Article

Discovery Medicine 2026; 38(211): 2418–2437
https://doi.org/10.24976/Discov.Med.202638211.191

Real-World Effectiveness of 5-Aminolevulinic Acid Photodynamic Therapy for HPV-Related Lower Genital Tract Lesions: A Single-Center Retrospective Cohort Study (2021–2024)

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Submitted: 29 April 2026 Revised: 8 June 2026 Accepted: 26 June 2026 Published: 24 August 2026

Background: Excisional procedures for high-risk human papillomavirus (HPV)-related lower genital tract lesions fail to eliminate the underlying HPV infection and carry fertility-related risks, leaving patients with persistent low- or high-grade lesions without an effective tissue-preserving option. Real-world data on 5-aminolevulinic acid photodynamic therapy (ALA-PDT) across the full lesion spectrum remain limited. This study evaluated composite and histological/cytological complete response rates of ALA-PDT and explored whether morphological and virological responses follow distinct temporal trajectories.

Methods: In this single-center retrospective cohort study conducted between 2021 and 2024, 176 patients were included after excluding isolated vulvar intraepithelial neoplasia (VIN), isolated condylomata acuminata, and HPV infection <12 months without morphological lesions. A total of 177 treatment episodes were recorded, as one patient received two independent ALA-PDT courses separated by >6 months, and only the first episode was retained in the primary per-patient analysis. Of these, 174 patients had confirmed cervical or vaginal intraepithelial neoplasia (with or without concurrent vulvar lesions or condylomata acuminata), and 2 had persistent HPV infection without morphological lesions. All patients received standardized ALA-PDT (20% ALA; 635 nm; 120 J/cm^2; 3–6 sessions). Of 174 patients with morphological disease, 147 had valid follow-up and constituted the primary efficacy population. The primary endpoint was composite complete response (CR), requiring concurrent morphological resolution, cytological normalization, and HPV clearance. A predefined secondary endpoint, histological/cytological CR (hCR), required morphological and cytological normalization irrespective of HPV status.

Results: Among 147 evaluable patients with morphological disease, composite CR was 68.7% (101/147), with hCR of 83.0% (122/147) and overall response rate of 93.2% (137/147). These estimates were robust across follow-up strata (CR ≥6 months: 70.8%; ≥9 months: 73.3%) and after inverse probability weighting (68.1%). CIN 2/3 achieved the highest composite CR (85.7%). Cross-sectional observations suggested that cytological normalization may plateau earlier than HPV clearance, which continued to increase through 12 months.

Conclusions: ALA-PDT demonstrated high efficacy across HPV-related lower genital tract lesions, with morphological response rates suggesting clinically meaningful disease control. These findings support ALA-PDT as an effective, tissue-preserving option for HPV-related lower genital tract lesions. Direct assessment of reproductive outcomes, sexual function, quality of life, and patient satisfaction was not feasible in this retrospective dataset and warrants future prospective studies.

Keywords: 5-aminolevulinic acid; photodynamic therapy; HPV; cervical intraepithelial neoplasia; lower genital tract; HPV clearance; fertility preservation; real-world evidence

Introduction

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections worldwide and is an established etiological agent of cervical intraepithelial neoplasia (CIN) and cervical carcinoma, as well as a major cause of HPV-related vaginal (VaIN) and vulvar (VIN) intraepithelial neoplasia, condylomata acuminata, and a subset of vaginal and vulvar invasive carcinomas [1,2,3,4]. Persistent high-risk HPV infection accounts for virtually all cases of cervical cancer, which was the fourth most common malignancy among women globally [5]. In China, a recent national summative analysis of over 2.7 million women reported an overall HPV prevalence of 17.70% and a high-risk HPV prevalence of 13.12%, with a bimodal age distribution peaking in women under 21 and over 61 years of age [6]. While HPV prevalence has remained relatively stable in recent years, age-standardized cervical cancer incidence increased by approximately 3.7% annually between 2006 and 2016 and is projected to continue rising

through 2030, with a widening urban-rural disparity among younger women [7].

Current management of cervical precancer is constrained by a fundamental paradox. Local excisional and ablative procedures, such as loop electrosurgical excision procedure (LEEP), cold knife conization, and laser ablation, effectively remove dysplastic tissue but do not reliably eradicate the underlying high-risk HPV infection that drives lesion persistence and recurrence [8,9]. Persistent postoperative high-risk HPV infection is a major driver of residual or recurrent disease, and recurrent CIN2+ is reported in approximately 5–25% of women after conization, with most recurrences detected within the first 2 years [9,10]. Meanwhile, the excisional approach itself carries risks of cervical insufficiency, second-trimester pregnancy loss, and preterm delivery, which are consequences of particular concern since CIN typically occurs in young women of reproductive age [11]. For low-grade lesions (CIN 1, VaIN 1), guidelines generally recommend expectant management or follow-up rather than immediate treatment, given their high spontaneous regression rates and low risk of progression [12,13,14]. However, persistent low-grade HPV-associated lesions pose a management dilemma: for persistent CIN 1, continued observation remains the preferred strategy, and VaIN 1 is likewise managed with follow-up [12,14]. Although several conservative therapies are under investigation, no conservative intervention has yet been established as standard of care in major guidelines [15]. Condylomata acuminata present a parallel challenge, whose recurrence remains common across treatment modalities [16,17]. These converging limitations, including surgical morbidity, failure to address the viral reservoir, and lack of effective non-excisional options, define the clinical need for a tissue-preserving treatment capable of both eliminating dysplastic lesions and promoting HPV clearance.

5-aminolevulinic acid photodynamic therapy (ALA-PDT) has emerged as a promising tissue-preserving, non-excisional therapeutic option for HPV-associated intraepithelial lesions [18]. ALA, a naturally occurring precursor in the heme biosynthetic pathway, is intracellularly converted to protoporphyrin IX (PpIX) and, after topical application, preferentially accumulates in dysplastic and other pathological cells, including HPV-associated lesions [19]. Illumination with red light (630–635 nm) generates reactive oxygen species through accumulated PpIX, inducing selective cell death with relative preservation of surrounding normal tissue [20]. Beyond direct cytotoxicity, ALA-PDT also appears to exert immunomodulatory effects by facilitating local antigen presentation and enhancing cytotoxic T-cell responses, which may contribute to HPV clearance beyond lesion regression alone [21,22]. This dual mechanism carries a clinically important implication that, because the immediate cytotoxic effects of ALA-PDT and the downstream immune processes associated with HPV control may evolve over different timescales, the morphological regression and HPV clearance may follow partially dissociated temporal trajectories after treatment [23].

Reported lesion remission or complete response rates after ALA-PDT have ranged from approximately 90–96% for CIN1 and 73–90% for CIN2/3, depending on the treatment protocol, comparator, and duration of follow-up [24,25]. However, notable limitations constrain the existing evidence. Most studies have focused on single lesion types, particularly cervical lesions, with comparatively sparse data on non-cervical sites such as VaIN, VIN, and condylomata acuminata [26,27]. Reported efficacy endpoints also vary widely, ranging from complete histological remission alone to composite outcomes requiring both lesion regression and HPV clearance, making cross-study comparison difficult and complicating direct assessment of whether the morphological efficacy of ALA-PDT is comparable to that of excisional procedures [28,29]. Further, the temporal relationship between cytological normalization and HPV clearance after PDT remains incompletely characterized, leaving uncertainty about the optimal timing of post-treatment HPV testing [30].

To address these gaps, we conducted a single-center retrospective cohort study evaluating the real-world effectiveness of ALA-PDT across the full spectrum of HPV-related lower genital tract lesions treated at our institution from 2021 to 2024. The primary aim was to determine the composite complete response rate using a stringent endpoint requiring concurrent cytological normalization, morphological resolution, and HPV clearance. We predefined a histological/cytological complete response (hCR) endpoint paralleling the success criteria conventionally used for LEEP to facilitate comparison with excisional surgery. Also, we examined the cross-sectional temporal profiles of cytological and virological response as an exploratory objective, testing the hypothesis that morphological response and HPV clearance follow distinct kinetic trajectories. The findings would have direct implications for the timing of post-treatment surveillance if confirmed.

Methods

Study Design and Patient Selection

This was a single-center, retrospective cohort study conducted at the Department of Obstetrics and Gynecology, The First Affiliated Hospital USTC, Hefei, China. We identified all consecutive patients with HPV-related lower genital tract lesions who received ALA-PDT between January 2021 and December 2024. The study was approved by the Ethics Committee of The First Affiliated Hospital of USTC (approval number: 2024-RE-137) and conducted in accordance with the Declaration of Helsinki. Written informed consent for retrospective extraction of routinely collected clinical data was waived by the ethics committee given the non-interventional nature of this retrospective review. Prospective written informed consent was

nonetheless obtained at the relevant clinical encounter, separately from this waiver, for the two patients who received ALA-PDT for the investigational indication of persistent HPV infection without morphological lesions, and for the patients whose colposcopic images are reproduced in this manuscript. This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cohort studies.

The baseline variables of parity, baseline cytology, and number of PDT courses completed had incomplete documentation in the source records. Parity was not systematically recorded in routine gynecology clinic notes during 2021–2023 (84 of 149 patients, 56.4%) and became part of standardized documentation only from 2024 onward (0 of 27 patients with missing parity). For baseline cytology, 10 patients (5.7%) had their cytology performed at referring institutions, with results documented narratively in the referral letter but not entered into the structured electronic medical record. The original cytology slides/reports were not available for re-review. Missing PDT course counts (n = 8, 4.5%) reflect 7 patients who were lost to follow-up before completing the planned treatment schedule and 1 patient who proceeded to LEEP after partial response. The actual number of administered sessions was recorded in all 8 cases (see PDT sessions row of Table 1), but these cases could not be categorized into the "1 course"/"2 courses" framework defined by completion of a full treatment cycle. All missing values are reported transparently in Table 1 as a separate category and were not imputed.

The inclusion criteria were:

• Female patients aged ≥16 years.

• Histopathologically or colposcopically confirmed cervical intraepithelial neoplasia (CIN, any grade) and/or vaginal intraepithelial neoplasia (VaIN, any grade). Patients with concurrent vulvar intraepithelial neoplasia (VIN) or condylomata acuminata in the setting of coexisting cervical or vaginal lesions were eligible provided that they had coexisting CIN and/or VaIN. Isolated VIN without concurrent CIN or VaIN were not eligible for inclusion.

