+ was

21.1 per 100 woman-years after cryotherapy and 14.0 per 100 woman-years after LLETZ [Greene S. *et al.* Cryotherapy versus loop electrosurgical excision procedure among HIV-infected women (JAMA (in press)).

2.5.2. Thermal ablation

Thermal ablation is another ablative treatment for CIN, also previously called 'cold coagulation'. The equipment is fairly simple and treatment is based on a 20-40 seconds application (multiple if needed) of a reusable metallic probe that is electrically heated to 100° Celsius, leading to epithelial and stromal destruction of the lesion. The equipment weighs less than 4 kg, which makes it easily portable to field clinics in developing countries. Recently light weight hand held and battery operated models have been developed. Like cryotherapy, thermal ablation can be provided by a variety of healthcare personnel including primary healthcare workers and is typically performed without anaesthesia. The WHO guideline review committee has recently approved recommendations for the use of TA, which will soon be officially published. Similar to cryotherapy, TA is recommended for CIN2/3 lesions, confirmed by histology or not in the context of screen and treat programs.

A previous meta-analysis by Dolman et al. [17] was recently updated by Randall et al. [18]. The update includes 23 reports among 6371 patients treated for histologically confirmed CIN2/3. Lesions were considered as cured if follow-up colposcopy/biopsy was <CIN2 or normal follow-up cytology. Overall cure rate for women treated for CIN2/3 or CIN3 were 94% and 89%, respectively.

The WHO guideline development group concluded that comparing the effects of thermal ablation to cryotherapy indirectly, there is moderate certainty evidence that the benefits and harms of these two treatments were similar. The great majority of these studies were carried out among HIV negative women.

In some studies, especially those from Scotland and UK, used a 2-probe method, in which treatment of the visible glandular epithelium with a small conical probe followed by treatment of the ectocervix with a flat probe. Other studies reported the use of a one-probe method. The WHO expert guideline development group concluded that although application methodology was not always clearly described in the reports,

evidence showed that more women may be cured with a 2-probe method (95%; 95%CI, 93-98%) than a 1-probe method (85%; 95%CI 80-90%).

Although the WHO TA recommendation extended to HIV positive women, it was recognized that any recommendation for this group was based on very low certainty evidence. In fact, there were no comparative studies evaluating the benefits and harms of thermal ablation compared to other treatment methods or no treatment in HIV positive women with histologically confirmed CIN 2-3. Assessment of cure of cervical lesions is complicated in HIV positive women and the utility of HPV testing in this context is limited. Thermal ablation, having a different mechanism of action compared to cryotherapy, may perform differently in this group. In fact, the efficacy of cryotherapy in HIV positive women has been shown to be limited. The recent WHO guidelines stress that the need for comparative studies is urgent particularly in women living with HIV, where there is little information about cures with thermal ablation, and no information about other important outcomes, such as HIV shedding or risk of transmission after treatment.

2.5.3. Large loop excision of the transformation zone (LLETZ) procedure

Excisional treatment by LLETZ uses a high-voltage electric current to excise the cervical neoplastic tissue and the transformation zone. The cutting electrodes are loops of very fine (0.2 mm) metal wire to achieve different widths, depths, and configurations of cut. LLETZ is the most used out-patient excisional treatment modality of CIN in HIC. The LLETZ equipment includes an electrical generator and a smoke evacuator to evacuate fumes that obliterate the view of the operating site and may contain the HPV virus particles. The wire loops are mostly dispensable single use and not always easily available in LMIC. Treatment by LLETZ requires much higher surgical skills on the part of providers and equipment infrastructure than required for ablation techniques such as cryotherapy or thermal ablation. They are mostly performed by doctors, although in some settings, specially trained nurses may provide LLETZ. Though this technique may be slightly more effective in the treatment of CIN2/3, it is more difficult to implement and therefore cryotherapy is currently more practiced in LMIC for the treatment of CIN2/3. In those countries, LLETZ is mostly used at a referral level for the treatment of cases that are not eligible for cryotherapy.

2.6. Smartphone enhanced visual inspection with acetic acid (SEVIA)

The performance of VIA is very subjective and variable and there is to date no standardized way to provide supervision after initial VIA training to the health care provider. The SEVIA platform is developed to manage and to send cervical VIA images to in a secure way to certified reviewers in order to provide feed-back and online supervision to the nurses [19]. The cervical images sent are reviewed within a few minutes by “expert” skilled health providers, so the health workers (in most cases, nurses) receive real-time supportive supervision. In this manner, nurse providers are able to offer high quality cervical cancer screening services without requiring in-person supervision. The non for profit platform was originally developed in partnership with the Ministry of Health in Tanzania and researchers from Queens University in Canada. The success of the SEVIA program has now resulted in its integration into the Tanzanian National Cervical Cancer Program for active (real-time) data management and related processes. SEVIA is now in high-demand, being adapted where necessary and trialed in six other countries in East and West Africa.

The costs of taking SEVIA to scale are modest, and when the platform is used at scale, the cost ‘per woman screened’ drops as screening numbers increase. SEVIA will soon be evaluated in clinical trials in Sudan, first with a demonstration site, followed by a scale-up program of VIA “screen and treat” by a multi-disciplinary cadre of community midwives, nurses, and medical assistants in nine states, in collaboration with the Ministry of Health and WHO.

