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Sinecatechins ointment as a potential novel treatment for usual type vulval intraepithelial neoplasia: a single-centre double-blind randomised control study

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Objective To compare the safety and efficacy of 10% sinecatechins (Veregen®) ointment against placebo in the treatment of usual type vulvar intraepithelial neoplasia (uVIN).

Design A Phase II double-blind randomised control trial.

Setting A tertiary gynaecological oncology referral centre.

Population All women diagnosed with primary and recurrent uVIN.

Methods Eligible patients were randomised 1:1 to receive either sinecatechins or placebo ointment (applied three times daily for 16 weeks) and were followed up at 2, 4, 8, 16, 32 and 52 weeks.

Main outcome measures The primary outcome measure, recorded at 16 and 32 weeks, was histological response (HR). Secondary outcome measures included clinical (CR) response, toxicity, quality of life and pain scores.

Results There was no observed difference in HR between the two arms. However, of the 26 patients who were randomised, all 13 patients who received sinecatechins showed either complete (n = 5) or partial (n = 8) CR, when best CR was evaluated. In placebo group, three patients had complete CR, two had partial CR, six had stable disease and two were lost to follow up. Patients in the sinecatechins group showed a statistically significant improvement in best observed CR as compared with the placebo group ($P = 0.002$). There was no difference in toxicity reported in either group.

Conclusion Although we did not observe a difference in HR between the two treatment arms, we found that 10% sinecatechins application is safe and shows promise in inducing clinical resolution of uVIN lesions and symptom improvement, thus warranting further investigation in a larger multicentre study.

Keywords Epigallocatechin-3-gallate, human papillomavirus, quality of life, vulvar intraepithelial neoplasia.

Tweetable abstract A randomised control study indicating that sinecatechins ointment may be a novel treatment for uVIN.

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Introduction

Usual type vulvar intraepithelial neoplasia (uVIN), a putative precursor lesion of VSCC (vulval squamous cell carcinoma), is associated with persistent high-risk HPV (HR-HPV) infection; predominantly HPV16.[1,2] The condition primarily affects young women, with a peak age incidence of 30–49 years. In recent years, the incidence of VIN has increased by more than three-fold.[3,4] Although the malignant progression of uVIN is significantly lower than

that of cervical intraepithelial neoplasia (CIN),[5] typically of the order of ~10% risk of cancer progression in untreated patients, unlike CIN, it often causes debilitating symptoms such as pruritus, pain and sexual dysfunction. To date, choices of non-surgical treatment remains limited[6] and there is a compelling need for new medical treatments which could interrupt the natural history of uVIN.

Sinecatechins ointment, with epigallocatechin-3-gallate (EGCG) as its primary bioactive green tea polyphenol, has been proven to be safe and effective in eradicating genital warts,[7] a low-risk HPV-associated proliferative disorder. Here, we report findings from a Phase II randomised placebo control study (EPIVIN) which evaluates the use of 10% sinecatechins (Veregen®) ointment in the treatment of women with uVIN, a hyperproliferative disorder caused by high-risk HPV infection.

Methods

The study was designed as a Phase II, single-centre, double-blind, randomised control trial. Inclusion criteria were all women ≥18 years of age who presented with histologically proven uVIN on biopsy, either as primary or recurrent disease. All uVIN lesions must be measurable with at least one lesion that can be accurately measured in one dimension with longest diameter ≥10 mm. Patients with recurrent disease must be treatment-free for at least 12 weeks and must be able to provide a written consent to participate in the study. Exclusion criteria were patients with suspected or histologically proven invasive disease; pregnant, breast-feeding or those trying to conceive; those with known allergies to any of Veregen® or placebo components; underlying immunosuppressive disease; those unable to comply with protocol; severe liver dysfunction or chronic liver disease; unable to provide written consent. This study was approved by the East Midlands - Derby Research Ethics Committee with study number 13/EM/0398. All patients provided written consent to participate in the study. Patients were not involved in the development of this study. Medigene AG, Germany, supplied 10% sinecatechins (Veregen®) and placebo ointments.