• Persistent high-risk HPV infection, defined as two or more positive high-risk HPV tests of the same genotype obtained at least 12 months apart (consistent with the WHO/ASCCP definition of persistent infection), without cytological or colposcopic evidence of morphological lesions, for whom ALA-PDT was offered as an investigational antiviral intervention. The inclusion of this subgroup was approved by the Ethics Committee as an exploratory cohort. All such patients provided prospective written informed consent at the time of treatment, in addition to the waiver of consent for retrospective data extraction, explicitly acknowledging the investigational nature of ALA-PDT for this indication. This subgroup is reported separately from the primary efficacy analysis, and no efficacy claims are made for this indication.

• Completion of at least one full course of ALA-PDT.

• Availability of at least one post-treatment follow-up record.

The exclusion criteria were:

• Pregnancy or breastfeeding.

• Known immunodeficiency or active immunosuppressive therapy.

• Prior invasive gynecologic malignancy within the preceding five years.

• Isolated VIN without concurrent CIN or VaIN (n = 8), as the resulting subgroup was too small for meaningful analysis.

• Isolated condylomata acuminata without concurrent intraepithelial neoplasia (n = 1).

• HPV infection of less than 12 months' documented duration in patients without morphological lesions (n = 5).

• Complete absence of post-treatment follow-up data.

One patient received ALA-PDT on two separate occasions for recurrent or persistent lesions during the study period, separated by more than six months. To avoid overestimating efficacy by double-counting outcomes from the same patient, the primary unit of analysis was the individual patient (N = 176), and only the first treatment episode was retained for this patient. This conservative approach reflects the true outcome of the initial treatment, given that the need for retreatment indicates persistent or recurrent disease. The episode-based analysis (N = 177), which includes both episodes from the retreated patient, is reported as a predefined sensitivity analysis. After applying inclusion and exclusion criteria, 176 patients were included in the baseline analysis (Table 1, Fig. 1). Two patients had persistent HPV infection without morphological lesions. Given the negligible sample size, these are reported descriptively and excluded from all efficacy denominators.

ALA-PDT Treatment Protocol

All treatments were performed using a standardized in-hospital protocol. A commercially available 5-aminolevulinic acid formulation (Aminolevulinic Acid Hydrochloride for Topical Solution; 118 mg ALA per unit; Fudan-Zhangjiang Bio-Pharmaceutical Co., Ltd., Shanghai, China) was applied topically to the lesion site at a concentration of 20% (w/v), with the dose adjusted according to the lesion area at approximately 38 mg/cm^2. The photosensitizer was left in contact with the tissue for 3 hours under light-protected conditions to allow intracellular PpIX accumulation. Photodynamic illumination was then delivered using a red-light laser device (wavelength 635 nm) at an irradiance of 80 mW/cm2 for 25 minutes, yielding a total light dose of 120 J/cm^2. Treatment sessions were scheduled at intervals of approximately 7–14 days with the menstrual period avoided. Each treatment course consisted of 3 sessions. Patients received 1 course (3 sessions) or 2 courses (6 sessions) based on interim clinical response, lesion grade, and treatment site. In general, patients with low-grade le-

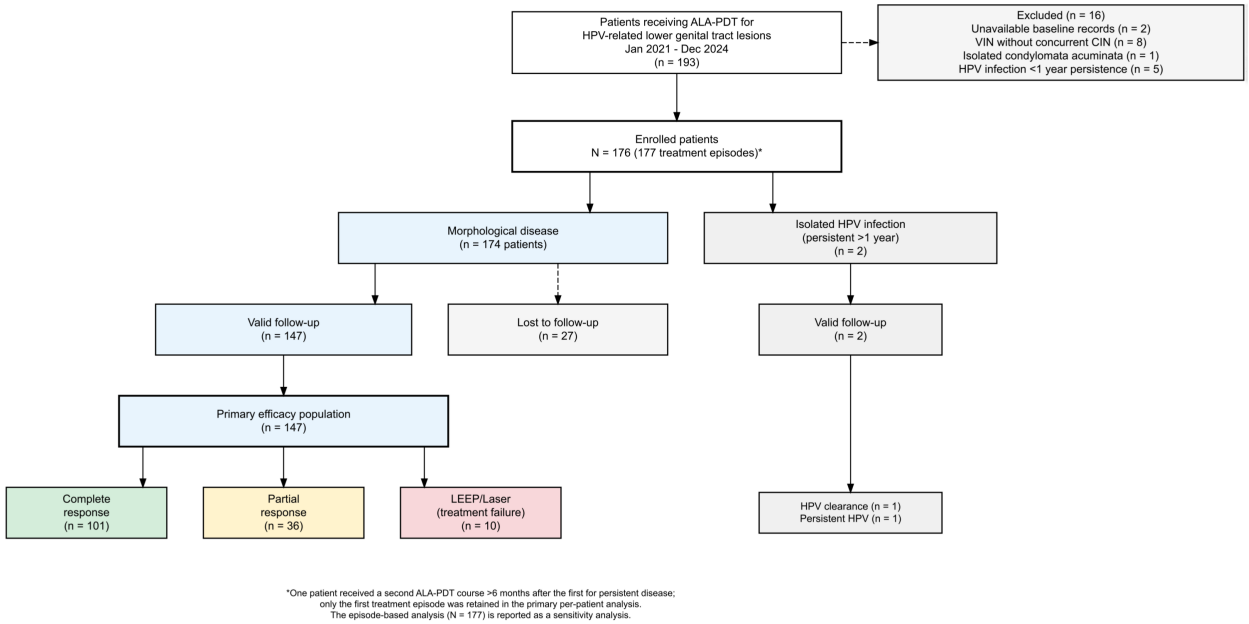


Fig. 1. STROBE flow diagram of patient selection. The diagram showing screening, enrollment, and follow-up status. The unit of analysis was the individual patient (N = 176). One patient received a second treatment course >6 months after the first for persistent disease. Only the first treatment episode was retained in the primary analysis. The episode-based analysis (N = 177) is reported as a sensitivity analysis. The 27 patients classified as lost to follow-up had no documented attendance at any scheduled post-treatment visit (3, 6, or 12 months). Individual reasons for non-attendance were not retrievable from routine clinical records. Solid arrows denote participants retained and carried forward through the analysis; dashed arrows denote participants not carried forward (excluded at screening or lost to follow-up). STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

sions or those demonstrating rapid clinical improvement received 1 course, whereas patients with high-grade or persistent lesions received 2 courses.

Follow-up Schedule and Outcome Definitions

Post-treatment follow-up was scheduled at 3, 6, and 12 months after the date of the last ALA-PDT treatment session. Each follow-up visit included liquid-based cytology (LBC; ThinPrep method), high-risk HPV genotyping, colposcopic examination, and targeted biopsy when indicated. HPV persistence was defined at the assay level (any high-risk genotype positivity at both timepoints). Genotype-specific concordance was not required, as clinical HPV genotyping panels do not consistently resolve individual genotypes across sequential visits in routine practice.

Colposcopic examinations included native (unmedicated) visualization, acetic acid application (5% acetic acid for 60 seconds), and Lugol's iodine staining. Colposcopic images were routinely captured at baseline and at follow-up visits. Representative pre- and post-treatment colposcopic images were selected to illustrate typical morphological response patterns (Fig. 2). Colposcopic images reproduced in this manuscript were obtained with prospective written informed consent for clinical documentation and publication at the time of image acquisition, in addition to the waiver of consent for retrospective data extraction. Identifying information was removed.

The following efficacy endpoints were defined.

Composite complete response (CR): Resolution of all visible lesions confirmed by colposcopy, with concomitant normalization of cytology (NILM on LBC) and clearance of the causative HPV genotype at the last available follow-up visit.

Histological/cytological complete response (hCR): Resolution of all visible lesions with normalization of cytology (NILM), irrespective of residual HPV positivity. This endpoint was predefined to facilitate comparison with the existing literature on excisional treatments, which typically define treatment success using histological or cytological criteria alone.

Partial response (PR): Morphological improvement without meeting the full CR criteria. This included two scenarios of histological downgrading, including the lesion (e.g., CIN 2/3 to CIN 1, or reduction in lesion size) regardless of HPV status, and complete morphological resolution with cytological normalization but without concurrent HPV clearance. The latter scenario was classified as PR rather than CR to maintain the stringency of the composite CR definition.

Overall response rate (ORR): Proportion of evaluable patients achieving either CR or PR.

HPV clearance: Conversion to negative high-risk HPV status at the last available follow-up visit with HPV testing, which may differ from the visit at which the com-

