Activity, safety, and feasibility of cidofovir and imiquimod for treatment of vulval intraepithelial neoplasia (RT³VIN): a multicentre, open-label, randomised, phase 2 trial

Amanda Tristram, Christopher N Hurt*, Tracie Madden, Ned Powell, Stephen Man, Sam Hibbitts, Peter Dutton, Sadie Jones, Andrew J Nordin, Raj Naik, Alison Fiander†, Gareth Griffiths†*

Summary

Background Vulval intraepithelial neoplasia is a skin disorder affecting the vulva that, if left untreated, can become cancerous. Currently, the standard treatment for patients with vulval intraepithelial neoplasia is surgery, but this approach does not guarantee cure and can be disfiguring, causing physical and psychological problems, particularly in women of reproductive age. We aimed to assess the activity, safety, and feasibility of two topical treatments—cidofovir and imiquimod—as an alternative to surgery in female patients with vulval intraepithelial neoplasia.

Methods We recruited female patients (age 16 years or older) from 32 centres to an open-label, randomised, phase 2 trial. Eligibility criteria were biopsy-proven vulval intraepithelial neoplasia grade 3 and at least one lesion that could be measured accurately. We randomly allocated patients to topical treatment with either 1% cidofovir (supplied as a gel in a 10 g tube, to last 6 weeks) or 5% imiquimod (one 250 mg sachet for every application), to be self-applied three times a week for a maximum of 24 weeks. Randomisation (1:1) was done by stratified minimisation via a central computerised system, with stratification by hospital, disease focality, and presentation stage. The primary endpoint was a histologically confirmed complete response at the post-treatment assessment visit 6 weeks after the end of treatment (a maximum of 30 weeks after treatment started). Analysis of the primary endpoint was by intention to treat. Secondary outcomes were toxic effects (to assess safety) and adherence to treatment (to assess feasibility). We present results after all patients had reached the primary endpoint assessment point at 6 weeks; 2-year follow-up of complete responders continues. This trial is registered with Current Controlled Trials, ISRCTN 34420460.

Findings Between Oct 21, 2009, and Jan 11, 2013, 180 participants were enrolled to the study; 89 patients were randomly allocated cidofovir and 91 were assigned imiquimod. At the post-treatment assessment visit, a complete response had been achieved by 41 (46%; 90% CI 37·0–55·3) patients allocated cidofovir and by 42 (46%; 37·2–55·3) patients assigned imiquimod. After 6 weeks of treatment, 156 (87%) patients (78 in each group) had adhered to the treatment regimen. Five patients in the cidofovir group and seven in the imiquimod group either withdrew or were lost to follow-up before the first 6-week safety assessment. Adverse events of grade 3 or higher were reported in 31 (37%) of 84 patients allocated cidofovir and 39 (46%) of 84 patients assigned imiquimod; the most frequent grade 3 and 4 events were pain in the vulva, pruritus, fatigue, and headache.

Interpretation Cidofovir and imiquimod were active, safe, and feasible for treatment of vulval intraepithelial neoplasia and warrant further investigation in a phase 3 setting. Both drugs are effective alternatives to surgery for female patients with vulval intraepithelial neoplasia after exclusion of occult invasive disease.

Funding Cancer Research UK.

Lancet Oncol 2014; 15: 1361–68

Published Online
October 8, 2014
http://dx.doi.org/10.1016/S1470-2045(14)70456-5

See Comment page 1287

*Joint first authors

†Joint last authors

HPV Research Group (A Tristram MD, Prof A Fiander DM, N Powell PhD, S Hibbitts PhD, S Jones MBBCh), **Wales Cancer Trials Unit** (C N Hurt MSc, T Madden PhD, Prof G Griffiths PhD, P Dutton MSc), **and Institute of Cancer & Genetics** (S Man PhD), **School of Medicine, Cardiff University, Cardiff, UK; University of Southampton Clinical Trials Unit, Faculty of Medicine, Southampton General Hospital, Southampton UK** (Prof G Griffiths); **East Kent Gynaecological Oncology Centre, Queen Elizabeth the Queen Mother Hospital, Margate, UK** (A J Nordin FRCOG); **and Northern Gynaecological Oncology Centre, Queen Elizabeth Hospital, Gateshead, UK** (R Naik MD)

Correspondence to:
Mr Christopher N Hurt, Wales Cancer Trials Unit, School of Medicine, Cardiff University, Cardiff CF14 4YS, UK
hurtcn@cardiff.ac.uk