Patients who fulfilled the eligibility criteria were randomised 1:1 into receiving sinecatechins (active) or a placebo ointment, randomisation was stratified on previous uVIN history using minimisation via a database implementation of the procedure. Sinecatechins 10% and placebo ointment were both manufactured and supplied by MediGene AG, Germany. All the components in the placebo ointment are identical to the verum, except that it lacks sinecatechins. Patients were to apply ointment thrice daily for 16 weeks, and the frequency of application was recorded in a diary by the patients. Follow-up visits were scheduled at 2 weeks (telephone call), 4, 8, 16, 32 and 52 weeks after starting treatment. Baseline biopsy for histological diagnosis was obtained prior to treatment and at 16 and 32 weeks after treatment. Recruitment to the trial was scheduled for 24 months. The study protocol is included in Appendix S1.

The primary objective was to evaluate whether application of 10% sinecatechins could induce histological resolution of uVIN when assessed up to 32 weeks following the start of treatment. Histological resolution is defined as the absence of uVIN lesions or invasive cancer. The secondary objectives were to assess clinical resolution (at least a partial response; ≥30% reduction in the sum of the longest diameter of all lesions when compared with baseline), treatment compliance, safety and tolerability, and quality of life using McGill's pain questionnaire[8] and Dermatology Life Quality Index (DLQI)[9] questionnaire. The primary endpoint, best histological response observed across the 32 weeks as established by blinded pathology review, was to be analysed as per Jung's design, an analogue of Simon's design, for randomised phase II trials. The trial was designed to have type I and type II error rates less than or equal to 0.15. Assuming that the VIN resolution rate in the control arm would be 10%, and that an improvement to 30% would be clinically important, a required total of 28 patients were to be randomised to each arm. At least three more cases of histological resolution on the experimental arm compared with the control arm of the trial were required to conclude sufficient activity.

Clinical resolution was assessed according to the criteria defined in our study protocol (see Appendix S1); the number of patients with clinical resolution, defined as at least a partial response, will be reported by treatment arm and will be compared using Fisher's Exact test. Time to clinical resolution was measured as the time from randomisation to clinical resolution, patients not having resolution were censored at the date last seen, and this will be presented in a Kaplan–Meier plot. Treatment compliance data were summarised at patient level taking into account both dose reductions and interruptions. Adverse event data was collected as per Common Terminology Criteria for Adverse Events (CTCAE v4.0) and summary tables were presented for this data. Quality of life questionnaire scores were presented using plots over time.

Results

A CONSORT diagram detailing the number of patients recruited into the study and their follow up for the primary analysis is presented in Figure 1. The recruitment target was not achieved within the allotted time due to lack of eligible patients and a slow recruitment rate within a single-centre setting. Analysis was performed after the study was closed to recruitment. A total of 26 patients with