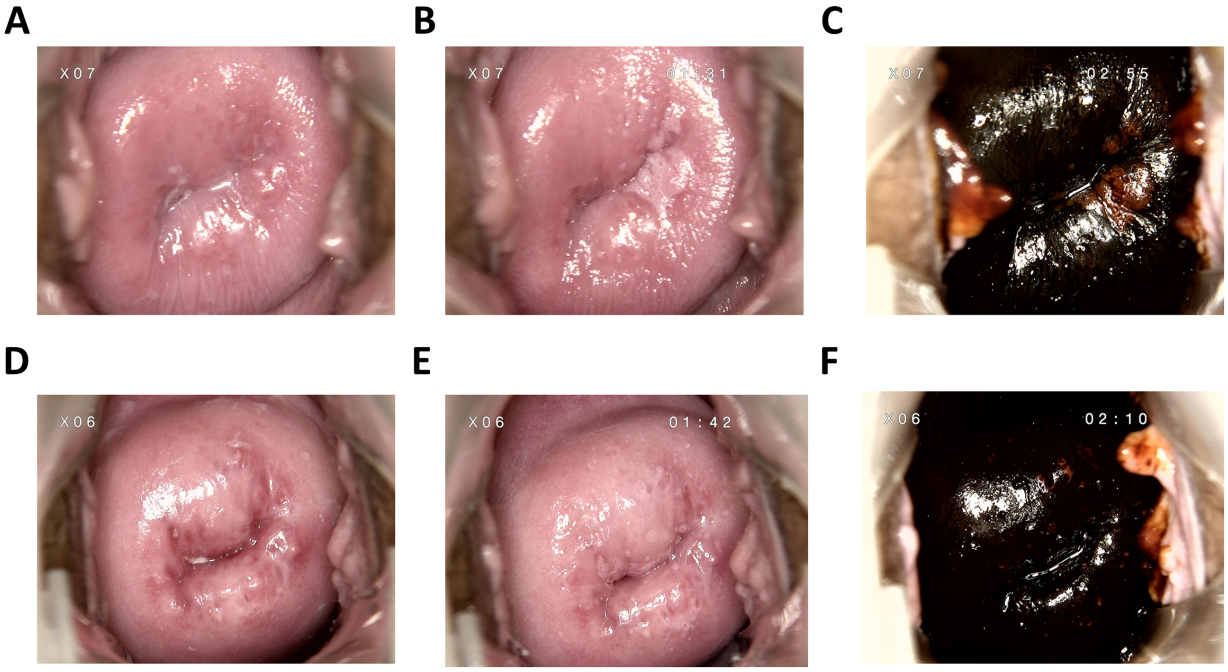


Fig. 2. Representative colposcopic images before and after ALA-PDT in a patient with CIN2. Upper panel: pre-treatment colposcopic findings. (A) Native view showing the cervical transformation zone. (B) After acetic acid application (5%), a dense acetowhite epithelium with well-defined margins is visible. (C) Lugol's iodine staining reveals a sharply demarcated iodine-negative (mustard-yellow) area corresponding to the dysplastic lesion. Lower panel: post-treatment colposcopic findings at 3 months. (D) Native view showing a smooth cervical surface. (E) After acetic acid application, no acetowhite changes were identified. (F) After Lugol's iodine staining, the previously iodine-negative lesion area now shows uniform mahogany-brown uptake, consistent with restoration of glycogenated mature squamous epithelium. A few faint punctate areas of attenuated iodine uptake remain visible within the otherwise uniformly stained field; these are small, scattered, and lack the sharply demarcated mustard-yellow borders or correlation with prior acetowhite changes that would characterize residual CIN, and were interpreted as immature squamous metaplasia in the early post-PDT re-epithelialization phase, which transiently lacks the full glycogen content of mature squamous epithelium. Camera light reflections from the moist mucosal surface are also visible and should not be confused with iodine-negative areas.

posite CR endpoint was formally assessed. A patient classified as PR (e.g., retaining HPV positivity at the visit used for outcome classification) may nonetheless be counted as achieving HPV clearance if a subsequent HPV test yielded a negative result. This conservative accounting framework means that the overall HPV clearance rate may exceed the composite CR rate. Follow-up HPV testing in routine clinical practice, at our institution, reported assay-level positivity for the panel of high-risk genotypes rather than genotype-resolved results at each visit. Accordingly, HPV clearance was operationalized at the assay level (negative for all tested high-risk genotypes at follow-up). This approach is more conservative than a genotype-specific definition, because any newly detected or persistent high-risk genotype at follow-up, including potentially newly acquired infections, is classified as not cleared. It is also consistent with the WHO and ASCCP framing of post-treatment HPV surveillance in routine clinical care [14,31], in which assay-level high-risk HPV status guides management. A per-genotype sensitivity analysis was not feasible, because genotype-resolved HPV results were not consistently available across sequential follow-up visits.

LBC negativity rate: Proportion of patients with NILM or negative cytology at each designated follow-up timepoint.

10 patients (5.7%) tested HPV-negative at baseline in the per-patient primary analysis (corresponding to 11 episodes, 6.2%, in the episode-based sensitivity analysis), having been referred primarily for colposcopically identified morphological abnormalities. For these patients, the HPV clearance component of composite CR was considered met by default, and confirmation of continued HPV-negative status at follow-up was required for inclusion in the HPV clearance numerator. Because all baseline HPV-negative patients who achieved CR maintained HPV-negative status at follow-up, these patients were counted in both the HPV clearance numerator and denominator. Treatment episodes in which the patient underwent LEEP or laser ablation were classified as treatment failures for efficacy endpoints. Post-procedural HPV test results from these

Table 1. Baseline characteristics of 176 patients receiving 5-ALA-PDT for HPV-related lower genital tract lesions.

Characteristic	n	All patients (N = 176)[1]
Age (years), mean ± SD	176	34.1 ± 10.8
Age group, n (%)	176	
<30 years		65 (36.9%)
30–39 years		72 (40.9%)
40–49 years		16 (9.1%)
≥50 years		23 (13.1%)
Parity, n (%)	92	
Nulliparous		33 (35.9%)
Parous		59 (64.1%)
Missing		84
Diagnosis, n (%)	176	
CIN 1		86 (48.9%)
CIN 2/3		52 (29.6%)
VaIN 1		13 (7.4%)
VaIN 2/3		10 (5.7%)
HPV infection only		2 (1.1%)
Combined lesions		13 (7.4%)
Treatment site, n (%)	176	
Cervix		135 (76.7%)
Vagina		26 (14.8%)
Vulva		1 (0.6%)
Multiple sites		14 (8.0%)
Baseline cytology (TCT), n (%)	166	
NILM/Negative		80 (48.2%)
ASC-US		31 (18.7%)
ASC-H		2 (1.2%)
LSIL		46 (27.7%)
HSIL		7 (4.2%)
Missing		10
HPV genotype, n (%)	176	
HPV 16 (without HPV 18)		46 (26.1%)
HPV 18 (without HPV 16)		14 (8.0%)
HPV 16/18 co-infection		9 (5.1%)
Other HPV		97 (55.1%)
Negative		10 (5.7%)
No. of PDT courses, n (%)	168	
1 course (3 sessions)		48 (28.6%)
2 courses (6 sessions)		120 (71.4%)
Missing		8
Follow-up duration among evaluable cases, n (%)	147	
≤3 months		37 (25.5%)
~6 months		16 (11.0%)
≥9 months		90 (62.1%)
Unclassified[2]		4 (2.7%)
Median (IQR), months	144	10.6 (6.3–15.4)

[1]Mean ± SD; n (%). Unit of analysis is patient. One patient contributed two treatment episodes (second course >6 months after the first), only the first episode was retained in the primary analysis. [2]Four evaluable cases could not be assigned to a stratum due to borderline timing relative to stratum boundaries. Their follow-up months were included in the median/IQR where available (n = 144 with documented follow-up duration). Parity was not systematically documented in routine clinical records during 2021–2023 (n = 84) and was added to standardized documentation only from 2024 onward. Baseline cytology was missing for 10 patients whose tests were performed at referring institutions and not entered into the structured electronic medical record. PDT course classification was unavailable for 8 patients (7 lost to follow-up before completing the planned schedule, 1 who proceeded to LEEP), although the actual number of administered sessions was recorded for all of them. Missing values were reported as a separate category and were not imputed. Abbreviations: TCT, ThinPrep cytologic test; CIN, cervical intraepithelial neoplasia; VaIN, vaginal intraepithelial neoplasia; HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion. Each treatment course consisted of 3 sessions at approximately 7–14 days of intervals.

Table 2. Comparison of baseline characteristics between patients with valid follow-up (n = 149) and those lost to follow-up (n = 27).

Characteristic	Follow-up status		Test statistic	*p*-value[2]
	Valid follow-up N = 149[1]	Lost to follow-up N = 27[1]		
Age (years)	33.9 ± 10.5	34.9 ± 12.7	W = 1956	0.821
Age group			χ^2 = 0.78, df = 3	0.813
<30 years	57 (38.3%)	8 (29.6%)		
30–39 years	60 (40.3%)	12 (44.4%)		
40–49 years	13 (8.7%)	3 (11.1%)		
≥50 years	19 (12.8%)	4 (14.8%)		
Parity			χ^2 = 2.05, df = 1; OR = 2.24	0.137
Nulliparous	21 (30.9%)	12 (50.0%)		
Parous	47 (69.1%)	12 (50.0%)		
Missing	81	3		
Diagnosis			χ^2 = 1.85, df = 5	0.875
CIN 1	74 (49.7%)	12 (44.4%)		
CIN 2/3	42 (28.2%)	10 (37.0%)		
VaIN 1	11 (7.4%)	2 (7.4%)		
VaIN 2/3	8 (5.4%)	2 (7.4%)		
HPV infection only	2 (1.3%)	0 (0.0%)		
Combined lesions	12 (8.1%)	1 (3.7%)		
Treatment site			χ^2 = 1.00, df = 3	0.840
Cervix	113 (75.8%)	22 (81.5%)		
Vagina	22 (14.8%)	4 (14.8%)		
Vulva	1 (0.7%)	0 (0.0%)		
Multiple sites	13 (8.7%)	1 (3.7%)		
Baseline cytology (TCT)[3]			χ^2 = 1.03, df = 4	0.930
NILM/Negative	67 (48.2%)	13 (48.2%)		
ASC-US	27 (19.4%)	4 (14.8%)		
ASC-H	2 (1.4%)	0 (0.0%)		
LSIL	37 (26.6%)	9 (33.3%)		
HSIL	6 (4.3%)	1 (3.7%)		
Missing	10	0		
HPV genotype			χ^2 = 6.71, df = 4	0.154
HPV 16 (without HPV 18)	39 (26.2%)	7 (25.9%)		
HPV 18 (without HPV 16)	12 (8.1%)	2 (7.4%)		
HPV 16/18 co-infection	5 (3.4%)	4 (14.8%)		
Other HPV	85 (57.1%)	12 (44.4%)		
Negative	8 (5.4%)	2 (7.4%)		
No. of PDT courses[4]			χ^2 = 6.37, df = 1; OR = 3.67	0.008
1 course	37 (25.0%)	11 (55.0%)		
2 courses	111 (75.0%)	9 (45.0%)		
Missing	1	7		

[1]Continuous variables: mean ± SD; categorical variables: n (%). [2]Continuous variables compared using the Wilcoxon rank-sum (Mann-Whitney U) test, reporting the W statistic. Categorical variables compared using Fisher's exact test, with the Pearson χ^2 statistic and degrees of freedom reported alongside as a descriptive companion. The 2 × 2 odds ratio is additionally reported for Parity and No. of PDT courses. [3]Missing values excluded from between-group comparison (n = 10 missing). [4]Difference reflects reverse causation: patients lost to follow-up by definition do not complete the planned treatment schedule (see Results text).

Table 3. Efficacy outcomes of ALA-PDT by diagnosis category.