Introduction

Vulval intraepithelial neoplasia is a chronic premalignant disorder that affects the vulval skin. The age-standardised incidence is about one case per 100 000 women per year, with a peak at 30–49 years of age, and incidence has been rising over recent decades.[1,2] Vulval intraepithelial neoplasia is graded as 1, 2, or 3 according to the proportion of the epithelium containing undifferentiated cells, with grade 3 disease showing full thickness neoplasia.[3] Symptoms can be severe and include pain, itching, and dyspareunia, with treatment generally needed on these grounds alone.[4] The disorder might be related to lichen sclerosus.[5]

More than 80% of women with vulval intraepithelial neoplasia have lesions associated with high-risk types of human papillomavirus (HPV), most typically HPV type 16. Persistent infection with high-risk types of HPV typically precedes deregulation of viral gene expression, leading to increased amounts of E6 and E7 oncoproteins and resulting in loss of activity of the tumour-suppressor proteins P53 and Rb, respectively. The rate of progression to invasive disease is difficult to estimate, because most patients undergo surgery to remove lesions, but it can be up to 5% per year, or 1–2% a year with surgery.[6] Spontaneous regression happens in 1·2% of patients and

usually takes place within the first 10 months.[6] Surgery (either excision or ablation) is the standard treatment, but this approach can be associated with substantial morbidity,[4,7] and recurrence is high (30–60%).[8]

Alternatives to surgery for vulval intraepithelial neoplasia are needed, and topical drugs and systemic treatments (eg, photodynamic therapy and immunotherapy) have been investigated. Two candidate drugs identified for topical use are cidofovir and imiquimod. Cidofovir is a nucleoside analogue with antiviral properties and proven activity in comparable disease (cervical intraepithelial neoplasia). In a pilot study of 15 women with cervical intraepithelial neoplasia type 3, seven (47%) of 15 women had a complete response after topical application of the drug.[9] In a pilot study of cidofovir for vulval intraepithelial neoplasia grade 3, four (40%) of ten women had a complete response at the end of follow-up.[10] Imiquimod is a drug that modifies the immune response. In previous small studies of topical imiquimod (n≤15), a complete response was reported in 28 (41%) of 67 women with vulval intraepithelial neoplasia, but with substantial variation among studies (0–73%).[11–16]

We aimed to assess the activity, safety, and feasibility of cidofovir and imiquimod in the Randomised Trial of Topical Treatment in Women with Vulval Intraepithelial Neoplasia (RT³VIN), an open-label, randomised phase 2 trial developed in the UK on behalf of the UK's National Cancer Research Institute.

For the **protocol of this trial** see http://www.wctu.org.uk/rt3vin/FINAL%20Signed%20Protocol%20Version%205.0%2018082011.pdf

