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# A randomised controlled non-inferiority trial to compare the efficacy of 'HPV screen, triage and treat' with 'HPV screen and treat' approach for cervical cancer prevention among women living with HIV

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We report results of a randomized controlled trial to compare 'HPV screen and treat' (Arm 1) and 'HPV screen, triage and treat' (Arm 2) in women living with HIV (WLHIV), using visual inspection with acetic acid (VIA) as the triaging test. Treatment was offered to all HPV-positive women in Arm 1 and to VIA-positive women in Arm 2 with either thermal ablation or large loop excision. All women underwent a repeat HPV test one year after randomization. The primary outcome was non-inferiority of HPV clearance of Arm 2 at one-year follow up when compared to Arm 1. Of 544 HPV-positive consenting WLHIV, 433 were randomised in a 1:1 ratio to trial arms. At baseline, CIN 2/3 lesions were detected in 16.7% and 13.3% women in Arm 1 and Arm 2 respectively. HPV clearance was observed in 56.6% (95%CI 48.9-64.1) women in Arm 1 and 41.4% (95%CI 34.3-48.7) women in Arm 2 at follow-up in the intention-to-treat population ($P = 0.004$). 'HPV screen, VIA triage and treat' strategy was non-inferior to the 'screen and treat' strategy as the lower bound of the 95% confidence interval from the regression model was greater than 0.49 in both intention-to-treat analysis (RR 0.73, 95%CI 0.59-0.91) and per-protocol analysis (RR 0.74, 95%CI 0.60-0.93) according to the pre-specified analysis plan. Clinical trial registration: CTRI/2020/02/023349.

Despite being eminently preventable, more than 600,000 new cases of cervical cancer occur every year in the world and almost 340,000 women die of it[1]. Women living with human immunodeficiency virus (WLHIV) have an increased risk of cervical cancer (RR 6·07, 95% CI 4·40–8·37) as compared to the women in the general population[2]. A synergistic association between human papillomavirus (HPV) and HIV[3] is responsible for the high burden of HPV-associated cancers including cervical cancer in WLHIV. Once WLHIV are persistently infected with

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high-risk HPV, they have an increased risk of progression to high-grade cervical intraepithelial neoplasia (CIN 2/3) and to cervical cancer[4]. Invasive cervical cancer is an AIDS-defining illness[5]. The lack of adequate facilities for cervical cancer screening and appropriate management of screen-detected precancers adds to the high burden of cervical cancer in countries with a high prevalence of HIV infection. The joint United Nations Programme on HIV/AIDS (UNAIDS) has estimated that 9 out of 10 women who die from this disease live in the low- and middle-income countries (LMICs)[6]. At the same time, experience from some of the high-income countries shows that WLHIV who undergo regular cervical screening may have the annual detection rate of high-grade CIN comparable to that of HIV-uninfected populations[7]. This shows the disparity between the low- and middle-income countries and it underscores the importance of regular screening of WLHIV and ensuring appropriate management of screen-positive women.

The overwhelming evidence supporting the superiority of the HPV detection tests over cytology or visual inspection of the cervix with acetic acid (VIA)[8,9] led the World Health Organization (WHO) in 2013 to recommend HPV detection as the primary screening modality of choice when resources are available[10,11]. In the 2013 guidelines, the WHO suggested 'screen and treat' or 'screen, triage and treat' options for women from the general population as well as those living with HIV, when HPV detection is used as the primary screening test. The subsequent WHO guideline published in 2021[12] recommended triaging of all HPV-positive WLHIV, and the tests for triaging could be either VIA or cytology, with or without partial genotyping. The primary consideration for selecting 'screen, triage and treat' over 'screen and treat' strategy was the low specificity of HPV detection tests in WLHIV resulting in a high rate of over-treatment with the 'screen and treat' strategy. A favourable benefit-to-harm ratio was observed with 'HPV screen, triage and treat' strategy based on modelling exercise[13]. At that time evidence could not be obtained from randomized controlled trials (RCTs) among WLHIV to substantiate this observation[10,11].

Both these strategies have their pros and cons. Apart from minimising loss-to-follow-up, the advantages of treating all HPV-positive women could be higher proportion of women clearing HPV infection, thereby being protected from future development of cervical neoplasia. On the other hand, triaging HPV-positive women avoids overtreatment of HPV infections that are unlikely to progress to cancer. This comes at the cost of loss to follow-up, VIA missing some of the high-grade lesions due to the subjective nature of the test and moderate sensitivity of VIA in triaging context[14], and additional expenses of repeating HPV test after one year in the triage-negative women. Considering the need for optimizing the management of HPV-positive WLHIV, we compared the 'HPV screen, triage with VIA and treat' with the 'HPV screen and treat' algorithm in WLHIV in a single centre, assessor-blinded, randomized controlled trial (RCT). The study was approved by the Institutional Ethics Committees for Research of Prayas.

At the time of designing our study, outcomes of the modelling exercise performed by the WHO guideline development group were not available. Hence, we hypothesised that the efficacy of the 'screen, triage and treat' strategy would be non-inferior to that of the 'screen and treat' strategy. All randomised women underwent a repeat HPV test one year after randomisation to evaluate clearance of HPV as the primary outcome. At follow-up, HPV-positive women in either arm were evaluated with colposcopy and biopsies and detection of CIN 2 or worse (CIN 2+) lesions as the secondary outcome to compare the two algorithms.

Ever sexually active and non-pregnant WLHIV aged 25–60 years attending two antiretroviral treatment (ART) centres of National AIDS Control Organisation (NACO) in Pune, India were screened with a high-risk HPV detection test [Hybrid Capture 2™ (HC2) assay; Qiagen INC, Maryland, USA] after they provided a written informed consent. HPV-positive WLHIV were randomised to either 'screen and treat' arm (Arm 1) or 'screen, triage and treat' arm (Arm 2) in a 1:1 ratio with allocation concealment (Fig. 1). Before randomization, a study nurse examined the cervix of the HPV-positive women to exclude any suspected invasive cancer, extensive acetowhite lesions extending to vagina that would be difficult to manage in an out-patient setting, or a cervix that was difficult to expose (e.g., due to vaginal atrophy). These cases were excluded from being randomized. Once randomized, neither the participants nor the investigators could be blinded to the allocation due to the nature of subsequent interventions.