Diagnosis	Enrolled (n)	Evaluable n (% of enrolled)	Complete response n (% of evaluable)	hCR n (% of evaluable)	Partial Response n (% of evaluable)	Morph CR, HPV+ n (% of evaluable)	Lesion downgrading n (% of evaluable)	Overall Response Rate n (% of evaluable)	LEEP/Laser n (% of evaluable)	HPV Clearance n (% of evaluable)
CIN 1	86	74 (86.0%)	52 (70.3%)	59 (79.7%)	18 (24.3%)	7 (9.5%)	11 (14.9%)	70 (94.6%)	4 (5.4%)	58 (78.4%)
CIN 2/3	52	42 (80.8%)	36 (85.7%)	39 (92.9%)	4 (9.5%)	3 (7.1%)	1 (2.4%)	40 (95.2%)	2 (4.8%)	36 (85.7%)
VaIN 1	13	11 (84.6%)	6 (54.5%)	9 (81.8%)	4 (36.4%)	3 (27.3%)	1 (9.1%)	10 (90.9%)	1 (9.1%)	6 (54.5%)
VaIN 2/3	10	8 (80.0%)	3 (37.5%)	7 (87.5%)	5 (62.5%)	4 (50.0%)	1 (12.5%)	8 (100.0%)	0 (0.0%)	4 (50.0%)
Combined lesions	13	12 (92.3%)	4 (33.3%)	8 (66.7%)	5 (41.7%)	4 (33.3%)	1 (8.3%)	9 (75.0%)	3 (25.0%)	4 (33.3%)
HPV infection only	2	2 (100.0%)	—	—	—	—	—	—	—	1 (50.0%)
Total	176	149 (74.7%)	101 (68.7%)[1]	122 (83.0%)[1]	36 (24.2%)	21 (14.1%)	15 (10.1%)	137 (91.9%)	10 (6.7%)	109 (73.2%)

Enrolled (n) indicates patients enrolled with each diagnosis (total N = 176). Evaluable n indicates patients with valid follow-up, and its percentage is calculated relative to enrolled. CR, complete response; PR, partial response; ORR, overall response rate; LEEP, loop electrosurgical excision procedure; hCR, histological/cytological complete response. hCR is defined in the Methods (resolution of all visible lesions with cytological normalization [NILM], irrespective of HPV status) and is presented here alongside composite CR to facilitate direct comparison across diagnoses. The same data are also displayed graphically in Fig. 3 and in Table 4 in numerical form. Morph CR, HPV+ = patients with complete morphological normalization and cytological clearance but retained HPV positivity. [1]Complete response (CR) and hCR percentages in the Total row are calculated using the 147 patients with morphological disease (primary efficacy population) as the denominator, excluding the 2 isolated HPV-infection patients for whom these outcomes are not applicable. Unit of analysis: patient. One patient contributed two treatment episodes (second course >6 months after the first); only the first episode was retained in the primary analysis. Combined lesions include CIN combined with VaIN and/or VIN. For HPV infection only, CR/PR/ORR not applicable; HPV clearance was the primary endpoint. Subgroup estimates for VaIN 1 (n = 11), VaIN 2/3 (n = 8), and combined lesions (n = 12) carry wide confidence intervals due to limited sample size and are presented as exploratory or descriptive observations only.

patients were not used for the HPV clearance calculation, as such results may reflect the excisional procedure rather than ALA-PDT efficacy. Two patients with isolated HPV infection, without morphological lesion, were excluded from all CR, PR, ORR, and hCR denominators by design and are reported as a separate descriptive subgroup.

Valid follow-up was defined as the presence of at least one post-treatment assessment including both LBC and HPV testing results. We deliberately adopted a stringent composite CR definition requiring concurrent cytological normalization, histological resolution, and HPV clearance because it better reflects true disease-free status. Because this composite endpoint is more conservative than definitions used in many surgical series, hCR was predefined as a secondary endpoint to enable direct comparison with excisional treatment literature.

Safety Assessment

Adverse events were documented from available procedure notes and follow-up records. Standardized grading criteria (e.g., CTCAE) were not prospectively applied; therefore, the frequency of mild events may be underestimated. The most commonly documented symptoms were mild to moderate local discomfort (burning or stinging sensation) during illumination, which typically subsided within hours after the session. Transient increase in vaginal discharge was noted in a subset of patients treated at cervical or vaginal sites, generally resolving within one week. No procedure notes documented severe adverse events, treatment discontinuation due to intolerance, or hospital admission related to ALA-PDT. No cases of cervical stenosis, significant hemorrhage, or infection requiring systemic antibiotics were identified.

Statistical Analysis

Elements were pre-specified before data analysis, including the per-patient unit of analysis, composite CR as the primary endpoint, hCR as the secondary endpoint, the inverse probability of censoring weighting (IPCW) sensitivity analysis with pre-specified covariates (age group, diagnosis, HPV genotype, treatment site, baseline cytology, and number of PDT courses), stratification by minimum follow-up duration (≥6 and ≥9 months), and the episode-based sensitivity analysis (N = 177). The following analyses were exploratory or post-hoc and are labeled as such in the relevant sections, including the cross-sectional temporal analysis of LBC negativity and HPV clearance at 3/6/12 months, the Firth penalized logistic regression for factors associated with CR, the diagnosis-stratified subgroup estimates and the analysis restricted to patients completing two treatment courses, and the post-hoc sensitivity analysis excluding patients with HPV-negative status at baseline. The study protocol and statistical analysis plan are available from the corresponding author upon reasonable request.

Patient characteristics and baseline variables were summarized using descriptive statistics. Normality of continuous variables was assessed using the Shapiro-Wilk test together with visual inspection of histograms and Q-Q plots. Continuous variables are presented as mean ± standard deviation (SD) when approximately normally distributed or as median (interquartile range) otherwise. Categorical variables are presented as frequencies and percentages. Given the small sample sizes in several subgroups, between-group comparisons of continuous variables used the Wilcoxon rank-sum (Mann-Whitney U) test, and between-group comparisons of categorical variables used Fisher's exact test. Efficacy outcomes of CR rate, ORR, and HPV clearance rate were calculated among treatment episodes with valid follow-up and presented with 95% confidence intervals (CIs) estimated using the Wilson score method. All tests were two-tailed, with $p < 0.05$ considered statistically significant.

As an exploratory observation, we examined cross-sectional temporal profiles of LBC negativity and HPV clearance at 3, 6, and 12 months after the last treatment session. These analyses used repeated cross-sectional denominators. The 3-month estimate included all evaluable patients with at least one valid post-treatment assessment using the earliest available result, whereas the 6- and 12-month estimates included only patients who attended the respective scheduled visit. Because HPV genotyping was performed more consistently than LBC across follow-up visits, the HPV denominator exceeded the LBC denominator at each time point; therefore, both denominators were reported separately. Paired longitudinal tracking was not feasible because only 7 of 147 patients had complete data at all three timepoints. Observed rates at each timepoint therefore reflect the available data cross-sectionally, and the apparent temporal trajectory should be interpreted as hypothesis-generating.

We also examined potential factors associated with CR using Firth penalized logistic regression using the logistf package in R (4.5.2 R Foundation for Statistical Computing, VIE, Austria). Standard maximum likelihood logistic regression was unsuitable due to complete or quasi-complete separation arising from 100% CR rates in several small subgroups. Variables with $p < 0.20$ in univariate analysis were considered for the multivariate model, subject to two additional pre-specified exclusion criteria intended to preserve the interpretability of the multivariate estimates. Variables exhibiting structural collinearity with another candidate predictor were not entered jointly, and variables representing post-baseline treatment-process events that lie on the causal pathway between baseline characteristics and the response outcome were not entered to avoid mediator or collider conditioning. Two univariate-significant variables were excluded on these grounds. Treatment site (cervical vs non-cervical, univariate $p = 0.006$) was excluded because it represents a coarser categorization of

diagnosis, and was therefore structurally collinear with it. Diagnosis was retained as the more clinically granular variable. Number of PDT courses (univariate $p = 0.073$ for 2 courses vs 1) was excluded because the number of courses administered is itself influenced by interim treatment response and physician-driven adjustments after the first course, placing it on the causal pathway between baseline characteristics and the final response outcome rather than acting as an independent baseline predictor. The robustness of the multivariate findings to these exclusions was assessed in a sensitivity analysis (described below). Treatment site was excluded from the multivariate model due to substantial collinearity with diagnosis category. The final multivariate model included age group and diagnosis category. Odds ratios (ORs) and 95% CIs are reported. A $p < 0.05$ was considered statistically significant.

To evaluate whether early assessment timepoints biased the composite CR estimate, we performed a predefined sensitivity analysis stratifying evaluable treatment episodes by minimum follow-up duration (≥6 months and ≥9 months) and recalculating composite CR and ORR within each stratum.

To assess the potential impact of informative loss to follow-up on the primary efficacy estimates, we performed an inverse probability of censoring weighting (IPCW) sensitivity analysis. A logistic regression model was fitted to predict the probability of having valid follow-up conditional on baseline covariates, including age group, diagnosis category, HPV genotype group, treatment site, baseline cytology, and number of PDT courses. Stabilized inverse probability weights $w_i = p(\text{follow-up})/p(\text{follow-up}|X_i)$ were calculated for each evaluable treatment episode and truncated at the 1st and 99th percentiles. The IPCW-adjusted composite CR and ORR were estimated among the 147 evaluable patients with morphological disease, with 95% CIs derived from 2000 bootstrap replicates. An episode-based sensitivity analysis (N = 177), which retained both treatment episodes of the one patient who received retreatment >6 months after the first course, was performed to confirm that the per-patient primary analysis did not substantially alter efficacy estimates. A further post-hoc sensitivity analysis was performed excluding patients who tested HPV-negative at baseline (n = 10 in the per-patient analysis, 8 of whom were in the evaluable population) to verify that the special handling rule for this subgroup did not influence the primary composite CR and HPV clearance estimates. An additional sensitivity multivariate Firth regression was performed including all four variables with univariate $p < 0.20$ (age group, diagnosis, treatment site, number of PDT courses) to verify that conclusions regarding the independent associations of age group and diagnosis were not driven by the pre-specified exclusions described above.

Results

Patient Characteristics

After applying the inclusion and exclusion criteria, 176 unique patients (177 treatment episodes in total) were included in the baseline analysis (Table 1, Fig. 1), of which 174 had morphological disease, and 2 had isolated persistent HPV infection. Overall, 149 patients (84.7%) had at least one valid post-treatment follow-up assessment, while 27 (15.3%) were lost to follow-up before any post-treatment evaluation could be performed. All 27 patients had no documented attendance at any of the three scheduled post-treatment visits (3, 6, or 12 months). Review of the available clinical records did not systematically identify documented reasons for non-attendance (e.g., transfer of care, geographic relocation, patient-reported withdrawal). Follow-up reminders in routine clinical practice were not logged in a structured manner, which precluded retrospective ascertainment of individual loss-to-follow-up reasons. Among episodes with valid follow-up, 147 had morphological disease and constituted the primary efficacy population; the remaining two had isolated HPV infection and are reported separately. The mean age was 34.1 ± 10.8 years (range 16–65). The largest age group was 30–39 years (n = 72, 40.9%), followed by patients under 30 years (n = 65, 36.9%). Parity information was available for 92 patients, among whom 33 (35.9%) were nulliparous.