See Online for appendix

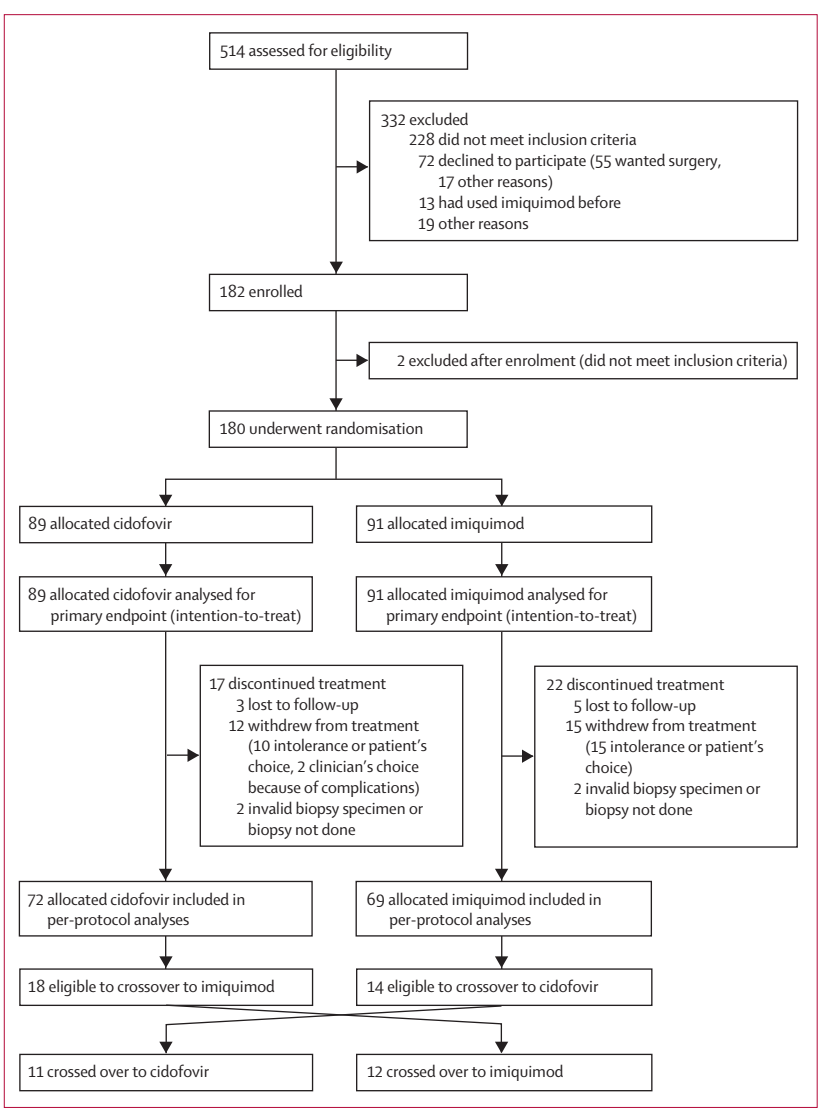

Figure: **Trial profile**

Methods

Study design and patients

We did an open-label, randomised phase 2 trial at 32 teaching and general hospitals located in Wales and England (appendix p 3). We included female patients who were age 16 years or older, had biopsy-proven vulval intraepithelial neoplasia grade 3 (including visible perianal disease not extending into the anal canal) within the past 3 months, and had at least one lesion that could be measured accurately with either a longest diameter of at least 20 mm or an area greater than 120 mm² (ascertained by measurement of two perpendicular dimensions). About halfway through recruitment (Sept 28, 2011), we expanded the inclusion criterion for size of lesion from "at least one lesion with longest diameter of at least 20 mm" to that described, to allow more patients to be entered into the study while ensuring that all lesions would be of sufficient size to allow a biopsy specimen to be taken. Clinicians with a special interest in vulval intraepithelial neoplasia recruited the participants.

We excluded early invasive disease by clinical and vulvoscopic examination (and, if necessary, biopsy), which was done by skilled clinicians. We also excluded pregnant women (by measurement of human chorionic gonadotropin in urine) and patients with impaired renal function (defined as serum creatinine >133 μmol/L). Moreover, we excluded individuals who had received active treatment for vulval intraepithelial neoplasia within the previous 4 weeks or who had a recorded failure on imiquimod or cidofovir after treatment three times a week for a minimum of 12 weeks. Finally, we excluded individuals with a known allergy to either of the topical treatments or any of their components.

All patients provided written informed consent before randomisation. We obtained appropriate regulatory approval from the UK Medicines and Healthcare Products Regulatory Agency (21323/0020/001-0001), the Office for Research Ethics Committees Northern Ireland (08/NIR03/82), and NHS research and development departments at participating sites.

Randomisation and masking

We randomly allocated patients in a 1:1 ratio to topical treatment with either imiquimod or cidofovir via a

central computerised system by stratified minimisation (with an 80:20 random element). We stratified the randomisation by hospital, unifocal or multifocal disease, and first presentation or recurrent disease. The study had an open-label design so we did not mask the treatment allocation from patients or investigators. Furthermore, masking was not possible because cidofovir gel has a very different appearance from imiquimod.

Procedures

A licensed pharmaceutical unit (St Mary's Pharmaceutical Unit [SMPU], Cardiff & Vale University Health Board) converted commercially available intravenous cidofovir into 1% topical gel (the same concentration as used in the pilot study),[10] which was supplied to recruiting centres in 10 g tubes. We asked patients allocated cidofovir to spread a thin layer of the gel over the whole affected area three times a week for a maximum of 24 weeks, with every 10 g tube to last about 6 weeks, requiring four tubes in total. We used commercially available sachets of 5% imiquimod. We asked patients assigned imiquimod to spread the contents of one sachet (250 mg) over the whole affected area three times a week for a maximum of 24 weeks, requiring 72 sachets in total. We advised patients to apply the treatment at night and wash with aqueous cream and water the next day.

If the patient was unable to tolerate three applications per week, we allowed them to reduce the dosage by one application a week, then two applications if intolerance continued. We stopped treatment if more than six consecutive applications were missed or if serum creatinine was more than 133 µmol/L. If new lesions arose during the trial they were also treated, but we did not include them in the assessments.