Women randomized to the 'HPV screen and treat' arm were assessed for eligibility for ablation by the study nurse after applying acetic acid for one minute to the cervix. Punch biopsies were obtained from any acetowhite area that was visible after acetic acid application. Those eligible for ablation were treated by the nurse with thermal ablation during the same sitting on the day of randomization. A portable, battery operated thermo-coagulator (WISAP®; Germany) was used for thermal ablation treatment. Depending upon the size of the lesion and the transformation zone, 4–5 multiple overlapping applications were made using a flat probe of 20 mm diameter. Women with lesions not eligible for ablation underwent large loop excision of transformation zone (LLETZ) at a later date by the study clinician at the main centre. LLETZ was performed under local anaesthesia.

Women assigned to 'HPV screen, triage and treat' arm underwent VIA triage by the study nurse and VIA-negative women were advised a follow-up HPV test after one year. VIA-positive women eligible for ablative treatment had biopsies taken from the lesion and were treated with thermal ablation by the study nurse at the same sitting or with LLETZ by the study clinician on a later date at the main centre. Women were advised to call the study coordinator for any adverse events following treatment and all calls were documented.

The women underwent a repeat HPV test (HC2) one year after randomization or treatment (for those who were treated with LLETZ at a later date) to evaluate clearance of HPV as the primary outcome. HPV clearance was defined as a positive HPV test report at baseline and a negative HPV test report at follow-up after one year of randomization or treatment. HPV-positive women at follow-up were evaluated with colposcopy when multiple punch biopsies were obtained from any lesion visible on colposcopy. Women with Type 3 TZ underwent endocervical curettage (ECC), even if they did not have any visible lesion on colposcopy.

## Results

Figure 1 describes the trial profile of study participants. Among the 2618 WLHIV consented and screened with an HPV test, 20.8% (544/2618) tested HPV-positive. Of the 544 HPV-positive women, 111 were not randomised (1 with suspected invasive cancer, 3 with large acetowhite lesions extending to the vagina, 30 with atrophic vagina/ cervix with inability to expose the cervix properly and 77 refused or did not return for test results). Of the remaining 433 women, 215 were randomly assigned to the 'HPV screen and treat' arm and 218 were assigned to the 'HPV screen, triage and treat' arm. The first and the last participants were randomised on 7th July 2020 and 9th April 2021 respectively. Follow-up of the randomized participants was completed in July 2022.

### Participant baseline characteristics

Baseline characteristics of the women by randomization arm are presented in Table 1. The demographic characteristics such as age, marital status, total number of pregnancies and HIV-related characteristics such as years since known to be HIV-positive, years since taking highly active antiretroviral treatment (HAART), CD4 cell counts at the time of onset of HAART and within 6 months of recruitment were comparable between the two arms. Baseline histopathology results based on biopsies obtained from acetowhite areas visible either during the assessment of eligibility for ablation for women in the 'HPV screen and

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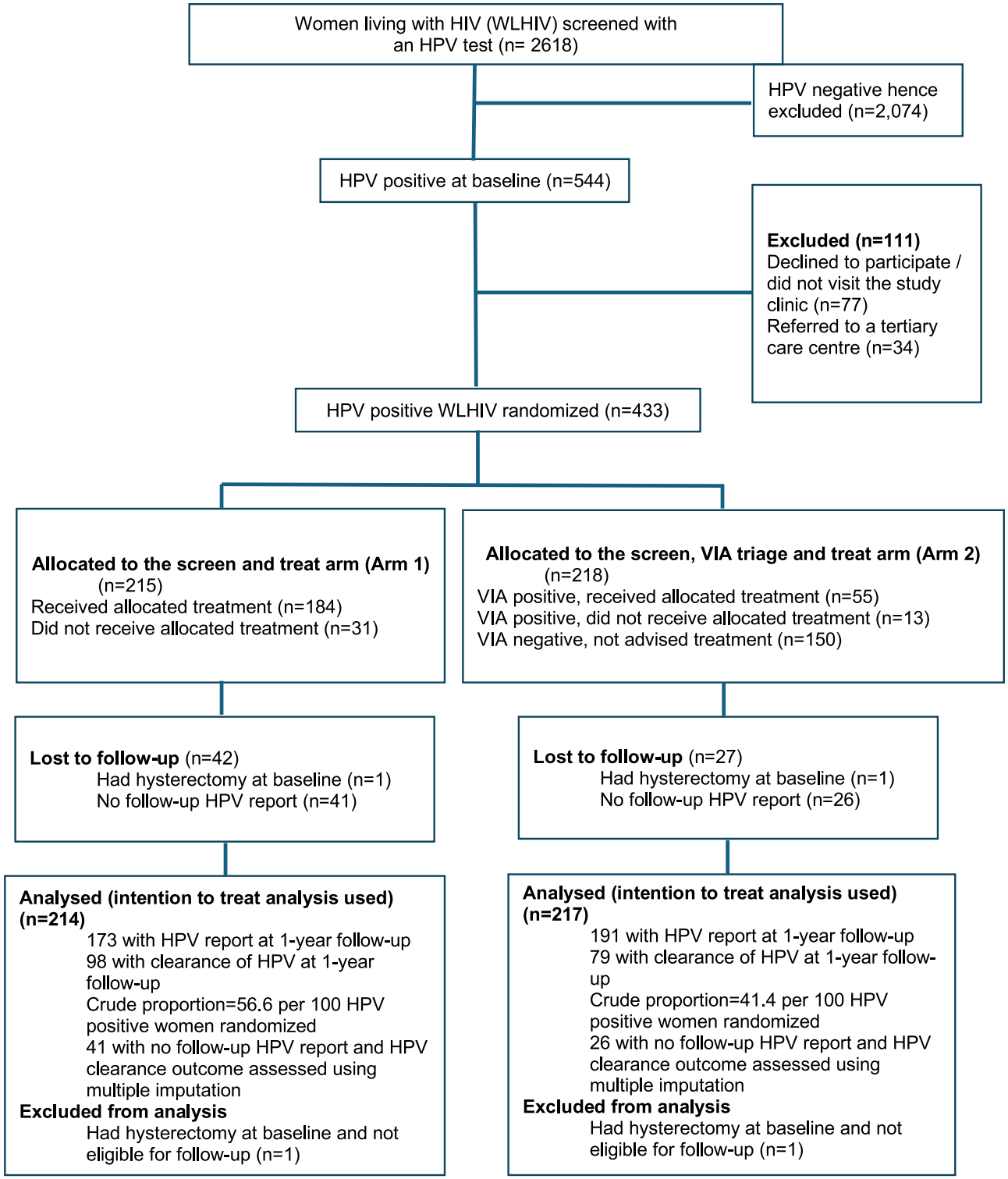


**Fig. 1** | Trial recruitment flowchart.

treat' arm or performing VIA for those in the 'HPV screen, triage and treat' arm are also shown in Table 1. CIN 2/CIN 3 lesions were detected in 36 out of 215 (16.74%) women randomized to Arm 1. In the other arm, CIN 2/CIN 3 lesions were detected in 29 of 218 (13.30%) women. No invasive cancer was detected at baseline among the women randomized to either arm.