The most common diagnoses were CIN 1 (n = 86, 48.9%) and CIN 2/3 (n = 52, 29.6%). Vaginal lesions (VaIN 1 or 2/3) accounted for 13.1% (n = 23). Combined multisite lesions were present in 13 cases (7.4%), and isolated HPV infection without morphological lesions in 2 (1.1%). The majority were treated at the cervix alone (n = 135, 76.7%), with 26 (14.8%) treated at vaginal sites and 14 (8.0%) at multiple anatomical sites.

Baseline cytology (LBC) was available for 166 patients, in which 80 (48.2%) had NILM or negative cytology, 46 (27.7%) had LSIL, and 31 (18.7%) had ASC-US. High-risk HPV genotyping showed that 26.1% harbored HPV 16 (alone), 8.0% harbored HPV 18, and 5.1% were co-infected with both HPV 16 and 18; other genotypes were detected in 55.1% of cases. 10 cases (5.7%) tested HPV-negative at baseline. Treatment session data were available for 168 of 176 patients. Among these, the majority (n = 120, 71.4%) received 2 treatment courses (6 PDT sessions), while 48 (28.6%) received 1 course (3 sessions).

Overall Treatment Efficacy

Among 176 enrolled patients, 149 (84.7%) had at least one valid post-treatment follow-up assessment (Fig. 1). The remaining 27 (15.3%) were lost to follow-up before any post-treatment evaluation could be performed (Fig. 1). Of the 149 evaluable patients, 147 had morphological disease and constituted the primary efficacy population. The remaining two had isolated HPV infection and are reported

separately (Fig. 1). The median follow-up duration among evaluable cases (4 patients had missing follow-up duration data) was approximately 10 months, with 37 (25.2%) having their last assessment at ≤3 months, 16 (10.9%) at 6 months, and 90 (61.2%) at ≥9 months (Table 1).

Comparison of baseline characteristics between the 149 patients with valid follow-up and the 27 lost to follow-up revealed no significant differences in age (p = 0.82, Wilcoxon rank-sum), age group (p = 0.81), diagnosis (p = 0.88), HPV genotype (p = 0.15), treatment site (p = 0.84), baseline cytology (p = 0.93), or parity (p = 0.14, restricted to patients with documented parity) (all Fisher's exact unless otherwise stated, Table 2). The number of completed PDT courses differed between groups (p = 0.008), but this reflects reverse causation rather than a baseline imbalance, since loss to follow-up by definition interrupts the planned treatment schedule. This variable is therefore not a true baseline characteristic and is reported in Table 2 for transparency only. The only significant difference was in the number of PDT courses (p = 0.008). Episodes with valid follow-up had a higher proportion receiving 2 courses, suggesting that greater treatment exposure was associated with higher follow-up adherence rather than differential dropout by disease severity.

Among the 147 evaluable patients with morphological disease (Table 3), 101 (68.7%; 95% CI 60.8–75.6%) achieved composite CR at the last available follow-up. An additional 36 (24.5%) achieved PR, yielding an ORR of 93.2% (137/147; 95% CI 87.9–96.3%). Among the 36 PR cases, 21 (58.3%) had achieved complete morphological normalization with cytological clearance (i.e., met the hCR criterion) but retained HPV positivity, while the remaining 15 (41.7%) had histological downgrading without complete resolution (Table 3). HPV clearance was documented in 109 of 149 cases (73.2%). Ten cases (6.8%) underwent LEEP or laser ablation for treatment failure or disease progression.

The IPCW-adjusted composite CR was 68.1% (95% CI 60.0–75.5%) and the IPCW-adjusted ORR was 92.3% (95% CI 87.3–96.7%), both closely approximating the unweighted estimates (CR 68.7%; ORR 93.2%; Table 4). The attenuation of approximately 0.6 percentage points indicates that differential dropout by baseline characteristics had minimal impact on the primary efficacy estimates. In a worst-case scenario assuming all 27 lost-to-follow-up patients with morphological disease were treatment failures, the composite CR rate would be 58.0% (101/174). This extreme assumption almost certainly underestimates true efficacy, and the IPCW-adjusted lower bound of 68.1% provides a more plausible conservative estimate.

In a post-hoc sensitivity analysis excluding the 8 baseline HPV-negative patients in the per-patient evaluable population, composite CR was 68.3% (95/139), HPV clearance was 73.4% (102/139), and ORR was 92.8% (129/139). The attenuation of ≤0.4 percentage points across all three estimates confirms that the special handling rule for baseline HPV-negative patients did not materially influence the primary efficacy results.

When the hCR endpoint was applied (cytological and colposcopic normalization regardless of HPV status), the hCR rate was 122/147 (83.0%; Table 5). The 14.3-percentage-point gap between hCR (83.0%) and composite CR (68.7%) was attributable to 21 patients who achieved complete morphological normalization but retained HPV positivity, consistent with the slower kinetics of viral clearance discussed below.

A sensitivity analysis restricted to evaluable episodes completing 2 treatment courses (n = 110; Table 6) yielded a composite CR rate of 72.7% (80/110), an ORR of 95.5% (105/110), and a LEEP/laser rate of 4.5% (5/110) (Table 7). When stratified by minimum follow-up duration (distribution shown in Table 1), composite CR rates were stable or slightly higher with longer follow-up, showing that 70.8% (75/106) among episodes with ≥6 months of follow-up (n = 106), and 73.3% (66/90) among those with ≥9 months (n = 90). ORR was similarly stable (94.3% and 94.4%, respectively) (Table 7). These findings indicate that early assessment did not inflate the primary CR estimate. Rather, the modest increase in CR during longer follow-up is consistent with progressive HPV clearance over time. Restricting the analysis to patients who completed 2 treatment courses attenuated the composite CR gap from 15.4 to 11.0 percentage points (CIN 1: 75.5%, 37/49; CIN 2/3: 86.5%, 32/37; Table 7).

A sensitivity multivariate Firth regression, including all four variables with univariate $p < 0.20$ (age group, diagnosis, treatment site, and number of PDT courses; n = 146), confirmed the robustness of the primary multivariate findings (Table 8). The independent associations of age group with composite CR were essentially unchanged in direction, magnitude, and significance. The direction of all diagnosis-level estimates was likewise preserved. Treatment site exhibited a markedly wide confidence interval in the full model (Non-cervical vs Cervix: OR 1.49, 95% CI 0.24–16.99), empirically confirming the structural collinearity with diagnosis that had motivated its pre-specified exclusion from the primary model. The number of PDT courses (2 vs 1: OR 1.95, 95% CI 0.81–4.70, p = 0.138) was not independently associated with composite CR after adjustment.

Among the two treatment episodes with isolated HPV infection, HPV clearance was achieved in one (50.0%). Given the small sample size and the investigational nature of this indication, these data are insufficient to support any efficacy conclusions and are reported for descriptive purposes only.

Representative colposcopic images from a patient with CIN2 illustrating the morphological response to ALA-PDT are shown in Fig. 2. Before treatment, acetic acid application revealed a dense acetowhite epithelium with

Table 4. Sensitivity analysis: IPCW-adjusted efficacy estimates compared with unweighted primary analysis and worst-case scenario.

Estimate	Unweighted (primary analysis)	IPCW-weighted (95% CI)	Worst-case scenario
Composite CR	68.7% (101/147)	68.1% (60.0–75.5%)	58.0% (101/174)
ORR	93.2% (137/147)	92.3% (87.3–96.7%)	78.7% (137/174)

Per-patient primary analysis (N = 176, 174 with morphological disease). The IPCW model included age group, diagnosis category, HPV genotype, treatment site, baseline cytology, and number of PDT courses as covariates. Stabilized weights were truncated at the 1st and 99th percentiles. Bootstrap 95% CIs were derived from 2000 replicates. The worst-case scenario assumes that all 27 lost-to-follow-up patients with morphological disease were treatment failures. IPCW, inverse probability of censoring weighting; CR, complete response; ORR, overall response rate; CI, confidence interval.

Table 5. Comparison of hCR and composite CR rates by diagnosis category.

Diagnosis	n (evaluable)	hCR n (%)	Composite CR n (%)
CIN 1	74	59 (79.7%)	52 (70.3%)
CIN 2/3	42	39 (92.9%)	36 (85.7%)
VaIN 1	11	9 (81.8%)	6 (54.5%)
VaIN 2/3	8	7 (87.5%)	3 (37.5%)
Combined lesions	12	8 (66.7%)	4 (33.3%)
Total	147	122 (83.0%)	101 (68.7%)

Both hCR and composite CR were calculated using the same denominator (n = 147). hCR: resolution of all visible lesions with cytological normalization, irrespective of HPV status. Composite CR additionally required HPV clearance. Per-patient primary analysis (N = 176 patients, 174 with morphological disease, 147 with valid follow-up). Combined lesions include CIN combined with VaIN and/or VIN.

Table 6. Distribution of PDT treatment sessions by diagnosis category (N = 176).

Diagnosis	N	1 course (3 sessions) n (%)	2 courses (6 sessions) n (%)	Missing
CIN 1	86	31 (36.9)	53 (63.1)	2
CIN 2/3	52	9 (18.8)	39 (81.3)	4
VaIN 1	13	3 (25.0)	9 (75.0)	1
VaIN 2/3	10	2 (20.0)	8 (80.0)	0
HPV infection only	2	1 (50.0)	1 (50.0)	0
Combined	13	2 (16.7)	10 (83.3)	1
Total	176	48 (28.6)	120 (71.4)	8

Each treatment course consisted of 3 sessions at intervals of approximately 7–14 days. Percentages calculated among patients with available session data (n = 168). Combined lesions include CIN combined with VaIN and/or VIN.

irregular surface contour in the transformation zone, and Lugol's iodine staining demonstrated a well-demarcated iodine-negative area corresponding to the dysplastic lesion. At 3 months after the last treatment session (6 sessions), colposcopic examination showed complete resolution of the acetowhite changes with restoration of a smooth, mature squamous epithelium, and Lugol's iodine staining demonstrated uniform iodine uptake across the previously affected area, consistent with complete morphological response. Small punctate areas of attenuated iodine uptake remained scattered within the uniformly stained field. These lacked the sharply demarcated mustard-yellow borders, defined size, and correlation with prior acetowhite changes that characterize residual CIN, and are interpreted as immature squamous metaplasia during early post-PDT re-epithelialization, which has not yet regained the full glycogen content of mature squamous epithelium.

