J Gynecol Oncol. 2024 Jul;35(4):e52
https://doi.org/10.3802/jgo.2024.35.e52
pISSN 2005-0380·eISSN 2005-0399

JOURNAL OF GYNECOLOGIC ONCOLOGY

Original Article

A phase 1/2a, dose-escalation, safety, and preliminary efficacy study of the RKP00156 vaginal tablet in healthy women and patients with cervical intraepithelial neoplasia 2

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Received: Jul 31, 2023
Revised: Dec 15, 2023
Accepted: Dec 31, 2023
Published online: Jan 24, 2024

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ABSTRACT

Objective: This study aimed to determine the safety and efficacy of the RKP00156 vaginal tablet, a CDK9 inhibitor, in healthy women and patients with cervical intraepithelial neoplasia grade 2 (CIN2).
Methods: We conducted a phase 1/2a clinical trial of RKP00156. In step 1, RKP00156 at a dose of 10, 25, or 50 mg or a placebo tablet was administered transvaginally to 24 healthy women. In step 2, RKP00156 at a dose of 10, 25, or 50 mg or a placebo tablet was administered once daily for 4 weeks in 62 patients with CIN2. The primary endpoints of this trial were the safety of RKP00156 and the change in the human papillomavirus (HPV) viral load.
Results: A total of 86 patients were enrolled and randomized. RKP00156 administration did not cause serious drug-associated adverse events (AEs). Although no significant difference in the HPV viral load was found between the experimental and placebo groups, a reduction in the HPV viral load was observed in the 25 mg-dose group (−98.61%; 95% confidence interval=−99.83%, 4.52%; p=0.046) after treatment completion in patients with a high HPV viral load, despite a lack of statistical power. No differences in histologic regression and HPV clearance were observed.
Conclusion: The safety of RKP00156 was proved with no serious AEs. Although the study did not show any significance in histologic regression and HPV clearance, our findings indicate that RKP00156 may have a possibility of short-term inhibitory effect on HPV replication in patients with higher viral loads.

Trial Registration: ClinicalTrials.gov Identifier: NCT02139267

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Trial Registration
ClinicalTrials.gov Identifier: NCT02139267

Funding
This study was supported by KinoPharma (Tokyo, Japan).

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Author Contributions
Conceptualization: K.Y.M., L.J.K.; Data curation: C.H.W., J.S., K.Y.T., K.J.W., K.Y.M.; Formal analysis: C.H.W., S.S.H.; Investigation: C.H.W., J.S., K.Y.T., K.J.W., C.C.H., H.S.Y., C.S.J.; Methodology: C.H.W., J.S., S.S.H., K.Y.M., L.J.K.; Project administration: L.J.K.; Resources: L.J.K.; Software: L.J.K.; Supervision: L.J.K.; Validation: K.J.W., C.C.H., H.S.Y., C.S.J., K.Y.M., L.J.K.; Visualization: C.H.W., J.S.; Writing - original draft: C.H.W., J.S.; Writing - review & editing: C.H.W., J.S., S.S.H., K.Y.T., K.J.W., C.C.H., H.S.Y., C.S.J., K.Y.M., L.J.K.

Keywords: Uterine Cervical Dysplasia; Human Papillomavirus Viruses; Viral Load; Viral Replication Compartments

Synopsis
RKP00156 vaginal tablet was well-tolerated by all patients. There were no significant results for the rates of human papillomavirus (HPV) clearance and histologic regression in overall cervical intraepithelial neoplasia grade 2 patient group. There was a significant reduction in HPV viral load in the high HPV viral load group immediately after the 4-week treatment.

INTRODUCTION

In 2018, >570,000 women were newly diagnosed with cervical cancer, and approximately 311,000 died of the disease worldwide. These numbers highlight the considerable burden that cervical cancer continues to place on the global public health system [1,2]. Persistent infection with human papillomavirus (HPV) increases the likelihood of the development of high-grade cervical intraepithelial neoplasia (CIN), which can be a precursor to invasive cervical cancer and is the most common important gynecologic disease in women of reproductive age [3,4].

The current standard of treatment for high-grade CIN is surgical excision, such as a loop electrosurgical excision procedure, which can lead to adverse pregnancy outcomes [4]. In addition, HPV vaccination to prevent HPV infection has some limitations, such as the need to keep the vaccine refrigerated and the lack of government-funded HPV vaccination programs in most Asian countries [5]. For these reasons, several studies have attempted to develop a non-surgical and non-invasive treatment for high-grade CIN; however, the results have been limited thus far [6,7].