Table 2 describes VIA test outcomes, treatment status and mode of treatment among the randomized women. Women in Arm 1 were either treated with thermal ablation following application of 5% acetic acid to determine eligibility for ablation or with excision when not eligible for ablation. Total 184 of the 215 women (85.6%) in Arm 1 received treatment (others refused);154 (83.7%) were treated with thermal ablation and 29 (15.8%) with LLETZ. VIA was positive in 31.2% (68/218) women in Arm 2 as they underwent triaging, and 80.9% (55/68) of the triage-positive women received treatment (others refused). In Arm 2, the mode of treatment was thermal ablation and LLETZ for 63.6% (35/55) and 34.5% (19/55) women respectively. One woman in each arm underwent hysterectomy outside the study after

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**Table 1 | Baseline characteristics of HPV positive women by randomization arm**

| | All | | Randomization arm | | | |
|---|---|---|---|---|---|---|
| | | | HPV screen and treat | | HPV screen, VIA triage and treat | |
| | *n* (%) | | *n* (%) | | *n* (%) | |
| Women assessed | 433 | | 215 | | 218 | |
| Completed age (years) | | | | | | |
| <40 | 186 | (43.0) | 91 | (42.3) | 95 | (43.6) |
| 40–49 | 182 | (42.0) | 96 | (44.7) | 86 | (39.4) |
| 50+ | 65 | (15.0) | 28 | (13.0) | 37 | (17.0) |
| Marital status | | | | | | |
| Married | 165 | (38.1) | 82 | (38.1) | 83 | (38.1) |
| Widowed or separated | 268 | (61.9) | 133 | (61.9) | 135 | (61.9) |
| Total number of pregnancies | | | | | | |
| 0–1 | 95 | (21.9) | 50 | (23.3) | 45 | (20.6) |
| 2–3 | 257 | (59.4) | 118 | (54.9) | 139 | (63.8) |
| 4+ | 81 | (18.7) | 47 | (21.9) | 34 | (15.6) |
| Years since known to be HIV positive | | | | | | |
| <1 year | 29 | (6.7) | 15 | (7.0) | 14 | (6.4) |
| 1–5 years | 80 | (18.5) | 41 | (19.1) | 39 | (17.9) |
| >5 years | 324 | (74.8) | 159 | (74.0) | 165 | (75.7) |
| On HAART | | | | | | |
| 1st line | 403 | (93.1) | 200 | (93.0) | 203 | (93.1) |
| 2nd line | 30 | (6.9) | 15 | (7.0) | 15 | (6.9) |
| 3rd line | 0 | (0.0) | 0 | (0.0) | 0 | (0.0) |
| Not yet initiated | 0 | (0.0) | 0 | (0.0) | 0 | (0.0) |
| Years since initiation of HAART | | | | | | |
| <1 year | 31 | (7.2) | 16 | (7.4) | 15 | (6.9) |
| 1–5 years | 122 | (28.2) | 61 | (28.4) | 61 | (28.0) |
| >5 years | 280 | (64.7) | 138 | (64.2) | 142 | (65.1) |
| CD4 count at the time of HAART initiation (cells per μL) | | | | | | |
| <200 | 176 | (40.6) | 88 | (40.9) | 88 | (40.4) |
| 200–499 | 200 | (46.2) | 102 | (47.4) | 98 | (45.0) |
| 500+ | 57 | (13.2) | 25 | (11.6) | 32 | (14.7) |
| CD4 count within 6 months of recruitment (cells per μL) | | | | | | |
| <200 | 45 | (10.6) | 20 | (9.5) | 25 | (11.7) |
| 200–499 | 154 | (36.3) | 76 | (36.0) | 78 | (36.6) |
| 500+ | 225 | (53.1) | 115 | (54.5) | 110 | (51.6) |
| Not available | 9 | (2.1) | 4 | (1.9) | 5 | (2.3) |
| Recent plasma HIV viral load within 6 months of recruitment (copies per ml) | | | | | | |
| <100 | 3 | (5.4) | 2 | (7.7) | 1 | (3.3) |
| 100–999 | 17 | (30.4) | 10 | (38.5) | 7 | (23.3) |
| 1000+ | 36 | (64.3) | 14 | (53.8) | 22 | (73.3) |
| Not available | 377 | (87.1) | 189 | (87.9) | 188 | (86.2) |
| **Baseline histopathology diagnosis** | | | | | | |
| **Not available**[a] | 311 | (71.8) | 147 | (68.4) | 164 | (75.2) |
| **Available** | 122 | (28.2) | 68 | (31.6) | 54 | (24.8) |
| **CIN detected among those who had histopathology report**[a] | | | | | | |
| Normal | 23 | (5.3) | 15 | (7.0) | 8 | (3.7) |
| CIN 1 | 34 | (7.8) | 17 | (7.9) | 17 | (7.8) |
| CIN 2 | 30 | (6.9) | 17 | (7.9) | 13 | (6.0) |
| CIN 3 | 35 | (8.1) | 19 | (8.8) | 16 | (7.3) |

*HPV* human papillomavirus, *VIA* visual inspection with acetic acid, *HIV* human immunodeficiency virus, *HAART* highly active antiretroviral therapy, *CD4* cluster of differentiation 4, *CIN* cervical intraepithelial neoplasia.

[a]For women randomized to HPV screen and treat arm, biopsies were collected only when there was an acetowhite lesion; for women in the HPV screen, triage and treat arm biopsies were obtained when the VIA was positive.

randomization but prior to the follow-up HPV test hence not included in the subsequent analysis.

### Primary outcome: HPV clearance at follow-up

The follow-up status of women randomised to the two arms and HPV clearance one year after randomization or treatment is presented in Table 2. Out of the 214 women eligible for follow-up in Arm 1, 173 (80.8%) underwent a repeat HPV test. In the other arm, follow-up compliance for the repeat HPV test was 88.0% (191/217). HPV clearance was observed in 56.6% women in Arm 1 and in 41.4% women in Arm 2 ($p = 0.004$).