Safety and Tolerability

Among 147 evaluable patients, the most commonly reported adverse events were increased vaginal discharge, local pain or burning sensation during illumination, minor vaginal bleeding, and mild lower abdominal distension. The majority of symptoms resolved within 1–3 days after each treatment session. No systemic severe adverse events, treatment discontinuation due to intolerance, cervical steno-

Table 7. Sensitivity analyses of efficacy estimates.

Analysis subset	n	Composite CR n (%)	ORR n (%)	LEEP/Laser n (%)
Primary analysis (all evaluable)	147	101 (68.7%)	137 (93.2%)	10 (6.8%)
Completed 2 treatment courses	110	80 (72.7%)	105 (95.5%)	5 (4.5%)
CIN 1, 2-course completers	49	37 (75.5%)	48 (98.0%)	1 (2.0%)
CIN 2/3, 2-course completers	37	32 (86.5%)	35 (94.6%)	2 (5.4%)
VaIN 1, 2-course completers	8	5 (62.5%)	8 (100.0%)	0 (0.0%)
VaIN 2/3, 2-course completers	6	2 (33.3%)	6 (100.0%)	0 (0.0%)
Combined lesions, 2-course completers	10	4 (40.0%)	8 (80.0%)	2 (20.0%)
Minimum follow-up ≥6 months	106	75 (70.8%)	100 (94.3%)	6 (5.7%)
Minimum follow-up ≥9 months	90	66 (73.3%)	85 (94.4%)	5 (5.6%)

All analyses are per-patient and restricted to the primary efficacy population (morphological disease with valid follow-up; n = 147). Analysis subsets are not mutually exclusive. The 2-course-completer subset retains episodes that completed 6 PDT sessions across two courses. Subgroup rows for VaIN 1, VaIN 2/3, and combined lesions are presented as exploratory/descriptive observations only, given the limited evaluable sample sizes (n ≤ 10). Minimum follow-up strata are defined as in the Methods (≥6 months includes the ~6-month and ≥9-month strata; ≥9 months is a subset of the ≥6-month category).

Table 8. Sensitivity multivariate Firth penalized logistic regression including all univariate-eligible variables (n = 146).

Variable	Main multivariate (Table 10)[1]			Sensitivity multivariate (full model)[2]		
	OR	95% CI	*p*	OR	95% CI	*p*
Age group (ref: 30–39 years)						
<30 years	0.41	0.16–0.99	0.048	0.39	0.15–0.95	0.039
40–49 years	0.42	0.12–1.57	0.189	0.38	0.10–1.51	0.165
≥50 years	0.18	0.04–0.69	0.012	0.15	0.03–0.60	0.007
Diagnosis (ref: CIN 1)						
CIN 2/3	2.46	0.93–7.24	0.069	2.04	0.75–6.15	0.167
VaIN	0.77	0.23–2.73	0.668	0.52	0.04–4.10	0.541
Combined	0.33	0.08–1.18	0.087	0.19	0.01–1.53	0.125
Treatment site (ref: Cervix)						
Non-cervical	—	—	—	1.49	0.24–16.99	0.689
PDT courses (ref: 1 course)						
2 courses	—	—	—	1.95	0.81–4.70	0.138

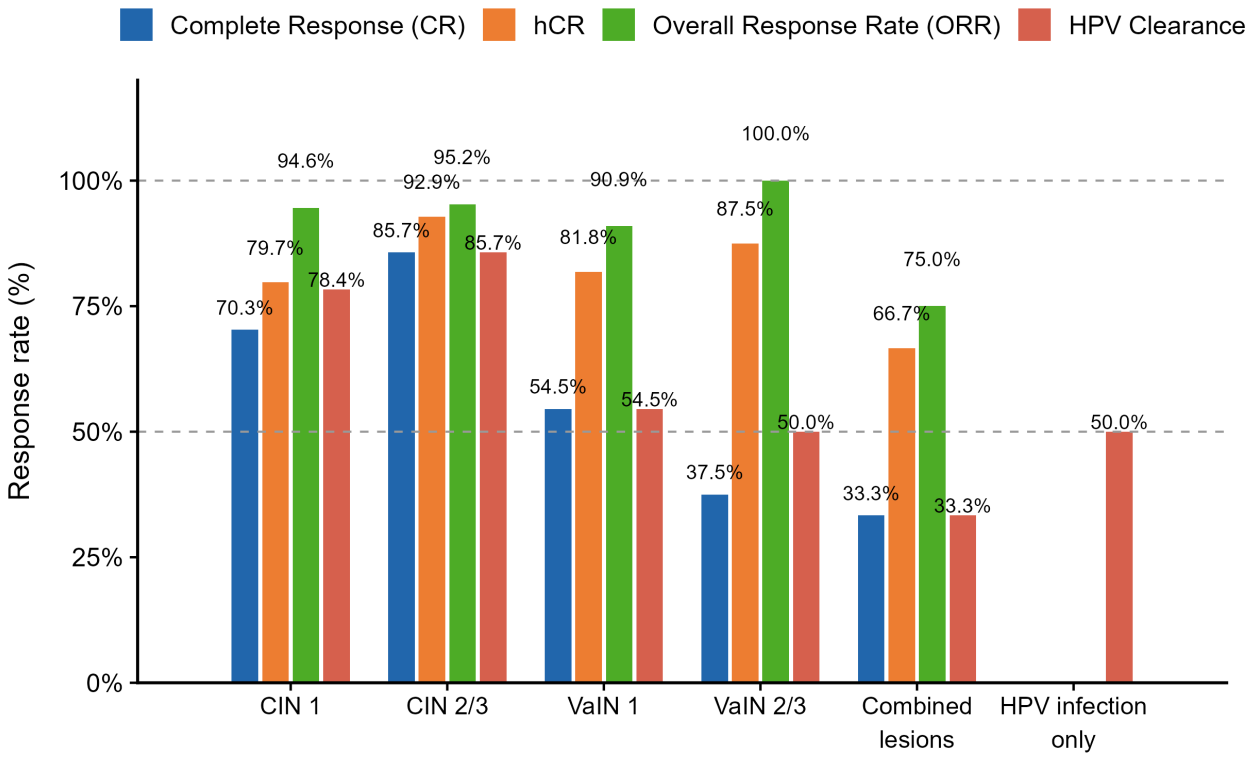
[1]Pre-specified primary multivariate model, including only variables not excluded by the structural-collinearity and mediator criteria described in the Methods. [2]Sensitivity multivariate model including all four variables with univariate $p < 0.20$ (age group, diagnosis, treatment site, number of PDT courses). The wide confidence interval for treatment site (0.24–16.99) directly illustrated the collinearity with diagnosis that motivated its exclusion from the primary model. Firth penalized logistic regression. Reference categories indicated for each variable. n = 146 after exclusion of episodes with missing PDT-course classification.

sis, significant hemorrhage, or hospital admission related to ALA-PDT were recorded.

Efficacy by Diagnosis Category

Efficacy outcomes varied across diagnosis categories (Table 3, Fig. 3).

Among cervical lesions, CIN 2/3 demonstrated a higher composite CR rate than CIN 1 (85.7%, 36/42 vs 70.3%, 52/74). ORR was 95.2% and 94.6%, respectively, and HPV clearance rates were 85.7% and 78.4%. When the hCR endpoint was applied, this gap persisted (CIN 2/3 92.9% vs CIN 1 79.7%), indicating that CIN 2/3 achieved superior rates of both morphological resolution and HPV clearance. Among the four CIN 2/3 PR cases, all had achieved complete morphological normalization with cytological clearance, but 3 retained HPV positivity and one had lesion downgrading. Among 18 CIN 1 PR cases, seven met hCR criteria, and 11 had histological downgrading without complete resolution. Among patients with available session data, CIN 2/3 patients received more PDT than CIN 1 patients (2 treatment courses in 81.3% vs 63.1%, respectively; Table 6). Restricting the analysis to patients who received 2 treatment courses attenuated the composite CR gap from 15.4 to 11.0 percentage points (CIN 1: 75.5%; CIN 2/3: 86.5%).



Percentages calculated among patients with valid follow-up within each diagnosis category (CIN 1, n = 74; CIN 2/3, n = 42; VaIN 1, n = 11; VaIN 2/3, n = 8; combined lesions, n = 12; HPV infection only, n = 2). CR, hCR, and ORR not applicable for the HPV infection only group.
CR, complete response; hCR, histological/cytological CR; ORR, overall response rate (CR + partial response); HPV clearance, conversion to HPV-negative at last follow-up. All values are also reported in Table 3.