RKP00156 (chemical name: N-[5-fluoro-2-(1-piperidinyl) phenyl] isonicotinthioamide) is a novel low-molecular compound that is being developed as an antiviral agent. RKP00156 selectively inhibits CDK9 activity, thereby exerting antiviral activity by preventing RNA polymerase II phosphorylation and suppressing viral DNA replication [8]. RKP00156 has also been shown to exhibit efficacy in suppressing the proliferation of HPV-infected cells [9]. When RKP00156 is administered transvaginally, it is expected to show its effect in the nearby uterine cervical epithelium at high concentrations, thereby treating CIN lesions by suppressing HPV DNA replication.

This phase 1/2a trial was designed to test the safety and clinical efficacy of RKP00156 in patients with HPV-induced cervical intraepithelial lesions. The aims of this study were (i) to evaluate the safety of RKP00156 in healthy women and patients with CIN2 and (ii) to assess the efficacy of RKP00156 in terms of HPV viral load change.

JOURNAL OF GYNECOLOGIC ONCOLOGY

MATERIALS AND METHODS

1. Study design and population

This prospective, randomized, multicenter, single-blinded, phase 1/2a trial was conducted at 9 sites in Korea. The trial was registered at ClinicalTrials.gov (No. NCT02139267). The protocol was approved by the Institutional Review Board or ethics committee at each study site, and written informed consent was obtained from each participant (2020GR0209). This study was conducted in accordance with the Declaration of Helsinki and all applicable laws. The trial consisted of 2 steps: step 1 was conducted to evaluate the safety of RKP00156 in healthy women, and step 2 was conducted to assess the safety, tolerability, and efficacy of RKP00156 in patients with CIN2. **Fig. 1** describes the full details of the study design. The study protocol is adherent to the Consolidated Standards of Reporting Trials (CONSORT) statement and guidelines.

Healthy premenopausal women aged 20–50 years, with no history of CIN lesions in Korea were included in step 1, and premenopausal women aged 20–40 years who were newly diagnosed of high risk HPV (hrHPV)-positive CIN2, including both single and multiple HPV infection, were recruited from 9 academic centers in Korea for step 2. Patients who had previous history of CIN or conization were excluded. Also, women who were pregnant; breastfeeding; co-infected with the hepatitis B virus, the hepatitis C virus, the human immunodeficiency virus, or syphilis; or diagnosed with any cancer or severe heart, liver, or kidney diseases were excluded in both steps. The study population were enrolled between July 2020 and October 2020.

2. Randomization and masking

The study participants were randomly assigned to receive either RKP00156 at different doses or a placebo. An independent statistician produced the randomization code before the start of the trial, and randomization was performed using block randomization at a 1:1:1:1 (RKP00156 10 mg:25 mg:50 mg:placebo) ratio. Because of the difference in color between the RKP00156 and placebo tablets, this trial was single-blinded, in which only the study participants were masked to the treatment group assignment.

3. Procedures

In step 1, healthy volunteers without any CIN lesion received one vaginal tablet of either 10 mg, 25 mg, or 50 mg of RKP00156 or a placebo, administered transvaginally by the investigators (day 1). The participants were hospitalized for 5 days for observation of any adverse events (AEs). Thereafter, they were discharged and scheduled for follow-up on the seventh day (day 8) after the administration (final visit). The escalation of the dose was confirmed by the principal investigators after the tablet with a smaller dose was deemed safe until the final visit.

In step 2, patients with CIN2 were randomly assigned to receive either 10 mg, 25 mg, 50 mg of RKP00156, or placebo vaginal tablets, administered transvaginally by themselves every day for a total of 4 weeks. The patients visited the study sites for safety and efficacy evaluation on days 1 (the starting day of treatment), 8, 15, 22, 29, and 43 (14 days after the end of the 4-week treatment). The final follow-up was conducted on day 85, 8 weeks after the end of the 4-week treatment. Dose-escalation was performed according to the judgment of the data monitoring committee in the absence of safety issues or any problems following the change in blood concentration levels after repeated administrations of the tablet with a smaller dose.