We performed an intention-to-treat analysis (in which all randomised participants were considered regardless of whether they received the allocated treatment or not), and a per-protocol analysis (which included only participants who followed the study procedures as per assigned treatment arm) for the primary outcome (Table 3). The effects of the study arms on the HPV clearance outcomes in the intention-to-treat and per-protocol cohorts are described in Table 3. On intention-to-treat analysis, HPV clearance was seen in 56.6% (95% CI 48.9–64.1) women in Arm 1 as compared to 41.4% (95% CI 34.3–48.7) women in Arm 2 at follow-up. We decided a priori that non-inferiority of the HPV screen, triage and treat arm (Arm 2) over the HPV screen and treat arm (Arm 1) would be inferred if the lower bound of the 95% confidence interval of the risk ratio (RR) from the regression model was greater than 0.49 (see statistical methods). In the intention-to-treat unadjusted regression analysis with multiple imputation, non-inferiority between the two arms was demonstrated as the lower bound of 95%CI of RR was greater than 0.49 (RR 0.73, 95%CI 0.59–0.91, $p$ value 0.005) (Table 3). Non-inferiority in HPV clearance between the two arms was also demonstrated in the per-protocol analysis (RR 0.74, 95%CI 0.60–0.93, $p$ value 0.008).

### Secondary outcome: detection of CIN 2+ at follow-up

Table 2 describes the CIN 2 or worse disease on histopathology among the randomised women with persistent HPV at follow-up according to their study arm. The proportion of women undergoing complete follow-up assessment (HPV test followed by colposcopy and biopsy for HPV positive women) was 70.6% in Arm 1 (screen and treat arm) versus 76.0% in the other arm (Arm 2; 'screen, triage and treat' arm) ($p = 0.2$). CIN2 or worse disease was diagnosed in 14.6% women in Arm 1 and 23.6% women in Arm 2 ($p = 0.043$).

The effect of the study arms on the CIN 2 or worse disease outcome in the intention-to-treat and per-protocol analysis are described in Table 3. On intention-to-treat analysis, CIN 2 or worse disease was diagnosed in 22/151 (14.6%, 95% CI 9.4–21.2) women in Arm 1 as compared to 39/165 (23.6%, 95% CI 17.4–30.9) women in Arm 2. In the per-protocol analysis, CIN 2 or worse disease was diagnosed in 20/141 (14.2%, 95% CI 8.9–21.1) women in Arm 1 as compared to 36/160 (22.5%, 95% CI 16.3–29.8) women in Arm 2.

In the unadjusted regression analysis, the risk ratio for CIN 2 or worse disease in the intention-to-treat analysis was 1.58; 95% CI 0.99–2.52 and in the per-protocol analysis it was 1.48; 95% CI 0.90–2.43 in the 'screen, triage and treat arm' (Table 3) but the increased risk of CIN2+ disease in the 'screen, triage and treat' arm was not statistically significant in both the per-protocol as well as the intention-to-treat analysis.

Two invasive cancers were detected in the 'HPV screen, triage and treat' arm (Arm 2) but none in the 'HPV screen and treat' arm (Arm 1) at follow-up. Age at randomization of the women detected with invasive cancers was 48 years and 52 years respectively. Both were triage-negative at baseline and, hence were untreated. A colposcopist reviewed post-hoc the cervical images of these two women obtained during VIA triage. Both women had Type 3 transformation zones (squamo-columnar junction was inside the cervical canal and not fully visible). The colposcopist felt that one of them should have been given

**Table 2 | Baseline treatment and follow-up details of women by randomization arms**

| | Randomization arm | | | | Pearson |
|---|---|---|---|---|---|
| | HPV and treat | | HPV, VIA triage and treat | | Chi-square |
| | *n* (%) | | *n* (%) | | *p* value |
| **Women randomized** | 215 | | 218 | | |
| **VIA triage result** | | | | | |
| Negative | NA[a] | | 150 | (68.8) | |
| Positive | NA[a] | | 68 | (31.2) | |
| **Women eligible for treatment** | 215 | (100.0) | 68 | (31.2) | |
| **Received treatment** | 184 | (85.6) | 55 | (80.9) | 0.351 |
| Thermal ablation | 154 | (83.7) | 35 | (63.6) | |
| LLETZ | 29 | (15.8) | 19 | (34.5) | |
| Hysterectomy | 1 | (0.5) | 1 | (1.8) | |
| **Women follow-up details for assessment of the HPV clearance outcome** | | | | | |
| Not eligible for follow-up due to hysterectomy done at baseline | 1 | (0.5) | 1 | (0.5) | |
| Eligible for follow-up | 214 | (99.5) | 217 | (99.5) | |
| Women with no follow-up HC II assessment | 41 | (19.2) | 26 | (12.0) | 0.040 |
| Women with follow-up HC II assessment | 173 | (80.8) | 191 | (88.0) | |
| Women with HPV clearance | 98 | (56.6) | 79 | (41.4) | 0.004 |
| Women with HPV persistence | 75 | (43.4) | 112 | (58.6) | |
| **Women follow-up details for assessment of CIN 2+ outcome** | | | | | |
| Not eligible for follow-up due to hysterectomy done at baseline | 1 | (0.5) | 1 | (0.5) | |
| Eligible for follow-up | 214 | (99.5) | 217 | (99.5) | |
| Women with no follow-up final diagnosis assessment | 63 | (29.4) | 52 | (24.0) | 0.20 |
| Women with follow-up final diagnosis assessment | 151 | (70.6) | 165 | (76.0) | |
| Women with no CIN 2 or worse disease at follow-up | 129 | (85.4) | 126 | (76.4) | 0.043 |
| Women with CIN 2 or worse disease at follow-up | 22 | (14.6) | 39 | (23.6) | |

*HPV* human papillomavirus, *VIA* visual inspection with acetic acid, *LLETZ* large loop excision of the transformation zone, *CIN* cervical intraepithelial neoplasia.
[a]VIA was not performed for women randomized to Arm 1, 5% acetic acid was applied to assess treatment eligibility.

a VIA-positive diagnosis and the acetowhite lesion was missed by the nurse performing VIA. In the second case, the endocervical lesion could be seen only on colposcopy.

### Safety: patient-reported side effects and complications

Participants undergoing treatment were advised to call the study coordinator on a dedicated mobile number for any complaints or side effects and data regarding the same was recorded on the case report form. Of the 154 participants in Arm 1 who received treatment with thermal ablation, pain/cramps and menorrhagia were reported by 2 (1.29%) each. Only one participant (0.65%) reported mild vaginal bleeding. Of the 29 participants in the same arm who were treated with LLETZ, only 1 (3.45%) reported pain/cramps and 2 (6.89%) reported mild bleeding. Of the 35 participants in Arm 2 who were treated by thermal ablation, only 2 (5.71%) reported more than average bleeding in the next menstrual cycle. None of the 19 participants in Arm 2 who were treated by LLETZ reported any side effects. All the side effects lasted for a few days and the women could be treated based on their symptoms (e.g., oral analgesics for pain and cramps and oral Tranexamic acid and Mefenamic acid (for bleeding) on out-patient basis only. No side effects related to treatment were reported at follow-up after one year by any of the participants.