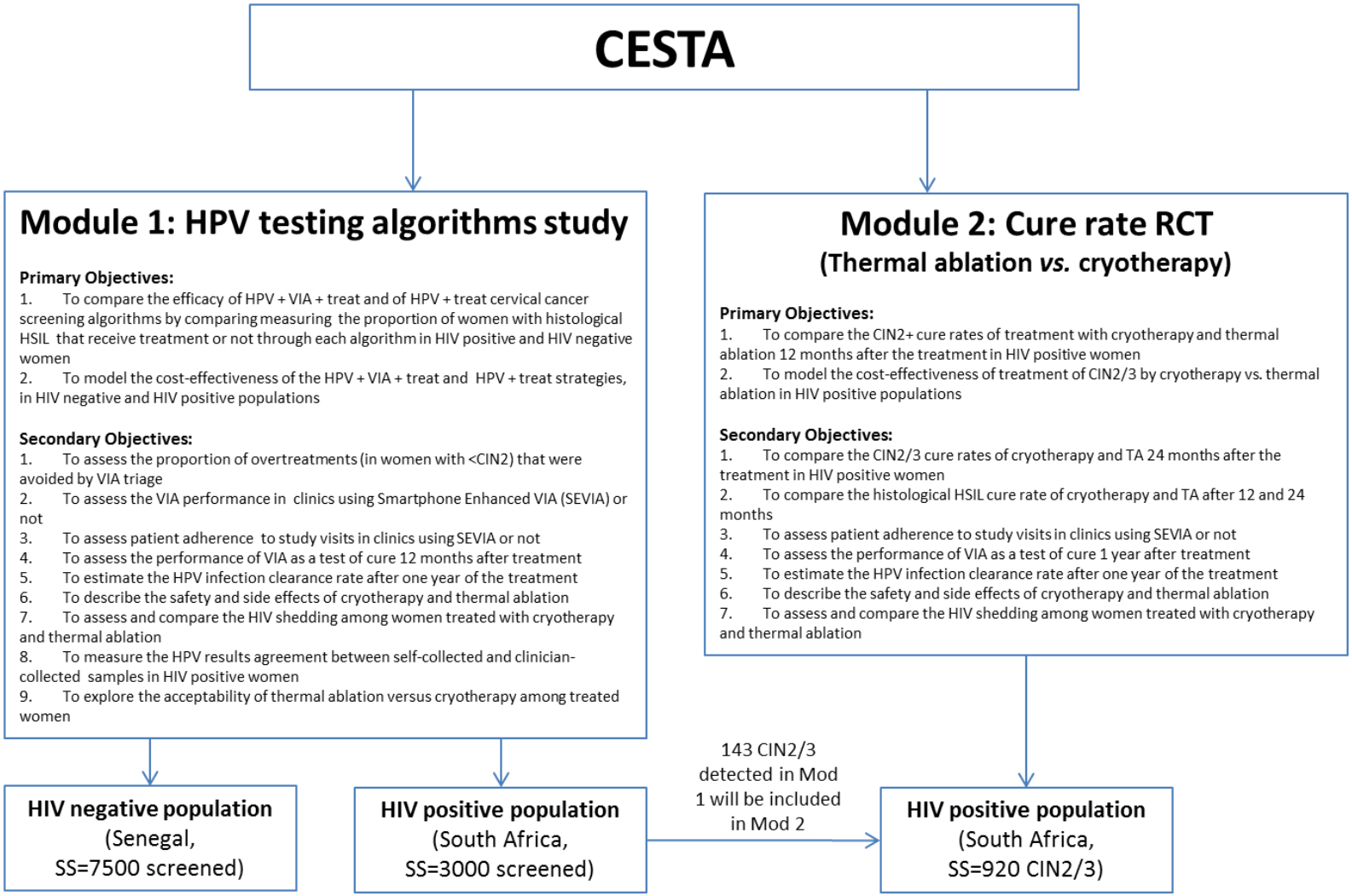
Novel “patient navigation” tools are being developed and implemented within the SEVIA platform. Patient navigation, first implemented in 1990, is a barrier-focused intervention that targets a defined set of health services required to complete a process of cancer-related care [20]. The provision of patient navigation services has been shown to reduce delays in patients accessing the continuum of cancer care services, with an emphasis on timeliness of diagnosis and treatment and a reduction in the number of patients lost to follow-up in high-income countries. The patient navigation tools for CESTA will be informed by the literature (scoping review in progress), and previous experience with mHealth navigation for breast cancer early diagnosis by community health workers in rural Bangladesh [21]. In our study, we will assess the difference in performance of VIA and VAT as well as attrition rates in clinics that use SEVIA, compared to clinics that do not.

2.7. The use of cervical intraepithelial neoplasia (CIN) and Lower Anogenital Squamous Terminology Standardization (LAST) terminology for cervical histology

Histology will is classified using the cervical intraepithelial neoplasia (CIN) classification. CIN1 corresponds to HPV infection, CIN3 is a highly reproducible cancer precursor and CIN2 is an intermediate and poorly reproducible category and represents a mixture of HPV infections (CIN 1) and true cancer precursors (CIN3). Despite the uncertainty associated with CIN2, it is recommended as the cut-off for treatment, that is, women with CIN2 or worse lesions (CIN2+) should receive adequate treatment [4]. The use of histological high-grade intraepithelial lesion (HSIL) has recently been proposed under the Lower Anogenital Squamous Terminology Standardization (LAST) project [22]. LAST incorporates the current knowledge of HPV biology and the use of biomarkers to improve diagnosis, such as *p*16 immunohistochemistry (IHC) particularly for evaluating whether a diagnosis of CIN2 represents true high-grade cervical disease. Histological HSIL includes CIN2 *p*16 positive, CIN3 and adenocarcinoma in situ (AIS), better reflecting precancer lesions. Therefore, the gold standard for final outcome analysis of this study will be histological HSIL to compare the performance of HPV testing with VIA triage followed by treatment with that of HPV followed by treatment, but we will use CIN2 as a cut-off for treatment as currently recommended.

3. Modules of the CESTA study

Figure 1: Modules of the Cervical cancer Screening and Treatment algorithms study using HPV testing in Africa (CESTA)



The CESTA study aims at comparing two algorithms for screening and treatment of high-grade cervical pre-cancer lesions in populations of HIV positive and HIV negative women; in addition the study aims to compare the efficacy (cure rate) of cryotherapy and thermal ablation in HIV positive women. The CESTA study is therefore comprised of 2 modules.

Module 1 is a randomised controlled trial of 2 screen and treat algorithms: screening women with HPV test followed by triage with VIA and treatment versus screening women with HPV test without VIA triage and followed by treatment in HIV positive women (in South Africa, women 25 – 54 years) and among HIV negative women (in Senegal, women 30 – 54 years).

Module 2 is a randomised controlled trial to compare the cure rates of thermal ablation versus cryotherapy in HIV positive women aged 25-54 years with CIN2/3; module 2 will also be carried out in South Africa.

3.1. CESTA Module 1: Randomised control trial of two algorithms, screening with HPV tests followed by VIA as a triage and treatment (HPV + VIA + treat), vs screening with HPV test followed by treatment (HPV + treat) among HIV-positive women in South Africa and HIV-negative women in Senegal

3.1.1. Primary Objectives:

1. To compare the efficacy of HPV + VIA + treat and of HPV + treat cervical cancer screening algorithms by comparing the proportion of women with histological HSIL that receive treatment or not through each algorithm in HIV positive and HIV negative women.
2. To model the cost-effectiveness of the HPV + VIA + treat and HPV + treat strategies, in HIV negative and HIV positive populations.

3.1.2. Secondary Objectives:

1. To assess the proportion of overtreatments (in women with no lesions or CIN1) that was avoided by VIA triage.
2. To assess the VIA performance in clinics using Smartphone Enhanced VIA (SEVIA) compared to clinics that don't.
3. To assess patient adherence to study visits in clinics using SEVIA compared to clinics that don't.
4. To assess the performance of VIA as a test of cure 1 year after treatment.

5. To estimate the HPV infection clearance rate after one year of the treatment.
6. To describe the safety and side effects of cryotherapy and thermal ablation.
7. To assess and compare the HIV shedding among women treated with cryotherapy and thermal ablation.
8. To measure the HPV results agreement between self-collected and clinician-collected samples in HIV positive women.
9. To explore the acceptability of thermal ablation versus cryotherapy among treated women.