https://doi.org/10.3802/jgo.2024.35.e52

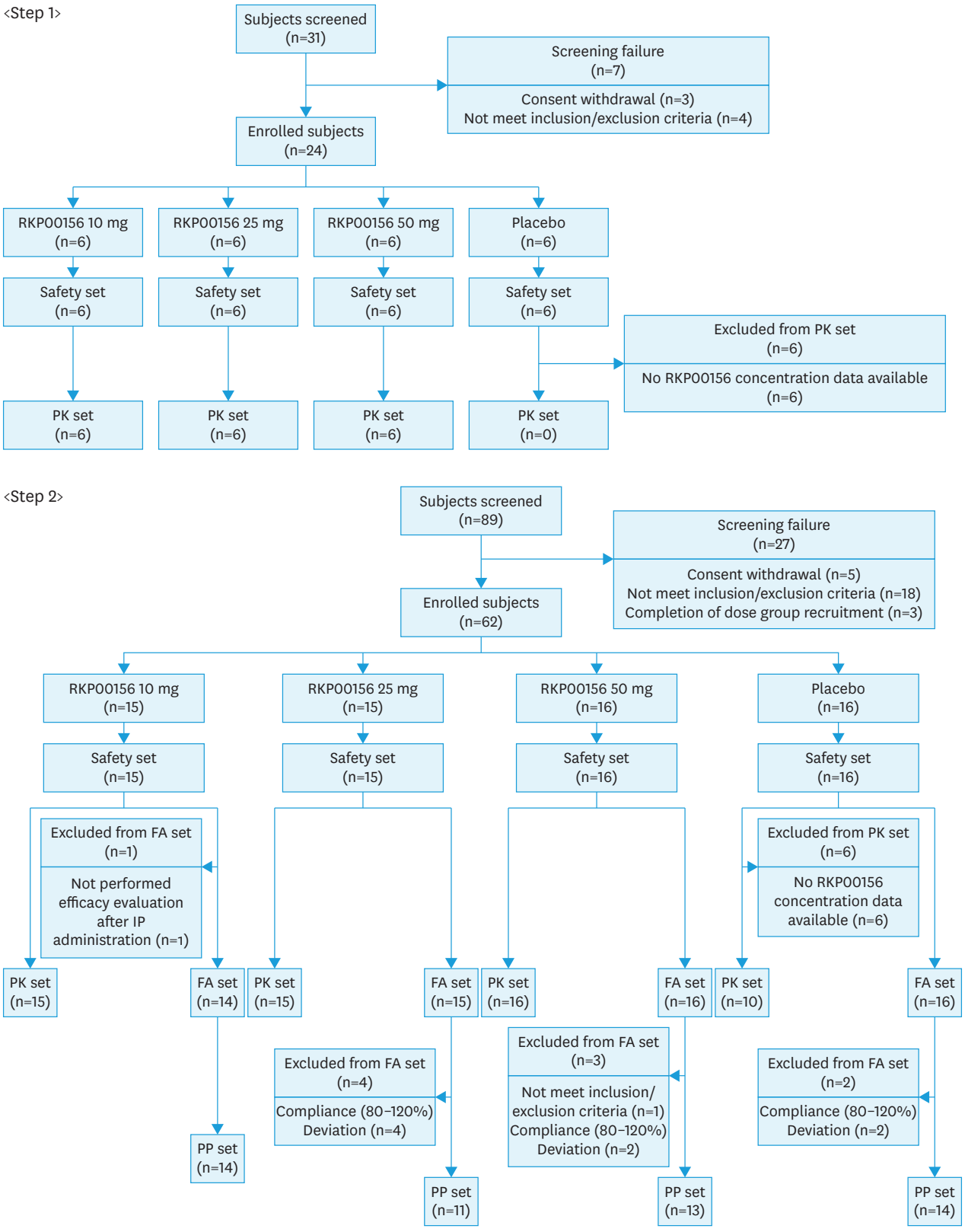

Fig. 1. Study flow diagram of the phase I/IIa clinical trial. In step 1, RKP00156 was assessed after the first dose of treatment and each subsequent participant was enrolled once each subject had completed the first dose of the treatment. In step 2, safety and efficacy ware assessed.
FA, full analysis; PK, pharmacokinetic; PP, per protocol.

https://doi.org/10.3802/jgo.2024.35.e52

Three mandatory colposcopies were performed on the participants in step 2: at baseline, on day 43, and on the final follow-up day (day 85). The cervix was inspected with colposcopy, and follow-up biopsies and cervical swabs were obtained for pathological review, HPV qualitative, and quantitative tests. At visits 1, 3, 5, and 7, cervical samples from patients were collected using a cervical brush; transported in a BD SurePath™ collection vial (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and maintained in a refrigerator for HPV investigation. DNA was extracted using a DNeasy® Blood & Tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, with modifications. DNA extraction and measurement of HPV viral load were performed at GeneticLab (Sapporo, Japan), using methods validated and established by the laboratory. The HPV genotypes identified in this study were 10 types of hrHPV (HPV 16, 18, 31, 33, 35, 39, 51, 52, 56, and 58). All the pathologic reviews were performed at each participating institution.