### Exploratory outcomes

We also analysed the outcomes of histopathology-proven CIN 2 and CIN 3 in WLHIV by the type of treatment. Overall in the two arms combined, out of the 27 women with CIN 2/ CIN 3 at baseline treated with thermal ablation, 13 (48.1%) had persistent CIN 2/CIN 3 lesions at one-year follow-up. The proportion of women having persistent CIN 2/ CIN 3 lesions was 31.6% (6/19) among those treated with LLETZ. The treatment success rates for CIN 2/3 lesions among WLHIV were only 51.9% and 68.4% with thermal ablation and LLETZ respectively however the difference was not statistically significant (*p* value 0.261).

## Discussion

Our randomized controlled non-inferiority trial compared the 'HPV screen and treat' strategy with the WHO recommended 'HPV screen, VIA triage and treat' strategy among WLHIV. HPV clearance and detection of CIN 2 or worse disease at one year after randomization / treatment were the primary and the secondary outcomes respectively. There was no difference between the participants in the two study arms in terms of demographic characteristics, HIV-related factors, VIA positivity and prevalence of CIN 2/3 at baseline suggesting that the two arms were evenly balanced. Our study has provided important evidence that the 'HPV screen, triage and treat' strategy is non-inferior to the 'HPV screen and treat' strategy in both, the intention-to-treat and per-protocol analyses with regard to the primary outcome of HPV clearance at follow up over a median duration of nearly 12 months. Although non-significant, there was 58% and 48% increased risk of CIN 2+ disease in the 'screen, triage and treat arm' when compared to the 'screen and treat' arm in the 'intention-to-treat' and 'per-protocol' analysis respectively.

Using a model platform (Policy1-Cervix-HIV) and the screening data from WLHIV in Tanzania, the 2021 WHO guideline suggested 'HPV screen, triage and treat' strategy in WLHIV[13]. Absence of any randomized study comparing head-to-head the two strategies compelled the WHO to depend only on modelling to arrive at a recommendation regarding management of HPV-positive WLHIV[12]. Outcomes of our RCT are well aligned with the latest WHO recommendation to follow screen, triage and treat strategy over screen and treat strategy to

**Table 3 | Effect of intervention on the primary and secondary outcomes among WLHIV randomized to 'screen and treat' or 'screen, triage and treat' arms (using multiple imputation to cater for missing information)**

| Baseline characteristics | Women HPV positive at baseline | Women HPV positive at baseline and assessed during follow-up | Women HPV positive at baseline with the endpoint of the particular outcome during follow-up | | | Unadjusted regression analysis with multiple imputation[c] | | |
|---|---|---|---|---|---|---|---|---|
| | *n* | *n* | *n* | Proportion (95% CI) | | Risk ratio (95% CI) | | *p* value |
| ***HPV clearance outcome (primary outcome)***[d] | | | | | | | | |
| **Women assessed (intention-to-treat analysis)**[a] | | | | | | | | |
| **Study arm (intention -to-treat analysis)**[a] | 431 | 364 | 177 | 48.6 | (43.4–53.9) | | | |
| HPV and treat | 214 | 173 | 98 | 56.6 | (48.9–64.1) | 1.00 | | |
| HPV, VIA and treat | 217 | 191 | 79 | 41.4 | (34.3–48.7) | 0.73 | (0.59–0.91) | 0.005 |
| **Women assessed (per-protocol analysis)**[b] | | | | | | | | |
| **Study arm (per-protocol analysis)**[b] | 387 | 345 | 168 | 48.7 | (43.3–54.1) | | | |
| HPV and treat | 183 | 160 | 91 | 56.9 | (48.8–64.7) | 1.00 | | |
| HPV, VIA and treat | 204 | 185 | 77 | 41.6 | (34.4–49.1) | 0.74 | (0.60–0.93) | 0.008 |
| ***CIN 2 or worse outcome (secondary outcome)*** | | | | | | | | |
| **Women assessed (intention-to-treat analysis)**[a] | | | | | | | | |
| **Study arm (intention-to-treat analysis)**[a] | 431 | 316 | 61 | 19.3 | (15.1–24.1) | | | |
| HPV and treat | 214 | 151 | 22 | 14.6 | (9.4–21.2) | 1.00 | | |
| HPV, VIA and treat | 217 | 165 | 39 | 23.6 | (17.4–30.9) | 1.58 | (0.99–2.52) | 0.053 |
| **Women assessed (per-protocol analysis)** | | | | | | | | |
| **Study arm (per-protocol analysis)**[b] | 387 | 301 | 56 | 18.6 | (14.4–23.5) | | | |
| HPV and treat | 183 | 141 | 20 | 14.2 | (8.9–21.1) | 1.00 | | |
| HPV, VIA and treat | 204 | 160 | 36 | 22.5 | (16.3–29.8) | 1.48 | (0.90–2.43) | 0.120 |

*HPV* human papillomavirus, *VIA* visual inspection with acetic acid, *HIV* human immunodeficiency virus, *CD4* cluster of differentiation 4, *CIN* cervical intraepithelial neoplasia.
[a]Included all women randomized regardless of whether they were appropriately managed or not as per the assigned treatment arm.
[b]Included only women who were appropriately managed as per assigned treatment arm.
[c]Multiple imputation used to cater for the missing data on outcome.
[d]Non-inferiority of the HPV screen, triage and treat arm to the HPV screen and treat arm was inferred when the lower bound of the 95% confidence interval from the regression model was greater than 0.49.

manage HPV positive WLHIV[12]. At the same time, our RCT also suggests that 'HPV screen and treat' strategy has certain advantages and may also be used in WLHIV. Efficacy of the screen and treat approach among WLHIV has been previously reported only from one study in South Africa, which showed that CIN2 or worse lesions were significantly reduced at month 36 among the HPV positive women receiving immediate treatment compared to the HPV positive women followed up with colposcopy and biopsy (RR = 0.20, 95% CI 0.06–0.69)[15].

The 2021 WHO guideline recommended in favour of triaging as the number needed to treat to prevent one cervical cancer death was lower with this strategy compared to treating all HPV positives, thus making the triage option more efficient as a public health strategy[13]. In certain regions in the world, particularly in Africa where HPV prevalence among WLHIV can be as high as 70%[16,17], many women have to be treated if the 'screen and treat' strategy is adopted. This high referral rate for treatment can sometimes overwhelm the health systems. Our RCT supports the 'screen, triage and treat' strategy in such settings. VIA triage in our study reduced the number of women requiring upfront treatment by nearly 70%. VIA triaging might improve the programme in regions with very high HPV prevalence.