3.2. Study outline CESTA Module 1

The CESTA Module 1 is a double-level randomized study with the main objective to evaluate the efficacy of two cervical screen-and-treat algorithms in HIV positive and HIV negative women separately.

3.2.1. CESTA Module 1 among HIV positive women in South Africa (see figure 2)

3000 HIV-positive women will be screened with HPV testing and HPV-positive women will be randomized in a 4:1 ratio to be triaged by VIA and treated (arm 1, HPV+VIA+treat) or treated right away (arm 2, HPV+treat).

The study will recruit women aged 25-54 years. Recruitment will be done mainly in HIV care clinics in South Africa. After informed consent, women will be administered short questionnaires on demographics and sexual history, and socio-economic characteristics. A health economics questionnaire focused on participant travel time and expenditures will also be administered. Women will then be asked to self-collect a vaginal sample and afterwards, a nurse will collect a cervical sample, both samples will be used for HPV testing. Any woman who refuses consent or opts out after consent will be referred to routine cervical cancer screening.

Participants will be given an appointment to the CESTA study clinics to collect their HPV result. HPV positive women will be randomised to arms 1 or 2 using the envelop method with pre-printed assignment cards.

ARM 1:

A nurse will carry out a speculum examination and collect an endocervical swab to measure HIV shedding. She will then perform VIA and in half of the CESTA study clinics, the nurse will take 2-3 pictures of the cervix using the SEVIA smartphone application. 2-4 biopsies will be collected at the squamo-columnar junction (SCJ) from all quadrants that show aceto-white changes or at 12 and 6 'o clock if no acetowhite changes are visible. All eligible women for ablative treatment will be further randomised to sub-arms A (cryotherapy) and B (thermal ablation).

VIA positive women not eligible for ablative treatment will be referred to the colposcopy clinic for appropriate referral and management.

The VIA examination is inadequate if the SCJ is not entirely visible. In this case, an endocervical brush will be collected for cytological examination and the woman will be referred to colposcopy once the cytology result is known.

Women with CIN2+ on biopsy who have not been treated based on screening results from arm 1 will also be randomised to sub-arms A and B if eligible for ablative treatment or referred to colposcopy otherwise.

ARM 2:

Study procedures in this arm are mostly similar to Arm 1. A nurse will carry out a speculum examination and collect an endocervical swab to measure HIV shedding. She will then perform a visual assessment for treatment using acetic acid (VAT, see section 2.4) to assess if the patient is eligible for ablative treatment and in half of the study clinics, 2-3 pictures will be taken with the SEVIA App. After VAT, 2-4 biopsies will be collected at the SCJ from all quadrants that show aceto-white changes or at 12 and 6 'o clock if no acetowhite changes are visible.

All women that are eligible for ablative treatment will be treated and randomised into sub-arm A (cryotherapy) and sub-arm B (thermal ablation) as in Arm 1; women with VAT not eligible for ablative treatment will be referred to the colposcopy clinic for appropriate referral and management.

Similar as for VIA, the VAT examination is inadequate if the SCJ is not entirely visible. In this case, an endocervical brush will be collected for cytological examination and the woman will be referred to colposcopy once the cytology result is known.

In both arms, endocervical sample collection for HIV shedding will be done after 1, 2, 3, and 4 weeks in 100 women (each arm) who received treatment (modes of selection for these women still needs to be decided). At those respective visits, women will answer a questionnaire on perceived side-effects and acceptability of treatment. Women who do not come back for HIV shedding sample collection will be called by telephone after 1 week and 1 month to assess perceived side-effects and acceptability of treatment. The nurse will take the necessary actions if serious side-effects are reported.
In addition, all treated women will be recalled at 12 months for a follow-up visit including HPV testing, VIA with colposcopy and biopsy on HPV positive women, and those with CIN2+ will be treated as appropriate.

The SEVIA App will only be applied in half of the study clinics, in order to allow to assess its effect on the quality of VIA and attrition rates. As described above in section 2.6, the nurses who use SEVIA will receive feed-back on their VIA or VAT assessment some minutes after they took the pictures and reported their own VIA/VAT assessment to the SEVIA platform. The original VIA/VAT assessment of the nurse will be used for study purposes, but we will assess at the end of the study if there was a difference in performance for the nurses that got supervision through SEVIA, compared to those who did not. The SEVIA application will also contain a patient navigation functionality. Women who agree will provide their telephone number and the SEVIA app will send automated reminders for their appointments. In case women are referred to colposcopy, the SEVIA system will also inform the staff at the colposcopy clinic about this referral and create alerts for women that missed an appointment.

Women who exit the study at any point will be counselled about the standard of care screening recommendations for HIV positive women in South Africa (3 yearly screening).

3.2.2. CESTA Module 1 among HIV negative women in Senegal (see figure 3)

The study among HIV-negative women in Senegal is similar to the study design described above for HIV positive women, with the exception that, given the lower prevalence of HPV infection and disease, 7500 women aged 30 -54 will be included and randomized in a 3:1 ratio

to arm 1 (HPV + VIA + treat) and arm 2 (HPV + treat) and that treatment will be done by thermal ablation only.

Women will be recruited from the communities around the CESTA study clinics and will be invited to attend cervical cancer screening at the study clinics. Community health education organisations will be involved to create cervical cancer awareness in the community. Study information leaflets will be developed and distributed in the communities by community health workers. Where possible, invitations to participate in the study will be handed out.

After informed consent, women will be administered short questionnaires on demographics and sexual history, and socio-economic characteristics. A health economics questionnaire focused on participant travel time and expenditures will also be administered. Women will then be asked to self-collect a vaginal sample and afterwards, a nurse will collect a cervical sample, both samples will be used for HPV testing. Any woman who refuses consent or opts out after consent will be referred to routine cervical cancer screening.

Participants will be given an appointment to the CESTA study clinics to collect their HPV result. HPV positive women will be randomized to arms 1 or 2 using the envelop method with pre-printed assignment cards.

ARM 1:

A nurse will carry out a speculum examination and perform VIA and in half of the CESTA study clinics, the nurse will take 2-3 pictures of the cervix using the SEVIA App. 2-4 biopsies will be collected at the SCJ from all quadrants that show aceto-white changes or at 12 and 6 ‘o clock if no acetowhite changes are visible. All eligible women for ablative treatment will be treated by thermal ablation.

VIA positive women not eligible for ablative treatment will be referred to the colposcopy clinic for appropriate referral and management.

The VIA examination is inadequate if the SCJ is not entirely visible. In this case, an endocervical brush will be collected for cytological examination and the woman will be referred to colposcopy once the cytology result is known.

Women with CIN2+ on biopsy who have not been treated based on screening results from arm 1 will also be recalled and treated with TA eligible for ablative treatment or referred to colposcopy otherwise.