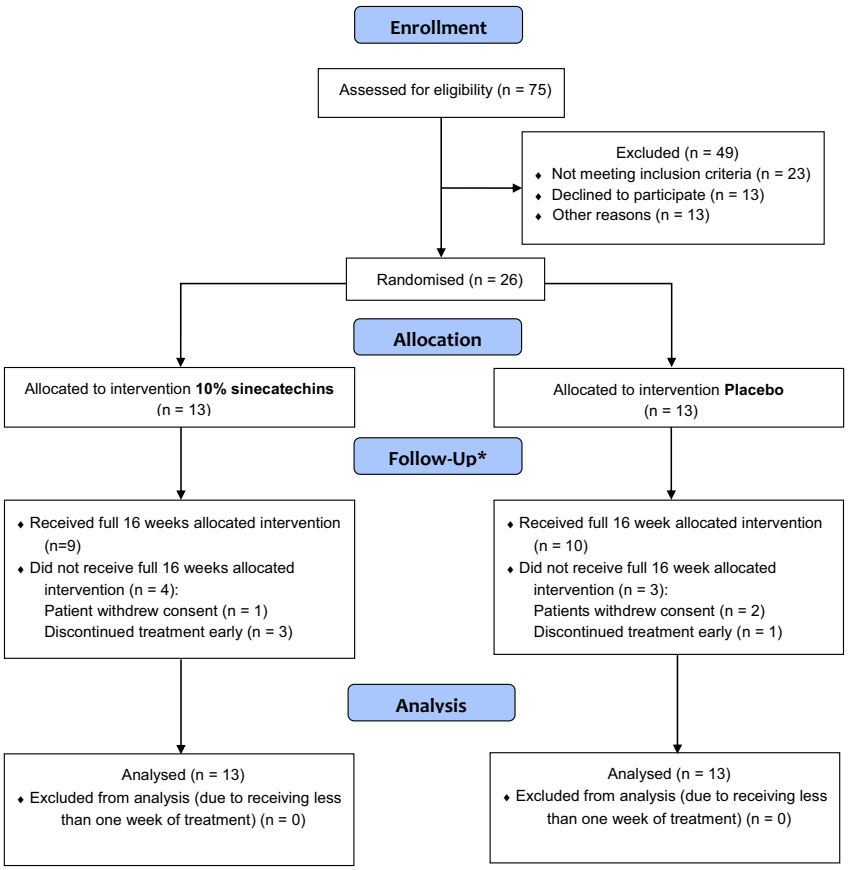

Figure 1. CONSORT *flow diagram depicting the progress through the phases of a 2-group parallel 1:1 randomised trial*

histological confirmation of uVIN were recruited into our study, with an equal number of patients randomised into the sinecatechins (n = 13) or placebo (n = 13) treatment arms.

Baseline characteristics of the study cohort

A summary of patient characteristics in the cohort is outlined in Table 1. The mean age of our cohort was 51 years old; 25 (96.2%) patients who were recruited, presented with recurrent disease, and only one (3.8%) patient had primary uVIN. Our patient cohort has a long-standing history of uVIN with a mean presentation of 10.70 years (range: 1.04–30.38 years); these patients also suffered from long-term symptoms with a mean duration of 22.78 months prior to randomisation. Over half of the patients (53.8%, $n = 14$) presented with a unifocal uVIN lesion, with the remainder ($n = 12$, 46.2%) presenting with multifocal lesions; two patients presented with five separate lesions. The majority of patients ($n = 21$, 80.8%) had received previous medical, surgical or both modalities of treatment; only three patients had received no prior treatment before being randomised into accepting sinecatechins.

There was no observed difference in histological response between the two study arms

All 26 patients randomised were included in the primary and secondary analyses as per the modified intention-to-treat (ITT) population, modified such that patients were required to have applied treatment for at least 1 week; patients lost to follow up were included as non-responders. Biopsies were obtained at 16 and 32 weeks after treatment to evaluate the histological resolution of uVIN and there was no observed difference in best histological response in these two groups. Three patients in each arm showed complete histology resolution; seven and six patients in the placebo and active arms, respectively, had persistent disease

Table 1. Patient characteristics

Variable	Level	Overall *n* (%)	Placebo *n* (%)	Sinecatechins *n* (%)
First VIN episode	No	25 (96.2)	12 (92.3)	13 (100)
	Yes	1 (3.8)	1 (7.7)	0 (0)
Prior treatment: imiquimod	No	18 (69.2)	8 (61.5)	10 (76.9)
	Yes	8 (30.8)	5 (38.5)	3 (23.1)
Prior treatment: laser/diathermy ablation	No	21 (80.8)	10 (76.9)	11 (84.6)
	Yes	5 (19.2)	3 (23.1)	2 (15.4)
Prior treatment: other topical cream	No	26 (100)	13 (100)	13 (100)
Prior treatment: other treatment	No	26 (100)	13 (100)	13 (100)
Prior treatment: surgery	No	6 (23.1)	2 (15.4)	4 (30.8)
	Yes	20 (76.9)	11 (84.6)	9 (69.2)
Smoking status	Current	15 (57.7)	9 (69.2)	6 (46.2)
	Never	2 (7.7)	1 (7.7)	1 (7.7)
	Previous	9 (34.6)	3 (23.1)	6 (46.2)