However, there are certain advantages of the HPV screen and treat strategy. We observed that significantly higher proportion of WLHIV in the 'screen and treat' arm could be treated with thermal ablation by a nurse at the ART clinic itself (no referral for LLETZ, no further visits required) as compared to the 'screen, triage and treat' arm (83.7% vs. 63.6%). The screen and treat strategy minimises loss to follow-up and treatment of HPV positive women offers an opportunity for HPV clearance before the lesions appear. The detection of two cases of invasive cancer at follow-up in the triaging arm, which were negative on VIA triage at baseline, is a matter of concern. This is in spite of the fact that the nurses in our study have been performing VIA for more than 10 years. WLHIV are at an increased risk of persistence of HPV infection, development of CIN and faster progression[4]. The HPV screen, triage and treat strategy relies on treating cervical lesions as they appear however the most pragmatic triaging option for the low- and middle-income countries with high burden of HIV and HPV is VIA which is not a perfect triaging test. These factors need to be considered while balancing the benefits versus limitations of the two algorithms as the ablative methods of treatment have minimal complications including pre-term delivery and are much less expensive than excisional treatment. The feasibility and safety of implementing HPV screening using a point-of-care X-pert HPV test and same day treatment in Khayelitsha in South Africa with high HIV and HPV prevalence has also been demonstrated earlier[18]. Hence countries in Africa including Zambia are now implementing[19] or planning to implement HPV screen and treat strategy for both, women in the general population as well as WLHIV.

It is also worth noting that the study was initiated when the first wave of Covid-19 pandemic started declining in western India. The study was continued throughout the 2nd and 3rd wave of Covid-19 pandemic[20] when screening was performed in a mobile screening unit[21] outside the ART centres and then in a makeshift clinic where cervix biopsies and ablative treatment were managed because the hospital was overflown with Covid-19 patients. This demonstrates the feasibility of implementing the 'screen and treat' strategy in the mobile screening unit as well as in a makeshift clinic.

Early initiation of antiretroviral treatment and appropriate monitoring of HIV disease reduces the risk of incident cervical cancer[17]. There is a compelling need for improving access to appropriate HIV disease monitoring as well as cervical cancer screening using an HPV test at the ART centres to avert preventable deaths due to cervical

cancer in the LMICs. Although Covid-19 pandemic could be the reason, despite the WHO recommendation to monitor HIV disease using HIV-1 viral load rather than CD4 count[22] more than 85% of the WLHIV in our study did not have their HIV viral load test report within 6 months of enrolment.

We based our primary outcome on detection of persistent HPV infection rather than CIN 2+ outcome. Our decision was based on the fact that HPV test is widely recommended as a 'test of cure' following cervical precancer treatment including in a 'screen and treat' setting[12,23–25]. Persistent HPV infection at follow-up can effectively predict the risk of disease recurrence. This is true for general population as well as WLHIV. In our previous longitudinal study among WLHIV, significantly higher risk of CIN 2+ disease was observed in women who cleared HPV infection (RR 23.95, 95% CI 2.40-661.07) and in those with persistent HPV infection (RR 138.18, 95% CI 20.30-3300.22) when compared with WLHIV who were HPV negative at baseline[26]. A Kenyan RCT observed that WLHIV with persistent high-risk HPV infection at follow up after cryotherapy or LLETZ had five times higher risk of recurrent CIN[27].

An exploratory but important finding of the study was the low success rate following treatment of cervical precancers in WLHIV, irrespective of whether it is ablative or excisional technique. This is already recognized as a major challenge among WLHIV for preventing subsequent risk of development of cervical cancers[27–29]. An RCT nested in 'screen and treat' programme in Zambia reported that treatment success among WLHIV following thermal ablation and LLETZ was only 44% and 54.5% respectively[30]. The Zambian study used a combination of VIA and HPV test to assess treatment success.

Although all the enroled participants in our study were on HAART, the impact of immune restoration by HAART in people living with HIV on HPV-induced disease is modest at its best[31]. Another possible explanation for persistent cervical HPV infection in women treated for CIN is cervical autoinoculation following treatment of CIN[32]. High prevalence and persistence of anal HPV infection among WLHIV has already been reported[33,34]. Women with anal HPV infection have a higher risk of recurrent cervical HPV infection in spite of successful treatment[35] and this association needs to be studied further among WLHIV.

Our exploratory analysis confirmed very low success rates in treating CIN 2/CIN 3 using histopathologic verification. Higher treatment failure rate is compounded by the second challenge that WLHIV have much faster progression compared to general population[36–38]. Hence, we should be following the most effective management approach and this is even more relevant for LMICs as recalling triage-negative women for a repeat HPV test after one year always has the risk of a significant number of them dropping out. This was observed even in a very controlled setting of our RCT. Very low rate of complication after treatment with either thermal ablation or LLETZ reported in our study is quite reassuring to promote widescale screen and treat strategy wherever feasible.

We used VIA as a triage test. WHO has recommended using either VIA or cytology for triaging. Due to the challenges of implementing quality assured cytology, most LMICs will have to depend on VIA. Although training of VIA improves the triaging outcome in HPV positive women, VIA is still challenging due to high variability of VIA performance[14]. Despite highly heterogeneous performance of VIA due to its subjective nature, WHO recommended the test considering its simplicity, feasibility and low cost[28]. Point-of-care nature of the test provides an opportunity to treat screen-positive women during the same visit, thus limiting the loss to follow-up[12]. There is evidence that VIA in a triaging setting performs better than in screening setting, since in the former setting smaller number of women with higher risk of harbouring lesions have to be examined[14]. Moreover, by using VIA to triage the HPV positive women many of the LMICs will be able to leverage the investment they have made to train a large number of providers to perform the test[13].