The safety of RKP00156 was assessed based on laboratory test results and physical examination findings. At each visit, the participants were asked about any adverse reactions that they experienced. The investigators evaluated the severity of the events and reported the results to the Institutional Review Board.

4. Outcomes

The primary efficacy endpoint for step 2 was the change in the HPV viral load among HPV-positive patients with CIN2 after the 4-week administration of the study drug. The safety profile of RKP00156 was analyzed for all participants who received at least one dose of the medication. The secondary outcomes were HPV clearance and histopathologic regression to CIN1 or normal pathology at the final visit.

5. Statistical analysis

The sample size was determined based on the primary efficacy endpoint. According to a previous study by Trimble et al. [10], the rate of spontaneous HPV 16 viral load reduction in CIN2 and CIN3 patients was 20.5%. We assumed HPV viral load reduction to 35% in the placebo group and 70% in the treatment group, and at least 13 participants were required in each treatment group to provide 80% power at a 2-sided α level of 0.05 with a standard deviation (SD) of 35%. Accounting for a 10% dropout rate, we recruited a total of at least 15 participants for each group. Student's t-test and Wilcoxon rank-sum test were performed to evaluate the statistical significance of the primary efficacy endpoint between the treatment and placebo groups. The same methods were used for secondary efficacy analyses and subgroup analyses. For continuous data, descriptive statistics such as n (number of observations), mean, and SD were used, and Student's t-test and Wilcoxon rank-sum test were performed to determine statistical significance. All analyses were performed using SAS statistical software (version 9.4; SAS Institute, Cary, NC, USA). Statistical significance was set at $p<0.05$.

RESULTS

1. Baseline demographics

In step 1, 24 of the 31 screened women were randomized into 4 groups, with 6 women each in the 10 mg, 25 mg, and 50 mg RKP00156 and placebo groups. In step 2, 62 of the 89 screened patients with CIN2 were randomized, with 15 patients in the 10 mg RKP00156 group, 15 patients in the 25 mg RKP00156 group, 16 patients in the 50 mg RKP00156 group, and 16 patients in the placebo group. **Table 1** summarizes the baseline characteristics of the

https://doi.org/10.3802/jgo.2024.35.e52

Table 1. General characteristics of study participants

Characteristics	RKP00156			Placebo	p-value*
	10 mg	25 mg	50 mg		
Step 1					
Number	6	6	6	6	
Age (yr)	39.00±7.75	30.00±6.99	29.33±4.76	32.17±8.50	0.108
Parity					
Yes	2 (33.33)	1 (16.67)	1 (16.67)	0 (0.00)	0.878
No	4 (66.67)	5 (83.33)	5 (83.33)	6 (100.00)	0.878
BMI (kg/m^2)	22.87±2.19	20.00±0.89	21.65±2.71	22.67±3.33	0.196
Step2					
Number	14	15	16	16	
Age (yr)	29.79±5.58	29.67±4.56	26.50±3.79	29.94±3.97	0.106
Parity					
Yes	1 (7.14)	4 (26.67)	0 (0.00)	2 (12.50)	0.099
No	13 (92.86)	11 (73.33)	16 (100.00)	14 (87.50)	0.099
BMI (kg/m^2)	21.17±2.31	22.81±4.54	21.49±3.82	21.79±2.48	0.597
Cytology					
NILM	3	3	4	5	0.916
ASCUS	5	5	7	6	0.967
LSIL	2	5	3	4	0.663
ASC-H	3	0	2	0	0.076
AGC	0	1	0	0	0.475
HSIL	1	1	0	1	0.794
HrHPV infection					
HPV 16	3	3	4	4	1.000
HPV 18	0	1	2	1	0.902
Other hrHPV	20	22	22	21	0.889
HPV titer	1,066,440.24±2,968,928.19	183,224.91±351,674.19	1,740,075.01±3,479,234.27	1,253,243.81±2,585,602.22	0.186
Histology (CIN2)	14	15	16	16	NA

Values are presented as mean ± standard deviation or number (%).
AGC, atypical glandular cell; ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion; BMI, body mass index; CIN2, cervical intraepithelial neoplasm grade 2; HPV, human papillomavirus; HrHPV, high risk human papillomavirus; HSIL, high grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NA, not applicable; NILM, negative for intraepithelial lesion or malignancy.
*These p-values were calculated by Fisher's exact test for nominal variables and by analysis of variance for continuous variables.

participants in steps 1 and 2. In step 1, the average age was 32.63±7.72 years and the average body mass index (BMI) was 21.80±2.56 kg/m^2. Among the women, 12.50% (3/24) were current smokers, and 33.33% (8/24) were current alcohol drinkers. In step 2, the average age of the patients was 28.89±4.59 years, and the average BMI was 21.77±3.39 kg/m^2. The proportions of current smokers and alcohol drinkers were 14.52% (9/62) and 61.29% (38/62) respectively. The average duration of disease (CIN2) was 1.60±4.05 months (minimum, 0.03 months; maximum, 30.69 months).