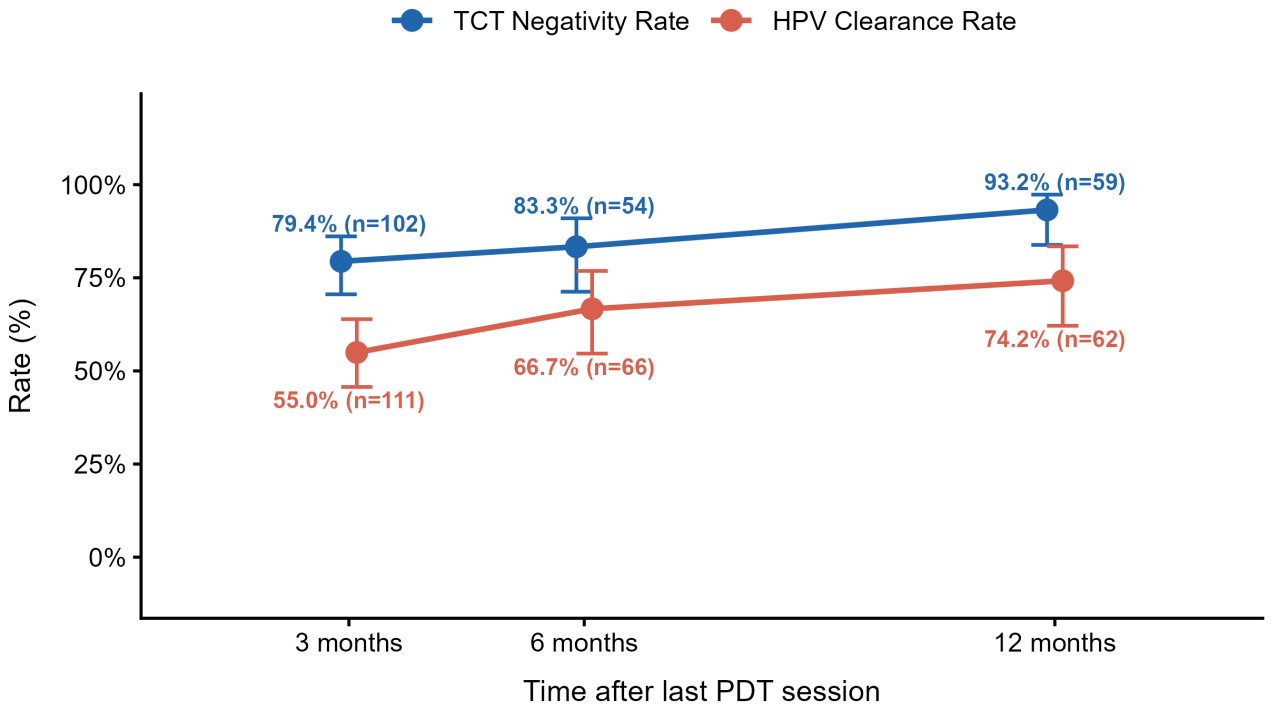
Fig. 3. Efficacy outcomes of ALA-PDT by diagnosis category. Bar graph showing composite complete response (CR) rate, histological/cytological CR (hCR) rate, overall response rate (ORR), and HPV clearance rate among patients with valid follow-up, stratified by diagnosis category. Subgroup estimates for VaIN 1 (n = 11), VaIN 2/3 (n = 8), and combined lesions (n = 12) carry wide confidence intervals due to limited sample size and are presented as exploratory or descriptive observations only. Pooled All-VaIN estimates (n = 19) are reported in the Results text as a clinically justified composite.

Among vaginal lesions, the hCR rates were substantially higher than composite CR rates (VaIN 1 81.8% vs 54.5%; VaIN 2/3 87.5% vs 37.5%), indicating that the lower composite CR was driven primarily by incomplete HPV clearance rather than failure of lesion regression. However, the small sample sizes (VaIN 1, n = 11; VaIN 2/3, n = 8) yielded wide confidence intervals, and these estimates should be interpreted with caution. As a clinically justified pooled estimate, combining VaIN 1 and VaIN 2/3 episodes (total n = 19 evaluable) yielded composite CR of 47.4% (9/19), hCR of 84.2% (16/19), ORR of 94.7% (18/19), and HPV clearance of 52.6% (10/19). Estimates for VaIN 1, VaIN 2/3, and combined lesions remain exploratory and descriptive only given the limited evaluable sample sizes (n = 11, 8, and 12, respectively).

Exploratory Temporal Observations in LBC Negativity and HPV Clearance

For the temporal analysis, the denominators at each timepoint reflect the available data cross-sectionally rather than a fixed longitudinal cohort (only 7 patients had data at all three timepoints). The 3-month data point reflects all evaluable patients with at least one valid post-treatment assessment (LBC: n = 103; HPV: n = 112). Subsequent timepoints (6 and 12 months) include only patients who attended the corresponding scheduled follow-up visits.

Among these patients, both LBC negativity and HPV clearance increased over time, but with different kinetic profiles (Fig. 4). LBC negativity was 79.4% (81/102; 95% CI 70.6–86.1%) at 3 months, rose to 83.3% (45/54) at 6 months, and remained stable at 93.2% (55/59) at 12 months. HPV clearance was 55.0% (61/111; 95% CI 45.7–63.9%) at 3 months, 66.7% (44/66) at 6 months, and 74.2% (46/62) at 12 months.



Error bars represent Wilson 95% confidence intervals.
Denominators vary by timepoint as not all patients had data at each visit.
TCT negativity: NILM or negative cytology; HPV clearance: conversion to HPV-negative.
Labels are colour-matched to their respective lines:
TCT negativity values above the upper 95% CI bound (blue),
HPV clearance values below the lower 95% CI bound (red).

Fig. 4. Temporal trends in LBC negativity rate and HPV clearance rate following ALA-PDT. Line graph depicting the proportion of evaluable patients achieving LBC negativity (negative/NILM) and HPV clearance at 3, 6, and 12 months after the last ALA-PDT session. Error bars represent 95% confidence intervals estimated using the Wilson score method. The denominators differed across timepoints (3-month: LBC n = 102, HPV n = 111; 6-month: LBC n = 54, HPV n = 66; 12-month: LBC n = 59, HPV n = 62). The difference in denominators reflects the higher frequency of HPV genotyping relative to LBC at each scheduled follow-up visit in routine clinical practice. These are repeated cross-sectional observations, not paired longitudinal measurements. The apparent temporal trajectory should be regarded as hypothesis-generating.

These cross-sectional observations suggest that cytological normalization consistently preceded virological clearance across all timepoints, with the gap between the two metrics persisting through 12 months. The observed pattern is consistent with the established biology of HPV, in which cell-mediated immunity, required for viral clearance, matures over months following photodynamic stimulation. However, these cross-sectional observations have important limitations: the denominators differed across timepoints, only 7 patients had complete data for all three visits, and differential retention bias cannot be excluded. Accordingly, the apparent temporal dissociation between cytological and virological response should be regarded as hypothesis-generating only and requires confirmation in prospective studies with paired longitudinal assessments.

Factors Associated With Complete Response

In exploratory Firth penalized logistic regression analysis (Tables 9,10), CIN 2/3 diagnosis showed a borderline positive association with composite CR compared with CIN 1 ($p = 0.069$ after adjustment), while combined multi-site lesions were associated with lower CR ($p = 0.016$, univariate; Table 9). Age ≥50 years was significant univariately ($p < 0.001$) and after adjustment for diagnosis ($p = 0.012$). Neither HPV genotype nor PDT course number was significantly associated with CR. Confidence intervals throughout this analysis were extremely wide due to near-complete separation in small subgroups, an expected consequence of Firth penalized estimation in sparse-data settings. All regression findings are exploratory and require validation in adequately powered prospective studies.

Table 9. Univariate analysis of factors associated with complete response after ALA-PDT (Firth penalized logistic regression).

	OR	95% CI	*p* value
Age (per year, continuous)	0.95	0.92–0.98	0.003*
Age group (ref: 30–39 years)			
<30 years	0.42	0.17–0.97	0.043*
40–49 years	0.33	0.09–1.20	0.091
≥50 years	0.11	0.03–0.34	<0.001*
Diagnosis (ref: CIN 1)			
CIN 2/3	2.41	0.95–6.81	0.063
VaIN	0.39	0.14–1.06	0.065
Combined	0.23	0.06–0.76	0.016*
HPV genotype (ref: Other HR-HPV)			
HPV 16+	0.98	0.46–2.13	0.957
Negative	1.21	0.29–6.89	0.803
Treatment site (ref: Cervix)			
Non-cervical	0.34	0.16–0.74	0.006*
PDT courses (ref: 1 course)			
2 courses	2.03	0.94–4.36	0.073

Firth penalized logistic regression was used to address complete or quasi-complete separation. Isolated vulvar lesions (VIN), isolated condylomata acuminata (without concurrent CIN/VaIN), and patients with HPV infection <12 months without morphological lesions were excluded from the study due to small sample sizes or insufficient documented persistence. Non-cervical sites include vagina, vulva, and multiple treatment sites. *$p < 0.05$. OR, odds ratio; CI, confidence interval; HR-HPV, high-risk human papillomavirus; PDT, photodynamic therapy.

Table 10. Multivariate analysis of independent predictors of complete response after ALA-PDT (Firth penalized logistic regression).

	OR	95% CI	*p* value
Age group (ref: 30–39 years)			
<30 years	0.41	0.16–0.99	0.048
40–49 years	0.42	0.12–1.57	0.189
≥50 years	0.18	0.04–0.69	0.012*
Diagnosis (ref: CIN 1)			
CIN 2/3	2.46	0.93–7.24	0.069
VaIN	0.77	0.23–2.73	0.668
Combined	0.33	0.08–1.18	0.087

Treatment site was excluded due to collinearity with diagnosis. Variables retained: age group and diagnosis (both $p < 0.20$ in univariate analysis). Firth penalized logistic regression was applied to address complete separation. *$p < 0.05$. OR, odds ratio; CI, confidence interval.

Discussion

In this real-world retrospective cohort of 176 patients, ALA-PDT demonstrated high overall efficacy across HPV-related lower genital tract lesions, with a composite CR of 68.7%, an hCR of 83.0%, and an ORR of 93.2%. Results showed that morphological efficacy approached the first-pass success rates of excisional surgery, and cross-sectional observations suggested a temporal dissociation between cytological normalization and HPV clearance.

The choice of response endpoint substantially influenced reported efficacy. Our composite CR definition is more stringent than the histological endpoints typically used to evaluate LEEP, which have traditionally focused on complete excision and the absence of residual or recurrent CIN2+ [9,32]. When the hCR endpoint was applied, the overall rate of 83.0% approached the approximately 95% disease-control rate reported 12 months after LEEP in a meta-analysis [33]; however, direct comparison is limited by differences in endpoint definitions, patient populations, and follow-up duration. For comparison, published studies of PDT for high-grade CIN/HSIL have reported response rates ranging from about 65% to 92.5%, depending on endpoint definition and follow-up duration [30]. Our higher figure may partly reflect the standardized 3- or 6-session protocol, the relatively younger patient population (mean age 34 years), and the potential for selection toward more treatment-responsive lesions in a retrospective setting.