ARM 2:

Study procedures in this arm are mostly similar to Arm 2 among HIV positive women (section 3.2.1). A nurse will carry out a speculum examination and perform a VAT to assess if the patient is eligible for ablative treatment and in half of the study clinics, 2-3 pictures will be taken with the SEVIA App. After VAT, 2-4 biopsies will be collected at the SCJ from all quadrants that show aceto-white changes or at 12 and 6 o'clock if no acetowhite changes are visible.

All women that are eligible for ablative treatment will be treated by TA; women with VAT not eligible for ablative treatment will be referred to the colposcopy clinic for appropriate referral and management.

VAT examination is inadequate if the SCJ is not entirely visible. In this case, an endocervical brush will be collected for cytological examination and the woman will be referred to colposcopy once the cytology result is known.

In both arms, women who receive treatment will be called by telephone after 1 and 4 weeks and a questionnaire on perceived side-effects and acceptability of treatment will be taken by the nurse. The nurse will take the necessary actions if serious side-effects are reported.

In addition, all treated women will be recalled at 12 months for a follow-up visit including HPV testing, VIA, colposcopy and biopsy on HPV positives, and those with CIN2+ will be treated as appropriate.

The SEVIA App will only be applied in half of the study clinics, in order to allow to assess its effect on the quality of VIA and attrition rates. Similar as described above in section 3.2.1, the nurses who use SEVIA will receive feed-back on their VIA or VAT assessment some minutes after they took the pictures and reported their own VIA/VAT assessment to the SEVIA platform. The original VIA/VAT assessment of the nurse will be used for study purposes, but we will assess at the end of the study if there was a difference in performance for the nurses that got supervision through SEVIA, compared to those who did not. The SEVIA application will also contain a patient navigation functionality. Women who agree will provide their telephone number and the SEVIA app will send automated reminders for their appointments. In case women are referred to colposcopy, the SEVIA system will also inform the staff at the colposcopy clinic about this referral and create alerts for women that missed an appointment.

Women who exit the study at any point will be counselled about the standard of care screening recommendations in Senegal and at which services they can be accessed.

3.3. Rationale for choice of target age for cervical screening

This study will target HIV positive and HIV negative women separately. The WHO recommends that: 1) cervical screening should target women 30 years of age and older because of their higher risk of cervical cancer, 2) priority should be given to women aged 30–49 years, and 3) for women of HIV positive status or unknown HIV status in high HIV endemic areas, screening should start as soon as a woman or a girl has tested positive for HIV and is sexually active (WHO screening recommendation update 2014). As the study aims to evaluate the efficacy of screen-and-treat cervical screening algorithms using HPV testing, the start age of HIV positive women to be included in the study will be 25 years of age, to avoid increasing the overtreatment rate as it is known that the prevalence of HPV is higher among young women (less than 30 years) and can be >50% among HIV positive women [23].

WHO recommends age 49 as the upper age limit for screening with VIA. VIA is less effective in women older than 50 years, because the squamocolumnar junction is less visible in post-menopausal women. In this study we want to include women up to the age of 54, in order to compare the performance of HPV testing with or without VIA triage including an age group above 50 years old.

3.4. Cost and Cost-effectiveness methods

3.4.1. Modelling approach

We will use the 'Policy1-Cervix' model platform developed by Cancer Council NSW to model the long-term impact and cost-effectiveness of the interventions. 'Policy1-Cervix' is an extensively validated dynamic model of HPV transmission, HPV vaccination, cervical precancer, cancer survival, screening, diagnosis and treatment. The model has been used for a number of HPV vaccine evaluations, including HPV vaccination in both girls and boys, catch-up vaccination, and the introduction of nonavalent vaccine. The model has also been used to evaluate screening implementation in organized national cervical cancer screening programs in high-income countries and in vaccination and screening in urban and rural areas of middle-income countries. In connection with the Cervical Cancer Elimination Initiative, the model

platform is being used to evaluate the potential impact of screening and HPV vaccination on a global scale.

In the context of this study, the model will incorporate a cervical cancer natural history specific to HIV positive women for application in South Africa. Each country model will be fit to epidemiological data collected from the clinical trial (e.g. age-specific HPV prevalence, HPV type prevalence and distribution, CIN2 prevalence and distribution) and where necessary, supplemental data from countries with similar cervical cancer outcomes and from the literature. Modelled natural history outputs will be validated against expected outcomes from the literature or other relevant sources.

The clinical trial patient pathways (i.e. the screen-and-treat algorithms) for each study arm respective to each study country will be modelled in the platform and country-specific costs collected alongside the trial will be integrated with each intervention. Modelled cost outputs will also be validated against expected values from the literature and relevant sources. Future costs will be estimated by applying official GDP inflators for each country. The patient-navigation component of the CESTA trial will not be included in the modelling.

Other model outputs will include counts of HPV tests, biopsies, laboratory thermoablation treatment, cryotherapy treatment, CIN2 detected and colposcopies.

3.4.2. Costs

The assessment of costs will be conducted from the health service provision and the patient perspective.

Table 1. Overview of costs and data collection methods

	Costs included	Estimation method	Data sources
Provider	*Direct medical costs* of cervical cancer screening and treatment and follow-up (HPV test, VIA, biopsy, lab fees, thermoablation, cryotherapy, colposcopy, clinical staff, medical supplies).	Ingredient approach	Observation, records, primary data collection
	Other recurrent and capital costs (overhead costs such as building, utilities), programme costs (e.g. costs of community recruitment in Senegal).	Step-down costing for overhead costs; ingredient approach for other	Health facility records and primary data collection
Patient/client	*Direct medical* (e.g. ancillary drugs) and non-medical costs (food and round-trip transportation)	-	Questionnaire
	Indirect costs (time costs)	Human capital method	Questionnaire

Health services costs: Direct medical costs on screening, diagnosis and treatment of precancerous lesions will be calculated using a micro-costing approach whereby quantities of resources used will be measured and multiplied by their respective unit cost or price. These will include the costs of staff (time and by type), consumables, equipment, quality assurance and control (for VIA) and transportation of cervical samples. We will use the micro-costing approach to estimate the cost of (i) HPV DNA testing, (ii) VIA testing, (iii) colposcopy, (iv) biopsy and (v) pre-cancer treatment (thermocoagulation and cryotherapy). Aggregate health facility expenditures (e.g. utilities) and capital costs (e.g. building, equipment and furniture) will be allocated to the different interventions provided using a step-down costing approach. Step-down costing will only be performed if required (e.g. if new facilities are set up). In addition to these costs, we will also consider programmatic costs. In South Africa this will include the cost of setting up recruitment at ART clinics and diversion to the appropriate clinical site. In the case of Senegal where recruitment will take place in the community, this will include the costs of information, education and communication (IEC) materials (e.g. advertisements on the radio, community sensitization activities), staff training, monitoring and evaluation and supervision.