2. Safety

The safety group comprised 24 healthy women in step 1 and 62 patients with CIN2 in step 2. The overall safety assessment results for each step are shown in **Table 2**.

In step 1, AEs were reported in 5 of the 18 participants (27.78%) in the RKP00156 groups and in none of the 6 participants in the placebo group. No serious AEs (SAEs) leading to the discontinuation of participation were observed. The most common AE was vaginal discharge. The other AEs were abdominal discomfort, nausea, vomiting, carbuncles, and headaches. Four cases of adverse drug reactions (ADRs) were reported in the RKP00156 groups. The most common ADR was vaginal discharge, followed by abdominal discomfort and headache.

https://doi.org/10.3802/jgo.2024.35.e52

Table 2. Safety assessment of the trial

Variables	RKP00156			Placebo
	10 mg	25 mg	50 mg	
Step 1				
Number of subjects	6	6	6	6
AE	1 (16.67) [0.42, 64.12]	1 (16.67) [0.42, 64.12]	3 (50.00) [11.81, 88.19]	0 (0.00) [0.00, 45.93]
ADR	1 (16.67) [0.42, 64.12]	1 (16.67) [0.42, 64.12]	2 (33.33) [4.33, 77.72]	-
SAE	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]
Adverse event that resulted in death	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]
Adverse event that caused dropout	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]	0 (0.00) [0.00, 45.93]
Step 2				
Number of subjects	15	15	16	16
AE	4 (26.67) [7.79, 55.10]	8 (53.33) [26.59, 78.73]	7 (43.75) [19.75, 70.12]	6 (37.50) [15.20, 64.57]
ADR	0 (0.00) [0.00, 21.80]	1 (6.67) [0.17, 31.95]	5 (31.25) [11.02, 58.66]	-
SAE	0 (0.00) [0.00, 21.80]	1 (6.67) [0.17, 31.95]	0 (0.00) [0.00, 20.59]	0 (0.00) [0.00, 20.59]
Adverse event that resulted in death	0 (0.00) [0.00, 21.80]	0 (0.00) [0.00, 21.80]	0 (0.00) [0.00, 20.59]	0 (0.00) [0.00, 20.59]
Adverse event that caused dropout	0 (0.00) [0.00, 21.80]	0 (0.00) [0.00, 21.80]	0 (0.00) [0.00, 20.59]	0 (0.00) [0.00, 20.59]

Values are presented as number (%) and [95% exact confidence interval].
ADR, adverse drug reaction; AE, adverse event; SAE, serious adverse event.

In step 2, 25 of 62 patients experienced various AEs: 4 patients in the 10 mg RKP00156 group (26.67%), 8 patients in the 25 mg RKP00156 group (53.33%), 7 patients in the 50 mg RKP00156 group (43.75%), and 6 patients in the placebo group (37.50%). The most common AE was a vaginal infection, in which the patient experienced symptoms accompanied by a positive vaginal culture result, indicative of organisms other than normal flora. The other AEs were dysmenorrhea, abdominal pain, seizure, cystitis, urticaria, vulvovaginal pruritus, anemia, pyrexia, contusions, insomnia, asthma, rash, headache, and papillary thyroid cancer. The ADRs for RKP00156, including vaginal infection and vulvovaginal pruritus, were all mild in severity. A patient diagnosed with papillary thyroid cancer had undergone a medical examination and a biopsy for a thyroid nodule before enrollment. As the histopathologic result of the biopsy was released after the treatment had started, we registered papillary thyroid cancer as an AE. However, we concluded that the patient's papillary thyroid cancer had no relation to the treatment. The incidence of SAEs was 6.67% (contusion in 1 of 15 patients, which was due to traffic accident, unrelated to the treatment) in the 25 mg RKP00156 group in step 2, and no other SAEs were reported. The patient was permanently withdrawn from the trial. Additionally, the administration of the study drug was discontinued in one patient owing to a seizure event that occurred during blood sample collection. The seizure was not related to the study drug and had a less-than-severe degree of severity.