Variable	Summary	Overall	Placebo	Veregen
Time: first clinical diagnosis to randomisation	Mean (SD)	10.70 (8.2)	12.66 (10.1)	8.90 (6.0)
	Missing	3	2	1
	n	23	11	12
	Range	1.04–30.38	1.04–30.38	1.28–25.41
Time: first histological diagnosis to randomisation	Mean (SD)	10.33 (8.5)	12.85 (10.5)	8.50 (6.7)
	Missing	7	5	2
	n	19	8	11
	Range	0.06–30.38	1.04–30.38	0.06–25.41
Time: first symptom to randomisation	Mean (SD)	12.20 (8.3)	14.43 (10.2)	10.19 (5.9)
	Missing	7	4	3
	n	19	9	10
	Range	3.01–30.38	3.26–30.38	3.01–25.41
Time: start of current VIN to randomisation	Mean (SD)	22.78 (33.3)	30.22 (44.0)	15.33 (16.0)
	n	26	13	13
	Range	0.47–148.97	0.70–148.97	0.47–45.87
Age (y)	Mean (SD)	51.01 (12.5)	49.40 (13.2)	52.62 (12.0)
	n	26	13	13
	Range	23.73–72.96	23.73–67.93	28.30–72.96

histologically, and three and four patients in the placebo and active arms, respectively, did not have post-treatment biopsies. One patient from the placebo group developed early-stage squamous cell carcinoma at 16 weeks and was subsequently withdrawn from the study and managed according to our local guideline for vulvar cancer.

Patients randomised into the sinecatechins arm showed a significantly better clinical response when compared with placebo

Clinical response was measured according to criteria set out in our study protocol (see Appendix S1, page 29): stable disease (no change in lesion size that would indicate a partial response or progressive disease); partial response (30% reduction in lesion size measured based on the sum of longest diameter of all baseline lesions); progressive disease (at least a 20% increase in sum of longest diameter of all baseline lesions); and complete response (no visible disease). Clinical response was measured at 4, 8, 16, 32 and 52 weeks after the start of treatment. Clinical responses for patients in the placebo and sinecatechin arms within the 52-week study period are detailed in Table S1. When comparing best clinical response between the two arms, all the patients in the sinecatechin arm show a significant improvement in lesion resolution when compared with the placebo arm ($P = 0.002$). Patients in the sinecatechin arm show a decreased time to clinical resolution as compared with placebo, both when clinical resolution is taken to be complete response and when it is taken to be complete/partial response (Figure 2A).