Our study has a few limitations. We did not collect cervical biopsies for women without any visible lesion after application of 5% acetic acid (for assessment of treatment eligibility in Arm 1 and for VIA triage in Arm 2). This might have underestimated the histopathological disease outcomes at baseline. However, this would have affected both the arms equally as the assessment was performed by the same study nurse. We used a hybridization assay (HC2) that did not provide partial genotyping information. Given the current landscape of available HPV tests, most programmes are likely to incorporate partial genotyping in the triaging strategies that would help predict the risk of women having a precancerous lesion more accurately. Considering this fact, the WHO is reviewing evidence favouring the use of extended genotyping as a triaging option for the living guideline. Although incorporating HPV 16/18 genotype information can improve the efficacy of the triaging algorithm, we have previously reported that HPV types other than HPV 16 and HPV 18 also contribute to a substantial number of incident CIN 2+ lesions among WLHIV[37]. Following the Indian national guidelines we extended screening to women up to 60 years of age. The WHO recommends screen, triage and treatment only up to 49 years as VIA triage is known to have lower sensitivity in women above 49 years due to non-visualization of entire transformation zone. However, only 15% of our study participants were above 49 years of age and the arms were comparable in this respect. In order to address the loss to follow-up and missing data in both the arms, we used multiple imputation in the regression analyses to reduce the impact of missing data on outcomes. Still we consider drop-outs from follow-up in either arm as a limitation. Considering the fact that about 50% of untreated CIN2 lesions regress over time[39], the ideal secondary endpoint could have been CIN3+ lesions however we considered CIN2+ lesions as the secondary endpoint since CIN2 is an accepted endpoint in studies in which all women with CIN2 or worse lesions are treated[40]. In spite of these limitations, our RCT has addressed some of the important knowledge gaps related to managing WLHIV detected to be positive on HPV test.

To conclude, wider access to HAART has improved life expectancy among WLHIV[41]. But they remain at a higher risk of developing cervical cancer compared to healthy individuals[42]. Screening women with an HPV test will provide large gains in objectively selecting WLHIV at risk of CIN, but these gains can be lost by failing to manage the HPV positive women appropriately. The LMICs introducing HPV detection-based screening for WLHIV need to ensure high quality of VIA tests and strengthen their recall systems to ensure the success of the screen, triage and treat strategy. At the same time, 'HPV screen and treat' strategy can also be used for managing HPV positive WLHIV whenever feasible. It is safe to be implemented in the out-patient setting and ablative treatment can be managed by trained nurses. A more sensitive and logistically simpler triaging test than VIA, possibly a biomarker such as p16/ki67 or methylation tests[43–45], may eventually tilt the balance further in favour of the 'screen, triage and treat' algorithm.

## Methods

The study was approved by the Institutional Ethics Committee for Research of Prayas, a non-profit organization in Pune, India. The International Agency for Research on Cancer (IARC-WHO), France provided technical support for sample size estimation, randomization sequence and data analysis. The trial has been registered with the Clinical Trial registry of India (Clinical trial registration: CTRI/2020/02/023349).

### Study setting and selection of participants

The RCT was conducted at two antiretroviral treatment (ART) centres of National AIDS Control Organisation (NACO), India. A written

informed consent was obtained by a trained study staff after counselling women on the importance of screening, early detection, and treatment of precancer and about the study procedures. Women were interviewed for socio-demographic, reproductive and HIV related information using a structured questionnaire. Consecutive WLHIV aged 25 to 60 years attending the ART centres who reported of ever having sex, were invited to participate in the study and were screened with an HPV test. Women who were pregnant or had any history of treatment of CIN or a hysterectomy were excluded. HPV-positive WLHIV were randomised to either 'screen and treat' or 'screen, triage and treat' arms (Fig. 1). While screening was performed at both centres, HPV-positive women were recalled to one centre for randomization and other procedures except large loop excision of transformation zone (LLETZ), which was subsequently performed at the clinic at Prayas.

### Sample collection and testing for HPV

WLHIV were screened using the HPV test by a study nurse after exposing the cervix with a bivalve speculum. Cervical sample for the HPV test was collected by a soft Christmas tree brush provided by the manufacturer and was placed in the Digene™ specimen transport medium (STM). The samples were sent to Genepath Diagnostics, Pune, which is a nationally accredited (MC-3361) laboratory and routinely participates in the College of American Pathologists proficiency testing.

Samples were analysed by the Hybrid Capture 2™ (HC2) assay (Qiagen INC, Maryland, USA) for 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) as per the manufacturer's instructions. A positive result was recorded for specimens with a ratio of relative light unit to a positive control (RLU/PC) of 1 or more, corresponding to 5000 or more viral copies.

Once the HC2 test reports were available, women who tested negative were advised a repeat HPV test after three years[10,11] and were excluded from the analysis (Fig. 1). HPV-positive women were recalled to the study clinic. They were informed about their HPV test report, the need for further evaluation and the study procedures to be followed. Women agreeing to continue participation in the study were further assessed before randomization as mentioned below.

### Randomization process

A study nurse performed speculum examination and applied 5% dilute acetic acid on the cervix to detect any suspected invasive cancer, extensive acetowhite lesions extending to vagina that would be difficult to manage in an out-patient setting, or a cervix that was difficult to expose properly (e.g., due to vaginal atrophy). After excluding these cases (and referring them appropriately), rest of the women were randomised to either 'screen and treat' (Arm 1) or 'screen, VIA triage and treat' (Arm 2) algorithm at a 1:1 ratio (Fig. 1). Serial numbers of the expected total number of participants to be recruited were randomly allocated to two arms using computer- generated simple randomization by the statistician at IARC. The numbers with allocation arm were distributed to the study site in opaque sealed envelopes by a staff not involved with the study implementation. The sealed, serially numbered randomization envelopes were kept in custody of the study coordinator at the clinic.

Once eligibility of a particular woman was confirmed by the nurse, the study coordinator opened the randomization envelope and informed the nurse about the assigned algorithm. Neither the participants nor the investigators could be blinded due to the nature of subsequent interventions.

### Triaging and treatment procedures

Women assigned to Arm 1 (screen and treat) were assessed for eligibility for ablation by the study nurse after applying acetic acid for one minute to the cervix. She determined suitability for ablation based on the following criteria: entire squamocolumnar junction (SCJ) was fully visible on the ectocervix and in presence of an acetowhite patch on the cervix, the lesion did not extend beyond three quadrants of the cervix or into the endocervix or vagina. Punch biopsies were obtained from acetowhite areas, if present. The histopathology report was evaluated on a later date. Those eligible for ablation were treated by the nurse with thermal ablation on the day of randomization. Thermal ablation involved application of a 20 mm flat probe electrically heated to 100 °C for 45 seconds to ablate the cervical transformation zone. We used a portable, battery-operated thermo-coagulator of WISAP® (Germany). If a single application did not cover the entire transformation zone, additional 4-5 overlapping applications of 45 seconds each were carried out. No local anaesthesia was administered prior to thermal ablation. Women with lesions not eligible for thermal ablation (large lesions involving more than 3/4$^{th}$ of the transformation zone, or lesions extending to the endocervix or vagina) underwent LLETZ at the clinic at Prayas at a later date.