3. HPV clearance and histologic regression

We analyzed HPV clearance associated with RKP00156 according to the dose administered to the patients in step 2. No significant difference in the rate of HPV clearance compared to placebo was observed in all hrHPV types (HPV 16, 18, 31, 33, 35, 39, 51, 52, 56, and 58) at 2, 4, and 12 weeks after the start of the medication at all doses ($p>0.05$). Furthermore, we did not find any increase in the rate of hrHPV clearance at the final visit for all doses compared to the placebo ($p>0.05$) (**Fig. 2**).

We evaluated the efficacy of each RKP00156 dose in terms of histologic regression. We observed a 40%–70% improvement in the rate of histologic regression regardless of the treatment (placebo or study drug); therefore, the effect of the drug was not clear ($p>0.05$) (**Fig. 2**). No case of progression to malignant lesions was observed after 12 weeks of treatment.

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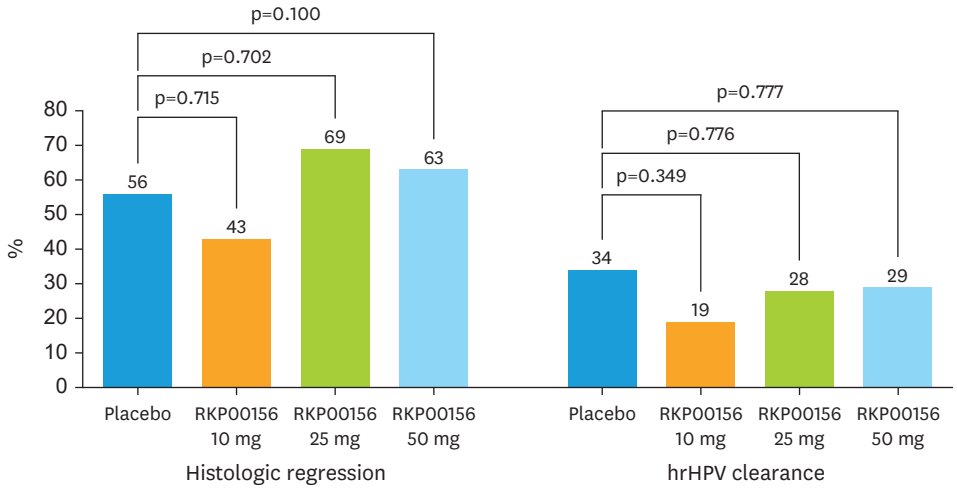

Fig. 2. Clinical efficacy: histologic regression, hrHPV clearance (placebo, 10 mg, 25 mg, 50 mg). The effect of RKP00156 was evaluated by histologic regression and hrHPV clearance. There was no significance between placebo and RKP00156 doses.
hrHPV, high risk human papillomavirus.

4. HPV viral load changes

The change in the HPV load according to the applied treatment was assessed (**Fig. 3**). Overall, no significant change in the HPV viral load was observed on days 15, 29, and 85. We performed a subgroup analysis in which all patients were divided into 3 subgroups according to their HPV viral load at baseline: low HPV viral load (<2,000 copies/cell), medium HPV viral load (2,000≤HPV<200,000 copies/cell), and high HPV viral load (>200,000 copies/cell). Similar to the overall group, the low and medium HPV viral load subgroups did not show any difference in viral load at each visit after the use of RKP00156, regardless of dose. However, in subgroup analysis, we observed a significant decrease in viral load in the high HPV viral load group immediately after the continuous administration of 25 mg RKP00156 for 4 weeks (p=0.046). The significance disappeared in the assessments during the other visits. We performed a subgroup analysis according to different HPV type, but we did not find any statistical significance (**Table S1**).

DISCUSSION

In this prospective, randomized, multicenter phase 1/2a study, we analyzed the safety and efficacy of the RKP00156 vaginal tablet in healthy women and patients with hrHPV-positive CIN2. The studied vaginal tablet was tolerated by all patients, and a single SAE, which was not related to the treatment, occurred. Although no significant results were obtained for the rates of HPV clearance and histologic regression in the overall group, subgroup analysis revealed a significant reduction in HPV viral load in the high HPV viral load group immediately after the 4-week administration of the RKP00156 tablet.