Woman's costs: For the patient perspective, we will collect data on direct medical, non-medical and indirect costs. Direct non-medical costs include the cost of transportation to and from home or work to the different health facilities and food. Indirect costs consist of time losses resulting from travelling to and from the health facilities, waiting and receiving care. These time losses will be valued with the human capital method using income data collected from women and/or published wage rates.

3.4.3. Data collection

Health services costs: Data on direct medical costs will be collected in association with 10% of the study participants. All costs incurred for the various interventions will be identified through observation of processes, time sheets where available and extensive consultation with the medical, laboratory and programmatic staff. Observations will include time-and-motion measurement of the various staff procedures and will be done by trained research staff. Data on overheads will be obtained from the health facilities directly.

Women's costs: To measure the costs incurred by study participants, repeated measurements on non-medical costs and travelling and waiting time will be carried out at each visit of the study participant to the health facility. Data will be collected through the administration of questionnaires by the research staff at each visit to the clinical sites. More in depth data to probe and validate time costs will be collected through convenience sampling of 10% of the participants using in-person interviews. During the first visit, the questionnaire will also include information on socio-demographic characteristics and income.

3.4.4. Effectiveness and cost-effectiveness evaluation

For each screening arm, we will calculate the predicted average lifetime reduction in the age-standardised rate of cervical cancer incidence and mortality, as well as the number of deaths averted and histologically confirmed high grade lesions.

In terms of cost-effectiveness, we will use a limited societal perspective with a lifetime time horizon and 3% discounting. The relative performance of the different screening strategies will be expressed in terms of the incremental cost-effectiveness ratio, representing the additional cost of a strategy divided by its additional benefit, compared to the next least expensive alternative and expressed as cost per quality-adjusted life year (QALY) and cost per life-years saved. Other outcomes include cost per CIN2 detected and cost per treatment

performed. We will also estimate the annual budget impact of the different interventions and annual resource utilisation in terms of number of colposcopies, biopsies, precancer treatments, HPV tests, triage tests and other relevant procedures when considering scaling-up of the interventions.

3.5. Statistical methods Module 1 Algorithms study

For main objective 1, we will use three different indicators: 1) percentage of HPV positive women with detected histological HSIL+ treated in each arm, 2) percentage of HPV positive women without histological HSIL+ lesions treated in each arm, and, 3) percentage of HPV positive women that are referred to colposcopy by VIA in arm 1 or VAT in arm 2. Proportions in each arm will be compared using Chi-square likelihood ratio tests. In addition, 95% confidence intervals will be estimated for each proportion and used graphically for comparison.

3.5.1. Sample size and power calculations

For the first objective, to compare the performance of the algorithms, the sample size has been calculated for the three indicators defined above. We used the percentage of HPV positive women with HSIL+ who were treated for the sample size calculation as it is the indicator that requires the largest sample size.

For HIV positive women, we assumed several features as follows: HPV testing sensitivity of 95%, 85% eligibility for ablative treatment and an overall attrition of 20% for both arms. Additionally, for Arm 1 (HPV+VIA), we assumed a VIA sensitivity among HPV positive women of 75% and 4% VIA result of suspected cancer or VIA inadequate. Using a randomisation ratio of 4:1 to Arm 1 and Arm 2, we will need to detect 125 histological HSIL+ cases in Arm 1 and 25 histological HSIL+ cases in Arm 2.

With 3000 HIV positive women attending screening, assuming 50% HPV positivity [24-26] and 50% VIA positivity, and a prevalence of 5% HSIL we will have at least 85% power to detect a difference of 24% (72.8% in Arm 1 vs 97% in Arm 2) with two-sided type error I 0.05 (see Table 2 and Table 3). In summary, under all assumptions stated, 1500 HPV positive women will need to be included.

Table 2. Differences expected according to number of histological HSIL+ cases treated and <HSIL women overtreated in HIV positive women

Outcomes	Total	Arm VIA (1)	Arm HPV (2)	Difference
Total HSIL+ cases (n)	150	120	30	
Total HSIL+ HPV positive cases (n)	143	114	29	
Total HSIL+ treated (n)	111	83	28	
Total <HSIL treated (overtreatment) (n)	742	487	254	
% of treatment among HSIL+ HPV positive women		72.8%	97.0%	24%
% of overtreatment among HPV positive women		41%	85%	44%

Table 3. Number of HIV positive women to be screened

Sample size	Parameter	n arm VIA (1)	n arm HPV (2)
Prevalence of HSIL+	0.05		
Expected number of HIV positive women (n)	3000	2400	600
Power	>85%		

Similarly for HIV negative women, randomising in a 3:1 ratio to Arm 1 or Arm 2, under the same assumptions for sensitivities (HPV 95%, HPV, VIA 75% in HPV positives, 85% eligibility for ablative treatment in Arms 1 and 2, and 20% attrition overall), we will need to detect 113 HSIL+ cases in Arm 1 and 38 in Arm 2 to compare the percentage of women with HSIL+ treated under each algorithm. With a sample size of 7500 HIV negative women, assuming 15% HPV prevalence [27], 50% VIA positivity, 2% prevalence of HSIL+, and two-sided type error I of 0.05, we will have at least 85% power to detect a difference of 24% (72.8% in Arm 1 and 97% in Arm 2) in the percentage of HPV positive women with HSIL+ treated (see Table 4 and Table 5). In summary, under all the assumptions stated, 1125 HIV negative HPV positive women will need to be included.

Table 4. Differences expected according to number of HSIL+ cases treated and <HSIL women overtreated in HIV negative women

Outcomes	Total	Arm VIA (1)	Arm HPV (2)	Difference
Total HSIL+ cases (n)	150	113	38	
Total HSIL+ HPV positive cases (n)	143	107	36	
Total HSIL+ treated (n)	112	78	35	
Total <HSIL treated (overtreatment) (n)	553	323	230	
% of treatment among HSIL+ HPV positive women		72.8%	97.0%	24%
% of overtreatment among HPV positive women		38%	82%	43%

Table 5. Number of HIV negative women to be screened and randomized

Sample size	Parameter	Arm VIA (1)	Arm HPV (2)
Prevalence of HSIL+	0.02		
Expected number of HIV negative women (n)	7500	5625	1875
Power	>85%		

3.6. Ancillary studies

We will obtain aliquots from HPV samples from participants in the study that will be stored at -80C. These samples can later be used to evaluate the performance of novel screening and triage methods for cervical cancer (HPV genotyping, oncoprotein tests, automated visual evaluation (AVE), and others).

3.7. CESTA Module 2: Randomized controlled trial to compare the cure rate of CIN2+ in HIV positive women treated by thermal ablation and cryotherapy (see figure 4)

3.7.1. Primary Objectives:

1. To compare the CIN2/3 cure rates of treatment with cryotherapy and thermal ablation 12 months after the treatment in HIV positive women.

2. To model the cost-effectiveness of treatment of CIN2/3 by cryotherapy *vs.* thermal ablation in HIV positive populations.