Women assigned to Arm 2 (screen, triage and treat) underwent VIA by the nurse using 5% acetic acid; and VIA-negative women were advised a repeat HPV test after one year. Women with a VIA positive outcome were assessed for eligibility for ablative treatment as mentioned above and were treated with thermal ablation during the same sitting after cervical punch biopsies. Women ineligible for ablative treatment were treated with LLETZ at a later date. The study nurses were trained in multiple workshops organized by IARC and had more than 10 years of experience in performing VIA. Cervical images were collected routinely for all women. The images were periodically reviewed by a colposcopist together with the nurses for quality control and also refresher training of the nurses.

### Follow-up procedures

All randomised women not treated at baseline were called one year after randomization and the rest were called one year after treatment for a repeat HPV test using nurse-collected sample analysed by HC2 testing. All women positive with the HPV test at follow-up underwent colposcopy at the clinic and biopsy from any visible lesion. Endocervical curettage was performed if the SCJ was not fully visible and there was no acetowhite lesion on colposcopy. Women with persistent HPV infection were offered treatment; thermal ablation or LLETZ was used to treat the cervix depending on eligibility. Women treated with ablation at baseline and having persistent HPV infection were treated with LLETZ.

### Histopathology

Cervical biopsy specimens were reported at the local laboratory at Prayas and CIN terminology was used for interpretation[46]. All the histology slides were re-reviewed by an experienced external pathologist from the Tata Memorial Centre, Mumbai, India and, in case of disagreement, a consensus diagnosis was reached. The final diagnosis was based on worst histopathology findings for those who had biopsies or excised LLETZ specimens or endocervical curettage. The pathologists were blinded to the randomization assignment at baseline or follow-up.

### Study outcomes

The first primary outcome was HPV clearance in the two arms which was defined as a positive HPV test report at baseline and a negative HPV test report at follow-up after one year of randomization or treatment. The secondary outcome was detection of CIN 2 or worse disease (CIN 2+) at follow-up based on biopsies obtained from colposcopically visible lesions in HPV-positive women or excised LLETZ specimens. In the absence of histopathological diagnosis, a colposcopy report not suggesting any neoplasia was considered as a proof of absence of CIN 2+ at follow up.

### Sample size estimation

The sample size estimation was done using following assumptions: 60% clearance of HPV in WLHIV in the 'screen and treat' arm based on previously published literature[26,47,48], 3% reduction in HPV clearance (58.2%) in the screen, triage and treat arm; a margin of non-inferiority of 15% absolute percentage points; 15% loss to follow-up; 2.5% one-sided level of significance; and power of 80%. Given these assumptions the sample size in both arms combined was estimated to be 446.

### Statistical analysis

Data were entered using Access 2000 software and analysed using STATA software, version 17.0 (Stata-Corp, College Station, TX, USA). The baseline characteristics of participants were presented as proportion and compared between the two study arms using the Pearson chi-square test. The baseline histopathology diagnosis and baseline treatment received by the participants were also shown as proportions, stratified by study arm. In addition, the primary and secondary outcomes were assessed in the two arms and provided as overall proportions, and stratified by baseline treatment status and baseline histopathological diagnosis categories.

To assess the effect of the randomization arm on the primary and secondary outcomes, both the intention-to-treat analysis (in which all randomised participants were considered regardless of whether they received the allocated treatment or not), and the per- protocol analysis (which included only participants who followed the study procedures as per assigned treatment arm) was performed. To reduce information loss due to missing data in the outcomes (e.g., drop-outs from follow-up) and some of the explanatory variables, multiple imputation using chained equations (MICE) was performed using the "mimpt chained" package in Stata software. In this MICE method, multiple variables are sequentially imputed using a Gibbs-like algorithm. The logit model was used for the two binary variables with missing data. Fifty datasets were imputed. The explanatory variables used in the imputation were: study arm, completed age, years since known to be HIV positive, CD4 count at the time of HAART initiation, CD4 count within 6 months of recruitment and baseline histopathology diagnosis. This was because in the univariate logistic regression analysis, the study arm, age and clinical variables were shown either to significantly affect the two outcomes due to missing data (study arm, age, CD4 count within 6 months of recruitment and baseline histopathology diagnosis) or their reduced or increased effect estimates (even though not statistically significant) were deemed to be clinically significant (Supplementary Table 1). The effect of the study arm on the study outcomes was evaluated using relative risks (RRs) and their 95% confidence intervals (CIs) obtained from the log-binomial regression using the "binreg" command in Stata in which a generalized linear model for the binomial family with the log link function was fitted. Finding based on multiple imputation are provided in Table 3 and those based on complete case analysis are given in the Supplementary Table 2.

Non-inferiority of the 'HPV screen, triage and treat arm' to the 'HPV screen and treat arm' was inferred when the lower bound of the 95% confidence interval from the regression model was greater than 0.49. This was obtained by taking the expected absolute difference in the proportion of HPV clearance between the study arms and the assumed margin of non-inferiority and converting them into expected maximum relative risk.

### Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

## Data availability

External researchers can make written requests to the corresponding author for sharing of the data after publication. A brief analysis plan and data request will be required and reviewed by the institutional ethics committee for approval of data sharing. When requests are approved, anonymised data can be shared electronically in password protected files. All data sharing will abide by rules and policies defined by the sponsor and the Ethics Committee regulations. Data sharing mechanisms will ensure that the rights and privacy of individuals participating in research will be always protected.

## Code availability

External researchers can additionally make written requests for code used in the statistical analysis from Dr. Richard Muwonge (email: muwonger@iarc.who.int) with a copy to Dr Smita Joshi (email: smita.j@prayaspune.org). These requests should include a brief explanation of what the code is going to be used for. Each request will then be discussed with and approved by the lead author and co-investigators. The approval will be done within a month of receipt of the request.

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## Author contributions

SJ was responsible for the conception, study design, conduct, acquisition of data, monitoring of the study, statistical analysis, interpretation of the data and manuscript preparation. RM and BKK were responsible for sample size, randomization, statistical analysis, interpretation of the data and manuscript preparation. RB, VK, PC, SK, NP, SR were responsible for acquisition, interpretation of data and manuscript preparation. MM and KD were responsible for histopathology reporting, analysis and manuscript preparation. RS and PB were responsible for study conception, data analysis, interpretation and manuscript preparation. All authors had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit the manuscript for publication.

## Competing interests

The authors declare no competing interests.

## Ethics and inclusion

This study was conducted among women living with HIV. All participants provided a written informed consent. The study protocol, participant informed consent document and case report forms were reviewed and approved by the Institutional Ethics Committee for Research, Prayas.

## Additional information

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