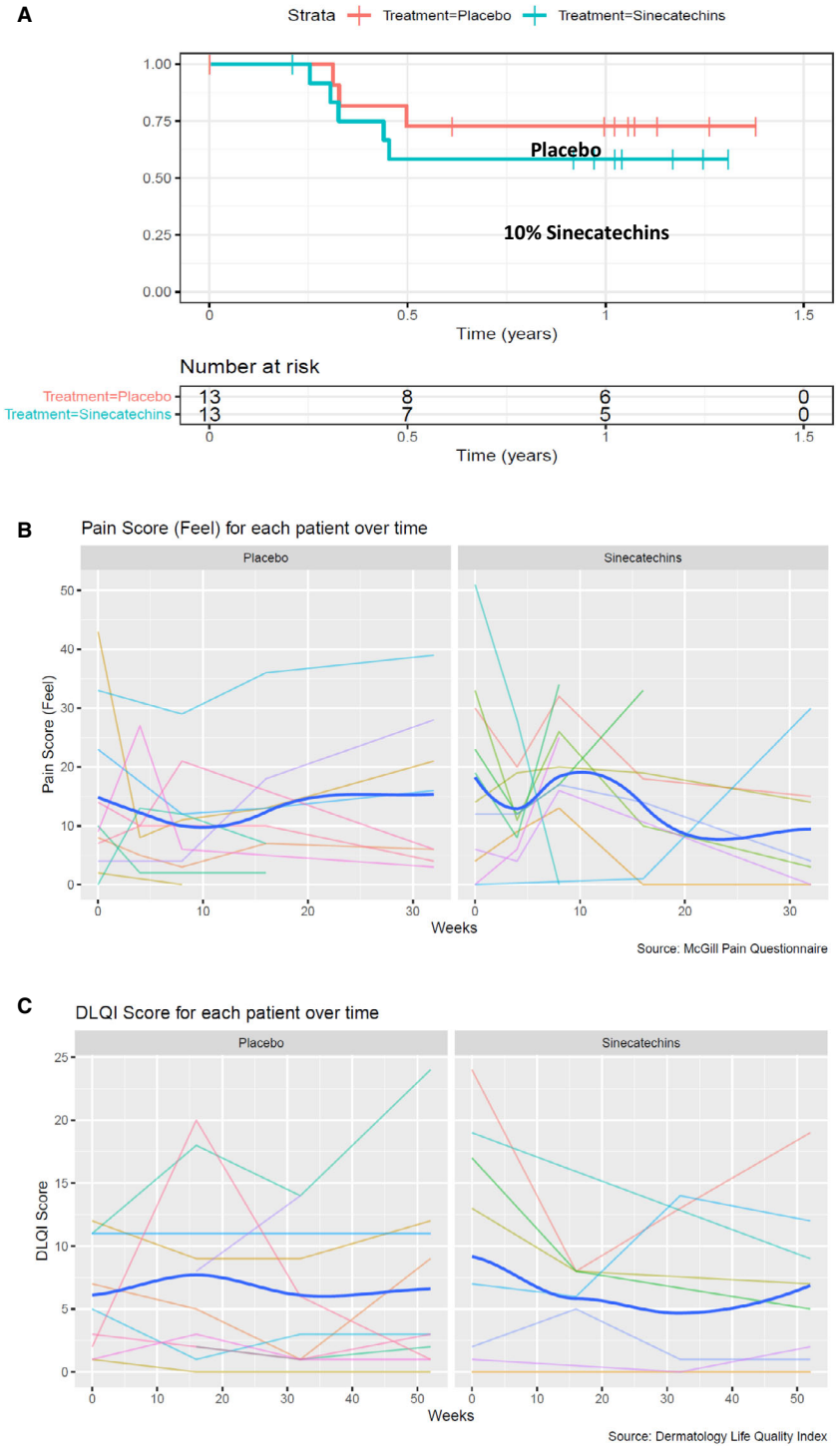

Figure 2. (A) Kaplan–Meier plot showing time to clinical resolution of uVIN lesions in 10% sinecatechins and placebo. (B) McGill Pain score reported by patients over time after 10% sinecatechins and placebo treatment. (C) Dermatology Quality of Life (QLDI) score reported by patients after 10% sinecatechins and placebo treatments

There was a trend towards improvement in baseline pain symptoms and quality of life scores related to uVIN following sinecatechins treatment.

McGill's pain questionnaire and DLQI questioners were used to assess changes in the symptoms of pain and quality of life (QoL) up to week 32 and 52, respectively. Baseline

questionnaires were completed prior to starting treatment, and there was no discernible difference in pain scores or QoL scores in the active group when compared with the placebo group. In the active group, symptoms of pain were reported to be lower than baseline after patients had completed 16 weeks of treatment, which remained stable at the 32-week follow up (Figure 2B). In the QoL index, there is an initial trend suggesting improvement in active group after 16 weeks of treatment and the overall scores, in general, remain lower than baseline at 32 weeks (Figure 2C).

Topical application of sinecatechins and placebo is relatively well tolerated by patients

Patients were advised to apply the ointment three times daily for 16 weeks. Nineteen patients continued the treatment for 16 weeks, 10 in the placebo arm and nine in the active arm. The percentage administered, taking into account reductions, interruptions and treatment discontinuation, was determined for each patient; the active arm administered a mean percentage of 58.31% for the full 16 weeks and the placebo arm 78.43%.

Overall, a higher number of patients reduced the frequency of ointment application in the active group than in the placebo group; 52 dose reductions in 11 patients and 67 dose reductions in 11 patients, respectively. Concomitant medications such as paracetamol, non-steroidal anti-inflammatory drugs and 1% topical lignocaine ointment were allowed to be used alongside the sinecatechins/placebo treatment to help alleviate symptoms caused by either the trial medication or uVIN. At baseline, only three patients in the active group and none in the placebo group were using concomitant medication. During the trial, two patients on the placebo arm used paracetamol and Sudocrem, respectively, whereas in the active arm, seven patients used lignocaine and one patient also took oral simple analgesia for pain control.

The adverse effects are detailed in Table 2. Overall, most adverse events reported were grade 1, 89% in the placebo and 74% in the active group. There were no Grade 3 and 4 adverse events reported in either study arm following treatment, the reported Grade 3/4 events were baseline uVIN symptoms. One patient did not report any adverse events; she was in the placebo group. There was a single serious adverse event, reported in the active group, of upper respiratory symptoms which was subsequently diagnosed as lower respiratory tract infection, an event unrelated to sinecatechins toxicity. Table S2 lists the adverse effects reported with the application of sinecatechins and placebo.