3.7.2. Secondary Objectives:

1. To compare the CIN2/3 cure rates of cryotherapy and TA 24 months after the treatment in HIV positive women.
2. To compare the histological HSIL cure rate of cryotherapy and TA after 12 and 24 months.
3. To assess patient adherence to study visits in clinics using SEVIA or not.
4. To assess the performance of VIA as a test of cure 1 year after treatment.
5. To estimate the HPV infection clearance rate after one year of the treatment.
6. To describe the safety and side effects of cryotherapy and thermal ablation.
7. To assess and compare the HIV shedding among women treated with cryotherapy and thermal ablation.

3.8. Study outline CESTA Module 2

The CESTA Module 2 is a randomised controlled trial to compare the cure rate of cryotherapy to thermal ablation HIV positive women. Women with diagnosed cytological LSIL+ (LSIL; HSIL; or ASC-H) at one of the estimated 8-9 involved ARV clinics in South Africa, all situated preferably around Durban, will be invited to one of the CESTA clinics. A total of 920 HIV positive women with CIN2/3 will need to be included in this module to obtain sufficient study power (see section 3.10.1). Some estimated 143 women will already be detected with CIN2/3 in the CESTA Module 1 and can therefore be invited to participate in the CESTA Module 2. This mean that we estimate that CESTA module 2 will need to identify another 777 women with CIN2/3.

Since 2018, women are routinely screened at ARV clinics with liquid based cytology every 3 years in South Africa. We will access the results of those women already screened at the clinics with permission of the clinics administration and the women themselves.

Women with LSIL+ will attend the closest CESTA study clinic. After informed giving written informed consent, the nurse will take some short questionnaires on demographics and sexual history, and socio-economic characteristics. A health economics questionnaire focused on

participant travel time and expenditures will also be administered. The nurse will collect 2-4 biopsies, with the same procedure as described under Module 1 (section 3.2.1.). Women will then receive an appointment for an additional biopsy results visit. At that visit, all women with CIN2 or 3 without glandular lesions and eligible for ablative treatment through VAT will be randomized in a 1:1 ratio into cryotherapy and thermal ablation, performed by the nurse at the same visit after taking 2-3 pictures with the SEVIA application. Ablation ineligible women will be referred to colposcopy and will be managed and treated appropriately including LLETZ. Women with normal or CIN1 biopsy results will be exited from the study and referred back to the ARV clinic to follow the South Africa standard care recommendations, i.e. rescreening after 1 year.

The VAT examination is inadequate if the SCJ is not completely seen and in that case, the nurse will obtain an endocervical brush for cytology and the women will be sent to the colposcopist when the cytology results are available.

Similarly as in Module 1 (section 3.2.1), women who receive treatment will be called by telephone 1 and 4 weeks after treatment and answer a questionnaire on perceived side-effects and acceptability of treatment. The nurse will take the necessary actions if serious side-effects are reported.

In addition, all treated women will be recalled at 12 and 24 months for a follow-up visit including HPV testing, VIA, colposcopy and biopsy on all HPV positive women, and those with CIN2+ will be treated as appropriate. The SEVIA application will also be used for quality control of the VAT by the nurse, and for patient navigation, as described in Module 1.

3.9. Statistical methods Module 2 Cure rate study

For primary objective 1, we will compare the CIN2/3 cure rates of treatment with cryotherapy (Arm A) or thermal ablation (Arm B) after 12 months of treatment among HIV positive women using Z-test for comparison of proportions in intention-to-treat and per-protocol analyses.

3.9.1. Sample size and power calculations

The sample size is based on: 1) the expected difference between the cure rates of cryotherapy and thermal ablation, 2) the lower of the two expected cure rates, and, 3) the prevalence of HSIL+ cases eligible for ablative treatment.

Larger samples sizes will be needed for comparing smaller differences and for comparing cure rates closer to 50%. It is expected that cure rates will be on the range of 70-75% in HIV positive women [28]. Assuming the lowest cure rate to be 70%, 10% difference between cure rates, 20% ineligible for ablative treatment in each arm, 20% attrition after 12 months of treatment, 920 HSIL+ HIV positive cases will be needed in total (387 cases per arm, 146 ineligible) to estimate the 10% difference with 80% power and two-sided type error I of 0.05. For larger differences or the lowest cure rate above 70%, the power to detect the differences will be higher.

Table 6. Number of HSIL+ cases and statistical power for the cure rate objective

Difference* (%)	N total	Attrition (+20%)	Subtotal	Total with 20% ineligibility	n per arm
8	942	236	1178	1472	618
8.5	830	208	1038	1297	545
9	734	184	918	1147	482
9.5	656	164	820	1025	431
10	589	147	736	920	387

Power = 80%. *Starting at 70%

From Module 1, we expect to identify 143 HSIL HPV positive cases (see Table 1). Therefore, we will need to include in this module 777 HSIL cases. Assuming a prevalence of 5% HSIL and a sensitivity of HPV test of 95%, 16,340 HIV positive women should be screened to complete the 920 HSIL cases.

4. Study Methods

For study methods specific for modules 1 and 2 we refer to the sections describing the study outline of the different CESTA modules (3.2.– 3.5; 3.9).

4.1. Eligibility and exclusion criteria for CESTA participation (Modules 1 and 2)

4.1.1. Eligibility criteria

1. Willing to disclose HIV status

2. HIV negative women aged 30-54 years; HIV positive women aged 25-54 years
3. Mentally competent to give informed consent
4. Physically able to have a pelvic exam

4.1.2. Exclusion criteria

1. Women reporting no previous sexual activity
2. History of cervical cancer
3. Treatment for cervical precancer in the last six months
4. Hysterectomy
5. Pregnancy
6. Serious pre-existing medical conditions (e.g. history of bleeding disorders, serious physical or mental disease)

4.2. Randomisation procedures

The method of randomization will be through the use of pre-printed cards. Each randomisation will have its own set of cards with allocations to one of the screen-and-treat study arms (1: HPV + VIA + treatment or 2: HPV + treatment) and to one of the treatment arms (A: cryotherapy or B: thermal ablation). The Soares big stick design (BSD) [29] algorithm will be used. This algorithm assures the best allocation randomness in a comparison of 14 methods [30]. Using R software, the output of this algorithm is a csv file with the participant number and the arm allocation. The same procedure will be applied on HIV positive and HIV negative women included in the study.

4.3. Duration of subject participation

For all women, the study will include one round of HPV testing. HPV negative women will finish their participation in the study after this first round.

In Module 1, all HPV positive women with or without VIA triage will have a follow-up HPV test at 12 months, and will end their participation if the HPV test at 12 months is negative or after <CIN2 histology on biopsy collected at the colposcopy visit or after treatment (LLETZ or other) for CIN2+.