RKP00156, regardless of the administered dose, was generally well tolerated by healthy women and patients with CIN2. Only one case of SAE (mild contusion) was identified in the RKP00156 group. The contusion was due to a traffic accident and was thus not related to the treatment. Vaginal infection and vaginal discharge, the most common AEs associated with RKP00156, were generally mild, had a short duration, and did not lead to discontinuation

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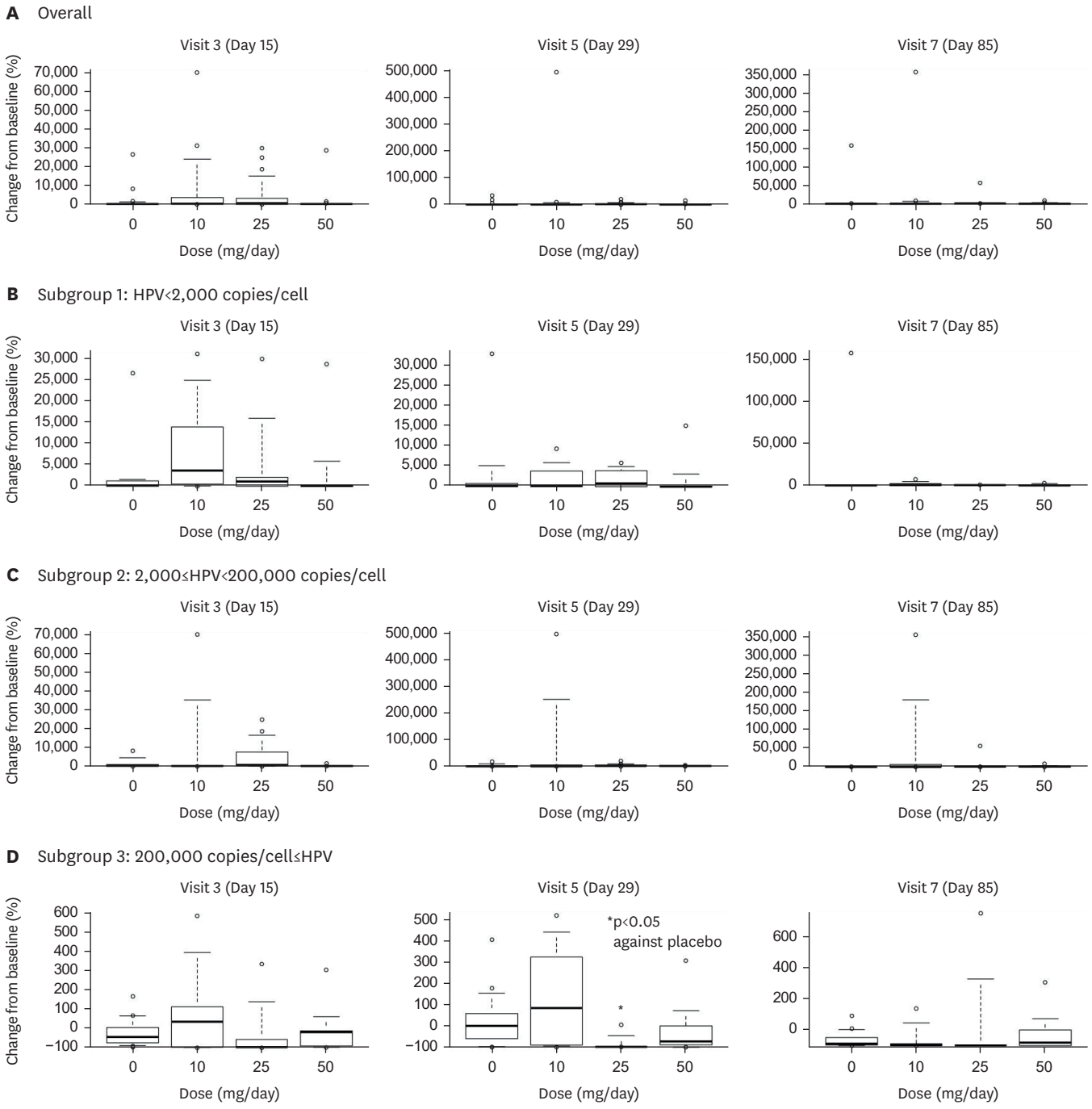

Fig. 3. Clinical efficacy: change of viral load. We observed a significant decrease in viral load in the high HPV viral load group immediately after the continuous administration of 25 mg RKP00156 for 4 weeks (p=0.046).
HPV, human papillomavirus.
*p<0.05.

of participation. Similar CDK9 inhibitors investigated in other studies did not elicit serious adverse reactions and showed good patient compliance; however, they were used for other diseases and administered differently from RKP00156 in this trial [10-13]. The results of the safety profile analysis were consistent between our study and previous studies.

https://doi.org/10.3802/jgo.2024.35.e52

We could not observe significant reduction of HPV viral load in the RKP00156 group when compared to placebo group. We believe that this finding was firstly, due to limited antiviral effect owing to the inclusion of the low viral load group. Among the total participants of 61 that were analyzed in step 2 for the drug efficacy, only 22 were classified into high viral load group. RKP00156, a CDK9 inhibitor, exhibits anti-HPV effect by suppression of the viral early promotor for E6 and E7 oncogene expression and viral replication [9]. According to a previous study, E6 and E7 oncoprotein expression is increased in high viral load patients [14,15]; thus, RKP00156 which targets E6 and E7 oncogene may possibly induce higher HPV inhibitory effect in patients with higher viral load. Also, the small sample size and relatively short treatment period may have resulted in lack of significant clinical response by RKP00156. A longer treatment period and larger sample size may provide a more response in overall viral load reduction.