Table 2. Adverse events reported by patients on either 10% sinecatechins or placebo

Grade	Overall events (patients)	Placebo events (patients)	Sinecatechins events (patients)
1	159 (24)	75 (12)	84 (12)
2	32 (14)	9 (5)	23 (9)
3	1 (1)	0 (0)	1 (1)
4	1 (1)	0 (0)	1 (1)
	4 (2)	0 (0)	4 (2)

Discussion

Main findings

There was no observed difference in histological resolution, the primary outcome, between the placebo and active group. Unfortunately, the numbers here are small, the accrual target was not met and there was only a single case of primary disease. However, all the patients in the active arm showed at least a partial clinical response, with only two patients showing disease progression or recurrence, respectively, at the 52-week follow up. Results from DLQI and McGill's pain scores suggest a trend towards symptom improvements from baseline following sinecatechins application when compared with placebo. Although approximately 40% of patients in the sinecatechins group did not adhere to treatment protocol or had to reduce treatment dose or prematurely stop treatment, the side effects profiles were reasonably good, with the majority of patients experiencing Grade 1 or 2 toxicity in the form of localised irritation. When compared with baseline or pre-treatment, many patients already had underlying symptoms of local irritation, and sinecatechins treatment per se did not worsen their symptoms substantially. Therefore, our study demonstrated that topical application of sinecatechins in patients with uVIN is safe and potentially effective in treating the disease and warrants further study in a larger multicentre study.

Strengths and limitations

The main strength of our study lies in the fact that our study was randomised double-blind controlled in nature and all histological assessments were reviewed and reported centrally by an accredited pathologist specialising in gynaecological oncology. Due to funding constraints and the rarity of the disease, we were not able to achieve our intended recruitment target in time and in a single-centre setting within the recruitment timeline. We were unable to extend our recruitment period further due to funding constraints. Furthermore, our patient cohort comprised mainly of those who have a refractory disease with previous multiple treatment failure, hence, there was lack of patients with primary uVIN in our cohort. Therefore, we believe that future study should be undertaken in a multicentre setting, as this will

circumvent the two main issues which our study encountered: lack of eligible patients and study cohort bias due to lack of participants with primary uVIN.

Interpretation

The treatment ointment is composed of 10% sinecatechins, with Epigallocatechin-3-gallate (EGCG) as its major active component, the primary bioactive polyphenol of green tea. EGCG has been shown to possess anti-carcinogenic effects in both cell culture systems in vitro and animal models of cancer in vivo.[10–12] A meta-analysis by Tzellos *et al.*[7] showed that application of sinecatechins ointment to genital warts, a hyperproliferative disease caused by persistent infection with low-risk HPV strains (LR-HPV), is effective in eradicating the lesions with a relatively low recurrence rate. In addition, sinecatechins, unlike imiquimod, is well tolerated by most patients, with minimal localised side effects such as skin irritation, which is reversed after treatment cessation.

Here, although there was no difference in histological response between the two treatment arms, we demonstrated that application of sinecatechins ointment led to at least partial clinical resolution of uVIN lesions. Nevertheless, our study did not meet the targeted accrual number and the study cohort was comprised mainly of patients with refractory disease and, hence, further study is required to validate our findings. Scientific study has shown that EGCG, the major bioactive component of sinecatechins, down-regulates expression of the high risk-HPV-encoded E6 and E7 proteins, which are required for cell growth transformation and efficient replication during the HPV life cycle.[11] Topical sinecatechins has been proven to be effective in the clinical treatment of HPV-induced hyperproliferative disorders and several in vitro studies have shown a marked reduction in proliferation of HR-HPV-driven cancerous cell lines when treated with purified EGCG[10], presumably through downregulation of the viral oncogenes[11,12]. This raises the question of whether prolonged-term treatment or maintenance therapy with sinecatechins could result in complete histological resolution of uVIN. With clinical evidence showing that patients with genital warts who achieved full disease resolution following sinecatechins treatment were less likely to experience disease recurrence,[7,13] it is worth exploring whether longer treatment duration with sinecatechins will result in histological resolution of uVIN.