In Module 2, women with CIN2/3 on biopsy will be randomized into TA or cryotherapy. Women that are ineligible for ablative treatment will be referred to colposcopy for appropriate management and all other women with LSIL+ cytology will be exited from the study and referred back to the ARV clinic for follow up according to South African standard of care. All treated women will attend a FU visit at 12 mo. and 24 mo. with HPV testing, VIA, and colposcopy-directed biopsies on HPV positive women to detect remaining/recurrent disease.

At the time of exit, all women will be given clear indications on how to continue with regular screening or with any needed follow-up under the local health system.

5. Ethical considerations

The study will be approved by the IARC and local ethical committees of participating countries: currently being the Biomedical Research Ethics committee (BREC) at the University of KwaZulu-Natal and the "Comité National d'Ethique pour la Recherche en Santé" under the Ministère de la Santé et de l'Action Sociale, Dakar. All women in the study will sign informed consent forms approved by IARC's and the local ethical committees.

The informed consent form will include details on the background, procedures of the study, risks and benefits, statement of confidentiality, specimen use and study staff to contact (informed consent document in annex).

The study is considered minimal risk and the procedures are standard practice in cervical cancer screening programs. The clinical procedures to be performed were developed by a team of clinicians including colposcopists mainly considering the safety of the participants in terms of reduction of cervical cancer risk, and all the procedures are consistent with standard medical practice and/or WHO recommendations, including thermal ablation.

We will make sure that women understand that they will continue to have access to their usual medical care even if they refuse to participate in the study. We will also assure that women who accept to participate are aware that they can withdraw from the study at any point without affecting their access to their regular cervical cancer screening. Also women will be informed that they can refuse to respond any question of the risk factor questionnaire or refuse to provide specimens without affecting their participation in the rest of the study.

Figure 2: Flowchart CESTA Module 1 Algorithms study in HIV POSITIVE women (South Africa)

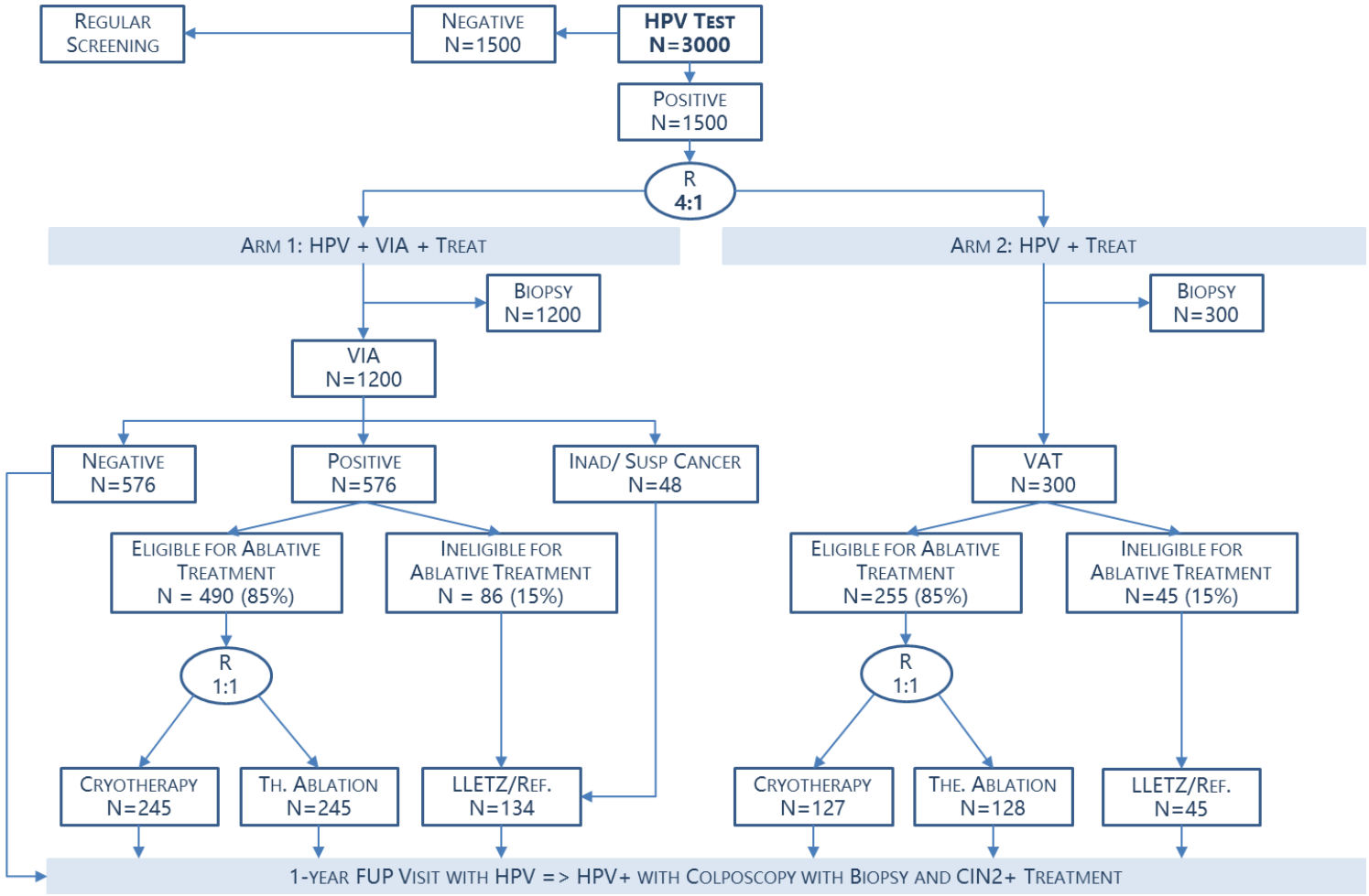


Figure 3: Flowchart CESTA Module 1 Algorithms study in HIV NEGATIVE women (Senegal)