In the subgroup analysis, the HPV viral load significantly decreased in the high viral load group immediately after 4 weeks of daily administration of 25 mg of RKP00156, which shows the viral suppression effect of the study drug. Previous studies have indicated that CDK9 inhibitors have antiviral effects against a wide spectrum of viruses [16-22], including HPV. Ajiro et al. [9] discovered that CDK9 inhibitor repressed HPV 18-induced dysplasia by reducing the viral load, using CIN model of an organotypic raft culture, and suppressed the growth of HPV 16 positive cervical cancer xenografts. However, the drug does not kill pre-existing DNA viruses; thus, the turnover time of infected cells is expected to be longer [11,12]. The viral load did not show a significant change after 4 weeks, which may be because the treatment ended after 4 weeks, and HPV DNA replication was no longer prevented. Thus, the result might change if the treatment period is extended, and the drug might continue to exert its effect after the point of turnover.

This study had several strengths. This was a prospective, randomized, single-blind, placebo-controlled study of non-surgical treatment for women with HPV-positive CIN2. As spontaneous regression of CIN lesions is possible [23,24], a randomized, placebo-controlled study is appropriate for the evaluation of the safety and effect of RKP00156 in patients with CIN2. This study included centrally determined information on HPV infection status before and after treatment to reduce bias. To our knowledge, RKP00156 is the first non-surgical transvaginal treatment for CIN2 that showed at least a short-term effect in reducing the viral load of HPV.

However, the exploratory nature and the lack of statistical evidence constrain our findings regarding this subgroup analysis. Nevertheless, this analysis points to a need for more data on the efficacy of the drug in CIN 2 patients with high viral load, and additional studies are planned in the future. Additionally, we could not find any significant outcomes in the analyses based on the types of HPV, because of small sample sizes of each group and due to its limitation as an exploratory analysis. However, RKP00156 is a CDK9 inhibitor and is thought to inhibit the growth of almost all DNA viruses, including HSV-1, HSV-2, HCMV, HADV [8]. Regarding HPV, there were significant effects on HPV 16 and 18, confirmed by previous study by Ajiro et al. [9] Its effects on other hrHPV types have not been investigated, but the effects have been indirectly examined on common warts in humans [12]. Warts are a disease mainly caused by infection with HPV2a, HPV27, and HPV57, which are low risk HPV types, and RKP00156 was shown to be effective in significantly reducing the wart area after only 2 weeks of administration. Based on these results, RKP00156 is believed to be effective against DNA viruses in general, and for HPV, it is considered to be effective not only against

HPV 16 or HPV 18 but also other HPV types. Additionally, the lack of supervision during the self-administration of the vaginal tablet by the patients might have resulted in some bias in the results of the efficacy analysis. Another limitation for our study would be the short duration of the treatment for 4 weeks, which made it difficult to determine the long-term safety of the drug. Also, follow-up biopsies were conducted on day 85, 8 weeks after the completion of the 4-week treatment. The relatively short follow-up period may account for the absence of observable histologic regression, as it may take an extended period after viral remission for such regression to become apparent.

RKP00156 may have the potential to be a novel medication that inhibits the replication of HPV. Our study confirmed the safety of the RKP00156 vaginal tablet in patients with CIN2 after 4 weeks of administration. However, owing to the limitations mentioned above, our study did not demonstrate the efficacy of RKP00156. Nevertheless, we identified the possibility that the drug can reduce the HPV viral load in CIN2 patients with high viral load. Therefore, further studies with longer treatment and follow-up durations are needed to determine the efficacy of RKP00156 in CIN2 patients with high viral load.

ACKNOWLEDGEMENTS

We thank the patients who participated in this study and the entire RKP001KA clinical team from the participating study sites; GeneticLab Co., Ltd (Sapporo, Japan). for developing the polymerase chain reaction method of measuring human papillomavirus viral loads.

SUPPLEMENTARY MATERIAL

Table S1

Change of HPV viral load assessed by the quantitative HPV test (step 2, full analysis set)

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