Among cervical lesions, CIN 2/3 demonstrated a higher composite CR than CIN 1 (85.7% vs 70.3%). This apparently counterintuitive finding does not reflect superior biological responsiveness of higher-grade lesions. Treatment intensity differed between the two groups: 81.3% of CIN 2/3 patients received 2 treatment courses (6 sessions), compared with 63.1% of CIN 1 patients. Restricting the analysis to patients who completed 2 courses attenuated the composite CR gap from 15.4 to 11.0 percentage points (CIN 1: 75.5%; CIN 2/3: 86.5%), confirming that differential treatment intensity was a measurable contributor. The residual difference is likely explained by clinical selection bias inherent in the study population. Patients referred for ALA-PDT with persistent CIN 1 represent a selected subgroup whose lesions have already resisted the spontaneous immune clearance that resolves most CIN 1 within two years. This referral pattern enriches the CIN 1 cohort for patients with relatively refractory HPV infections, which would selectively reduce HPV clearance rates without necessarily impairing morphological response. This interpretation is supported by the hCR data. When assessed by morphological and cytological criteria alone (irrespective of HPV status), the gap narrowed to 13.2 percentage points (CIN 2/3: 92.9% vs CIN 1: 79.7%), indicating that the composite CR difference was driven primarily by differential HPV clearance rather than morphological failure. Among the 22 CIN 1 patients not achieving composite CR (18 PR + 4 LEEP), seven had complete morphological normalization with residual HPV positivity, 11 had histological downgrading without complete resolution, and four required rescue LEEP; only two CIN 2/3 patients required

rescue LEEP. One possible mechanistic explanation is that persistent CIN 1 patients may harbor HPV infections inherently more resistant to immune-mediated clearance, which would selectively impair the immunomodulatory component of ALA-PDT while leaving direct PpIX-mediated cytotoxicity intact. However, this hypothesis remains speculative and would require prospective assessment of HPV-specific T-cell responses for validation.

For vaginal and combined multi-site lesions, composite CR rates were lower than for cervical lesions (VaIN 1: 54.5%; VaIN 2/3: 37.5%; combined: 33.3%). However, hCR rates were substantially higher (VaIN 1: 81.8%; VaIN 2/3: 87.5%), indicating that the lower composite CR was driven primarily by incomplete HPV clearance rather than failure of morphological response. The rugated vaginal epithelium may impede uniform photosensitizer distribution and light delivery, although this hypothesis remains speculative given the small sample sizes (VaIN 1: n = 11; VaIN 2/3: n = 8; combined: n = 12). These subgroup estimates carry wide confidence intervals and should be interpreted as descriptive observations only. Whether modified treatment protocols could improve virological outcomes at vaginal sites requires evaluation in larger dedicated studies. The ability to treat multiple anatomical foci within a single treatment course remains a practical advantage of ALA-PDT over staged surgical approaches.

The cross-sectional temporal analysis suggested that cytological normalization may precede virological clearance, with LBC negativity appearing to plateau by 6 months, while HPV clearance continued to increase through 12 months. This pattern is consistent with the established biology of HPV clearance, in which cell-mediated immune responses mature over months following photodynamic stimulation. The observation that composite CR was stable or slightly higher at longer follow-up (≥6 months: 70.8%; ≥9 months: 73.3%) further supports the interpretation that extended follow-up captures additional HPV clearance events rather than revealing late treatment failures. However, because the patient samples differed across timepoints and the overlap was minimal (n = 7 with complete data at all three visits), this apparent temporal dissociation should be interpreted with caution. These observations raise the question of whether HPV testing at 6 months may underestimate eventual virological clearance, a hypothesis that warrants prospective evaluation with paired longitudinal follow-up.

HPV clearance in the isolated HPV infection subgroup (n = 2 evaluable, 50.0%) was limited. Given the very small sample size and investigational nature of this indication, these data do not support the routine use of ALA-PDT for HPV clearance in the absence of morphological disease outside formal clinical trials.

In the exploratory regression analysis, CIN 2/3 diagnosis showed a borderline association with higher CR, while age ≥50 years was significant univariately and after adjustment with a little attenuation, suggesting partial confounding. Neither HPV genotype nor session number was significantly associated with CR. As discussed in the Methods section, the wide CIs throughout reflect the expected consequence of near-complete separation in small subgroups when applying Firth penalized regression. These associations are hypothesis-generating only and should not be interpreted as evidence of independent predictive effects or as grounds for patient selection. The borderline *p*-values, wide confidence intervals, reliance on Firth penalization in sparse-data settings, and the absence of adjustment for multiple testing collectively preclude clinical inference. Validation in independent, adequately powered prospective cohorts is required before any of these candidate associations, including the borderline signal for CIN 2/3 and the inverse association with age ≥50 years, can be considered actionable.

These findings, while subject to the limitations of indirect comparison, carry several practical implications. The hCR rate of 83.0% supports ALA-PDT as a potentially viable fertility-preserving alternative for CIN, particularly relevant given that 77.8% of our cohort was under 40 years and 35.9% were nulliparous. We note, however, that the fertility-preserving framing in the present study is inferred from the favorable structural safety profile (absence of cervical stenosis, significant hemorrhage, or structural cervical damage) rather than from direct patient-reported or reproductive outcome data. Subsequent pregnancy and delivery outcomes, sexual function, health-related quality of life, and patient satisfaction were not systematically captured in routine clinical records and cannot be derived from this dataset. The ability to simultaneously treat multiple anatomical sites (8.0% of our cohort) is a distinctive advantage over staged surgical approaches. Also, the progressive HPV clearance through 12 months suggests that post-treatment HPV surveillance should be maintained for at least 12 months before concluding that virological clearance has failed.

The safety profile observed in this cohort was favorable, with only mild, self-limited adverse events documented and no cases of cervical stenosis, significant hemorrhage, or structural cervical damage. The absence of structural complications is particularly relevant for this population, of whom 77.8% were under 40 years and 35.9% were nulliparous. However, these observations were limited by the absence of prospective standardized grading, and mild events were likely underreported. These safety findings, if confirmed by prospective studies with systematic CTCAE grading, would represent a clinically meaningful advantage over excisional procedures, which carry well-documented risks of cervical shortening and adverse obstetric outcomes.

This study still has several limitations. The retrospective single-center design without a concurrent control group precludes direct causal inference or head-to-head comparison with excisional procedures. Also, 15.3% of patients

(27/176) were lost to follow-up before any post-treatment evaluation. Although this rate remains below the conventionally acceptable 20% threshold for cohort studies, any loss to follow-up introduces the potential for bias. Specifically, individual patient-level reasons for non-attendance were not systematically captured in routine clinical records, an inherent limitation of retrospective single-center data. Several predefined sensitivity analyses were performed to evaluate the robustness of the primary estimates. The IPCW-adjusted composite CR (68.1%) closely approximated the unweighted estimate (68.7%), and baseline characteristics did not differ significantly between those with and without valid follow-up (Table 2). Composite CR rates were stable or slightly higher when restricted to episodes with longer follow-up (≥6 months: 70.8%; ≥9 months: 73.3%), confirming that early assessment did not inflate the primary estimate. Even under a worst-case assumption that all lost-to-follow-up patients were treatment failures, the composite CR would be 58.0% (101/174), which almost certainly underestimates true efficacy. Additionally, the timing of the last available assessment varied across treatment episodes (range 3–12 months), introducing heterogeneity in endpoint ascertainment. A prospective design with fixed assessment windows would provide more uniform endpoint evaluation. Further, several subgroups had limited evaluable sample sizes (VaIN 2/3, n = 8; combined lesions, n = 12), and efficacy estimates for these subgroups should be interpreted with particular caution. The maximum follow-up of 12 months for most patients precludes assessment of long-term recurrence. Since 25.2% of patients had a last follow-up at ≤3 months and HPV clearance continued to accumulate through 12 months, the composite CR rate may be conservatively underestimated. Although treatment-related adverse events were documented from procedure notes and follow-up records, standardized prospective grading (e.g., CTCAE) was not applied, and the frequency of mild events may be underestimated. Finally, the temporal analysis of LBC negativity and HPV clearance was based on repeated cross-sectional observations with non-identical denominators at each timepoint, and should be regarded as hypothesis-generating. Future prospective studies should incorporate standardized longitudinal follow-up with paired assessments at predefined timepoints, systematic CTCAE-graded safety monitoring, adequate sample sizes for non-cervical lesion subtypes, and standardized response definitions distinguishing morphological and virological endpoints to enable meaningful cross-study comparisons. Patient-reported outcomes, including health-related quality of life, sexual function, subsequent pregnancy and delivery outcomes, and patient satisfaction, were not systematically collected in this retrospective dataset and are therefore not reported. The claim of fertility preservation in this study rests on the absence of structural complications rather than on direct measurement of reproductive outcomes, a meaningful limitation given that fertility preservation is one of the central rationales for tissue-preserving therapy in this population. Future prospective studies should incorporate standardized longitudinal follow-up with paired assessments at predefined timepoints, systematic CTCAE-graded safety monitoring, adequate sample sizes for non-cervical lesion subtypes, standardized response definitions distinguishing morphological and virological endpoints, and validated patient-reported outcome instruments together with structured tracking of subsequent pregnancy and delivery outcomes to establish the patient-centered advantages of ALA-PDT directly. Follow-up HPV testing was reported at the assay level rather than per genotype, precluding a formal per-genotype clearance sensitivity analysis. However, the assay-level approach is conservative, as it classifies any high-risk HPV positivity at follow-up, including potentially newly acquired infections, as not cleared.

Conclusions

In this real-world cohort study, ALA-PDT demonstrated high and clinically meaningful efficacy across the spectrum of HPV-related lower genital tract lesions, with a composite CR of 68.7%, an hCR of 83.0%, and an ORR of 93.2%, estimates that were robust across follow-up strata and after inverse probability weighting. When assessed by hCR, morphological response rates suggest clinically meaningful efficacy in the range reported for excisional procedures, although formal comparison awaits prospective head-to-head studies with harmonized endpoint definitions. The ability to treat multi-site disease simultaneously is a distinctive practical advantage. The observation that composite CR was stable or increased with longer follow-up, coupled with the apparent temporal dissociation between morphological and virological response, supports extending post-treatment HPV surveillance to at least 12 months before concluding virological clearance has failed. These findings support ALA-PDT as an effective, tissue-preserving treatment for HPV-related lower genital tract disease, particularly for patients with persistent low-grade lesions who occupy the current treatment vacuum between observation and excision. The favorable structural safety profile is compatible with, but does not directly demonstrate, preservation of reproductive function; direct evidence will require prospective studies incorporating patient-reported and reproductive outcome measures. Prospective controlled studies with standardized protocols, systematic safety monitoring, and longer follow-up are needed to confirm these findings and optimize patient selection.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

ZZC designed the research study. ZZC, HH, XLW and XYZ performed the research. ZZC and HH analyzed the data. ZZC drafted the article. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of The First Affiliated Hospital of USTC (approval number: 2024-RE-137) and conducted in accordance with the Declaration of Helsinki. Written informed consent was waived given the retrospective nature of the study.

Acknowledgment

Not applicable.

Funding

This work was supported by 2024 Open Research Project of the Key Laboratory of Tumor Invasion and Metastasis (Ministry of Education) [No.: 2024KFKT014].

Conflict of Interest

The authors declare no conflict of interest.

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