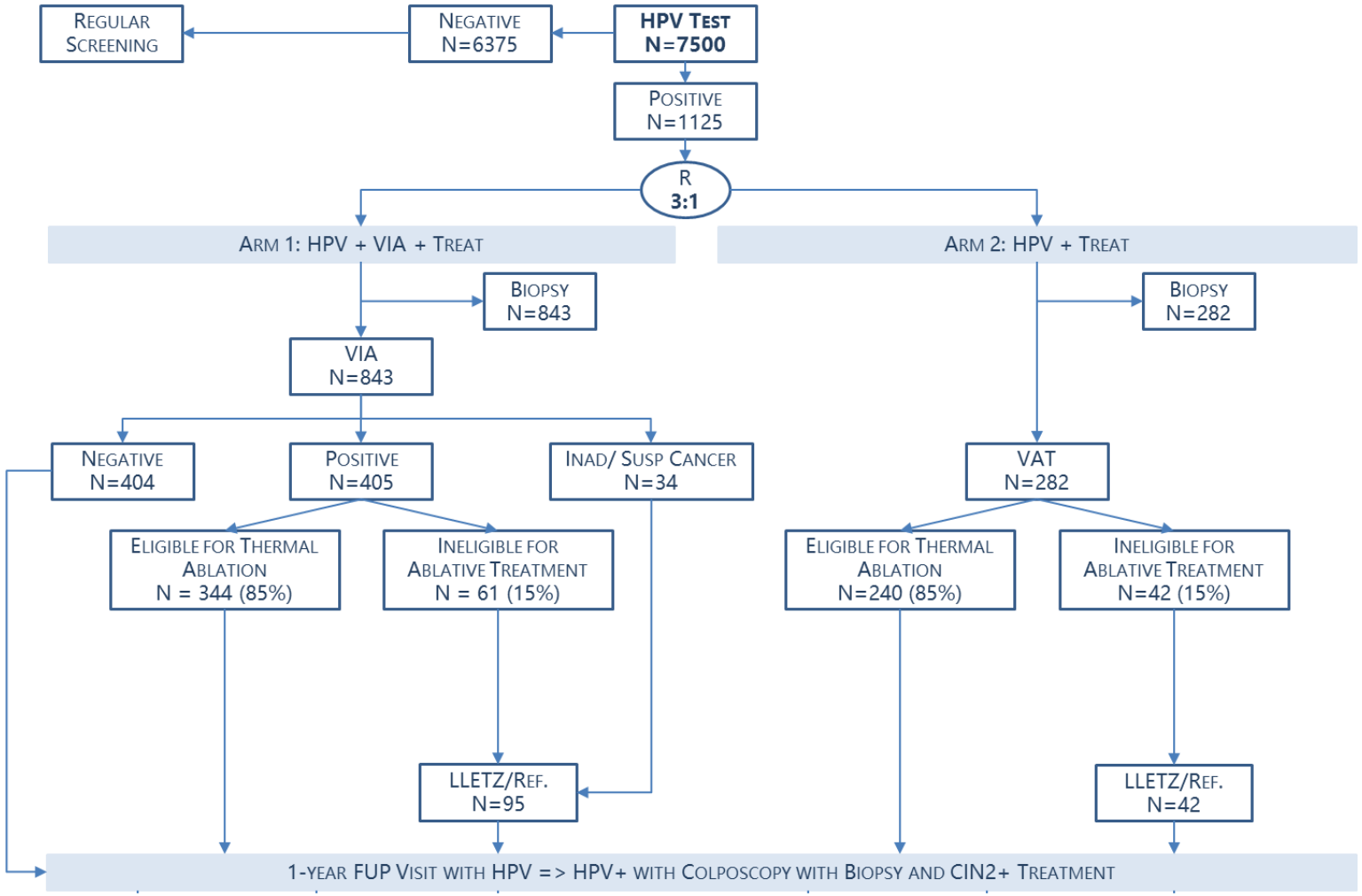
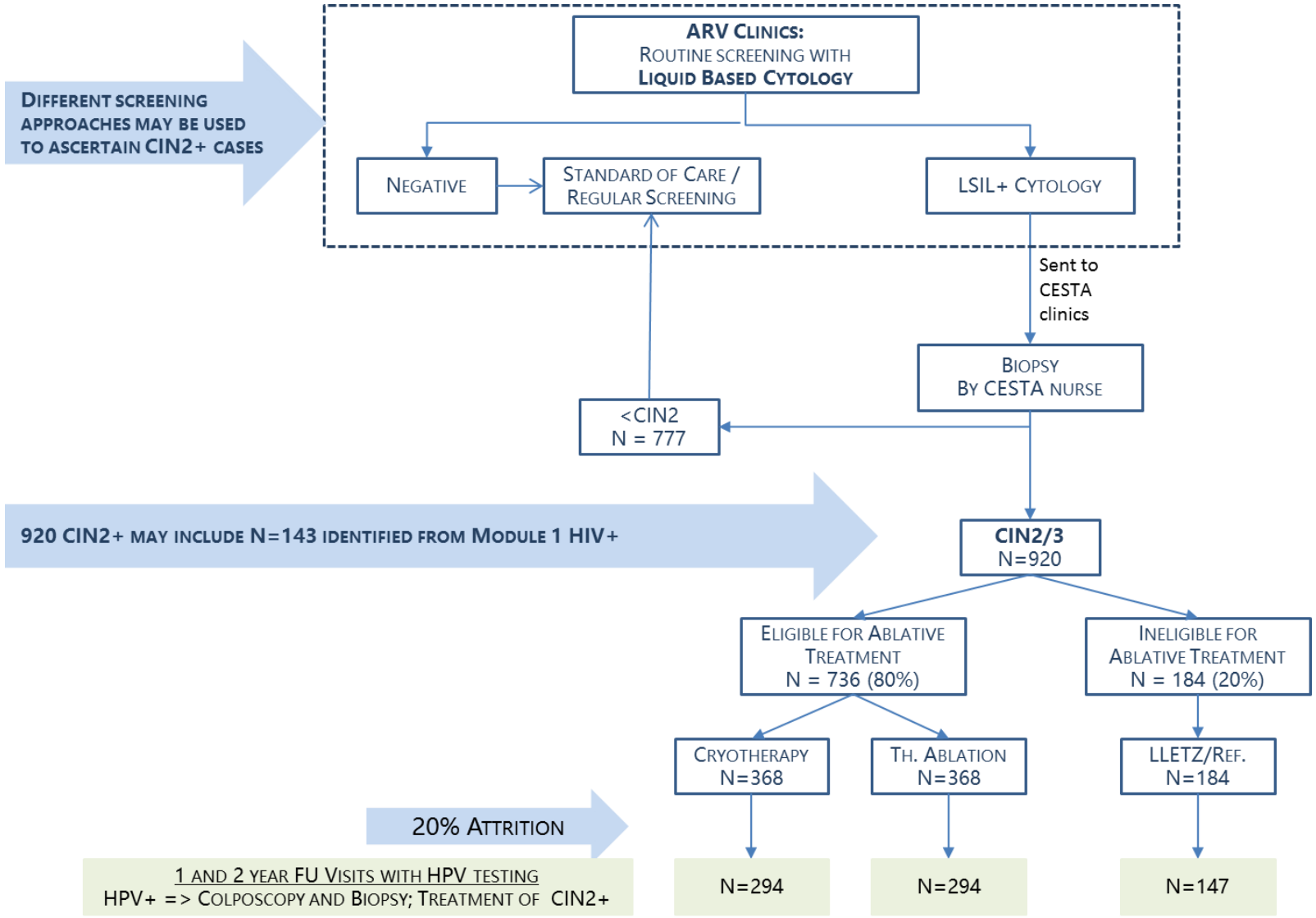


Figure 4: Flowchart CESTA Module 2 Cure rate study (HIV positive women, South Africa)



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