There were a number of reasons we believe may have contributed to the lack of observed histology response in our cohort following sinecatechins treatment. As previously discussed, our study did not achieve the intended recruitment target within the allotted time and there was a lack of patients with primary disease. The majority of our patients had a refractory disease and had experienced multiple treatment failures in the past. Whether patients with primary disease may show a better histological response to sinecatechins treatment warrants further investigation. Two previous independent randomised control studies that evaluated the use of imiquimod versus placebo[14] and imiquimod vs cidofovir (RT3VIN study),[15] respectively, examined patients with VIN grade 1, 2 and 3. In the former study, there was a significant reduction in disease severity/grade following imiquimod treatment, an observation that we were not able to undertake in our study as histological assessment in our study was based on The International Society for the Study of Vulval Disease, which uses morphological criteria to classify vulval intraepithelial neoplasia into usual-type (HPV-related) or differentiated (non-HPV-related).[16] Thus, women with low grade squamous intraepithelial neoplasia (LSIL) and VIN2 were excluded from our study; hence, the lack of observed difference in histological resolution, despite clinical symptoms and lesion size improvements, may be because our patients had a higher-grade disease. Moreover, the RT3VIN study found that fewer than half of the patients treated with either imiquimod or cidofovir showed a complete histological response, thus highlighting that longer treatment duration or maintenance treatment may be required to facilitate histological resolution. Potentially, future study should consider including patients with LSIL and VIN2, as these patients also suffer from similar debilitating symptoms to that of VIN3 and surgery might be avoided in this group of patients.

As the risk of progression from uVIN to vulval cancer is relatively low,[5] and symptom control is often the primary treatment aim for these women, sinecatechins ointment may potentially be an alternative long-term treatment to surgery or act as an adjuvant treatment for surgery. Surgical treatment does not always offer a cure, as optimal surgical resection margin is not often achieved, and most patients will recur within 3 years even if the disease has been completely resected, and further surgery is associated with psychosexual comorbidities.[17] Thus, it is worth evaluating in a future study whether sinecatechins treatment could provide better symptom control and reduce the need for surgical intervention.

Conclusion

Although our study did not show an observed difference in histology response (primary outcome) between the placebo and active arm, we found that topical sinecatechins treatment is relatively well tolerated; leads to at least a partial clinical resolution of uVIN lesions in all patients; and potentially offers symptom improvement. However, further, larger multicentre study is required to validate our finding given that our study did not achieve the accrual target and the study cohort was biased towards patients with refractory disease. As sinecatechins ointment is relatively well

tolerated with minimal side effects, there is a possibility for increasing treatment duration beyond the currently recommended 16 weeks to examine whether prolonged treatment may lead to a histological resolution of uVIN.

Disclosure of interests

This study was part funded by Medigene AG, Germany, who supplied 10% sinecatechins ointment (Veregen®) and placebo ointment. Medigene AG did not participate in designing or conducting the clinical trial or recruiting patients into the study, nor did it have any input in reporting the study outcome or writing the manuscript. None of the authors have any role in Medigene AG. This report is independent research commissioned by the National Institute for Health Research (Research for Patient Benefit Programme). The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health. All authors declare no other disclosures. Completed disclosure of interest forms are available to view online as supporting information.

Contribution to authorship

JY, CD, SV and DL conceived the idea for the study, participated in its design and coordination, and provided final approval of the version to be published. JY, DL and BS recruited patients into the study. RG reviewed histological diagnoses. HG, AH and BK undertook data management. DS performed statistical analysis. JY, CD, DS and DL wrote the manuscript and all authors read and approved the final manuscript.

Details of ethics approval

This study is approved by the East Midlands - Derby Research Ethics Committee on 20 December 2013 with study number 13/EM/0398. All patients provided a written consent to participate in the study. Clinical trial registration details: EudraCT number: 2013-003107-19; ISRCTN number: 98495886; Trail open date: 1 January 2014; first recruitment date: 13 October 2014; URL: http://www.isrctn.com/ISRCTN98495886.

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Data availability

Data available on request from the authors.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Clinical responses of patients on placebo and 10% sinecatechins.

Table S2. Adverse events by event and grade.

Appendix S1. EPIVIN clinical trial protocol. ■

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