At baseline, we recorded the patient's medical history, did a clinical assessment of the lesion with adapted Response Evaluation Criteria in Solid Tumors (RECIST; appendix pp 1–2), did a pregnancy test, assessed toxic effects with the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 3.0, analysed urine for protein, and did a 4 mm punch biopsy for HPV testing. We marked lesions present at baseline on a case report form (CRF) and recorded on the CRF the lesion from which the diagnostic biopsy specimen was taken. We specifically sought details about other previous anogenital neoplasia and whether the patient was immunocompromised.

We analysed biopsy samples taken at baseline for the presence of HPV 16, using type-specific E6 PCR.[17] To detect non-HPV 16 types, we also tested all samples with the Greiner PapilloCheck assay (Greiner Bio-One, Frickenhausen, Germany), a PCR-based assay designed to detect 18 high-risk HPV types.

We assessed patients at 6, 12, 18, and 24 weeks of treatment. We did a clinical assessment of the lesion using adapted RECIST, did a pregnancy test, and analysed a urine sample. Furthermore, we assessed adherence to treatment by collection of diary cards and we recorded toxic effects (defined by CTCAE version 3.0). Reporting of serious adverse events was real-time until 30 days after the last participant received their last dose of treatment.

We asked patients to attend a post-treatment assessment visit either 6 weeks after the end of treatment (a maximum of 30 weeks after treatment started) or 6 weeks after a complete response or disease progression

	Cidofovir (n=89)	Imiquimod (n=91)
Age (years)	48 (42–52)	46 (41–55)
Immunocompromised		
Yes	3 (3%)	6 (7%)
No	86 (97%)	85 (93%)
Smoking status		
Current	50 (56%)	56 (62%)
Previous	24 (27%)	18 (20%)
Never	15 (17%)	17 (19%)
Disease focality		
Unifocal	44 (49%)	45 (49%)
Multifocal	45 (51%)	46 (51%)
Lesion size (mm)*	40 (30–60)	40 (30–65)
Time from current diagnosis of VIN to randomisation (days)	44 (21–73)	36 (16–73)
Recurrent VIN		
Yes	42 (47%)	40 (44%)
No	47 (53%)	51 (56%)
Time from first diagnosis of VIN to randomisation (months)†	58·0 (19·8–114·8)	79·4 (22·0–117·8)
Number of previous treatments†		
0	2 (2%)	6 (7%)
1	16 (18%)	11 (12%)
2–4	20 (22%)	19 (21%)
≥5	1 (1%)	4 (4%)
Unknown	3 (3%)	0
Previous other anogenital neoplasia		
Cervical intraepithelial neoplasia	29 (33%)	23 (25%)
Vaginal intraepithelial neoplasia	6 (7%)	3 (3%)
Anal intraepithelial neoplasia	6 (7%)	5 (5%)
None	48 (54%)	59 (65%)
Unknown	0	1 (1%)
HPV DNA positive		
Yes	75 (84%)	76 (84%)
No	8 (9%)	7 (8%)
Missing biopsy findings	6 (7%)	8 (9%)
HPV 16 DNA positive‡		
Yes	68 (76%)	67 (74%)
No§	15 (17%)	16 (18%)

Data are number of patients (%) or median (IQR). VIN=vulval intraepithelial neoplasia. *Sum of longest diameters. †Applicable to patients with recurrent disease only. ‡Applicable to patients who are HPV DNA positive. §Includes patients who are HPV DNA negative.

Table 1: **Baseline characteristics**

	Cidofovir (n=89)		Imiquimod (n=91)	
	Grade 1–2	Grade 3	Grade 1–2	Grade 3
Expected adverse events				
Fatigue	19 (21%)	3 (3%)	21 (23%)	4 (4%)
Pruritus	46 (52%)	3 (3%)	43 (47%)	6 (7%)
Ulceration	3 (3%)	0	6 (7%)	0
Pain in vulva	33 (37%)	2 (2%)	21 (23%)	1 (1%)
Headache	14 (16%)	1 (1%)	12 (13%)	3 (3%)
Muscle pain	11 (12%)	0	13 (14%)	0
Proteinuria	4 (4%)	0	9 (10%)	0
Other adverse events*				
Neurological event	2 (2%)	0	2 (2%)	1 (1%)
Pulmonary or upper-respiratory event	0	1 (1%)	0	0

Data are number of patients (%). Adverse events defined by Common Terminology Criteria of Adverse Events, version 3. No grade 4 adverse events were reported. *Included if at least one patient had an event of grade 3 or higher, or if grade 1–2 toxic effects in more than 10% of the population were present in any column.

Table 2: **Adverse events at baseline**

	Cidofovir (n=89)	Imiquimod (n=91)
First 6 weeks of the study		
Patients meeting 6-week adherence endpoint	78 (88%)	78 (86%)
Lost to follow-up	0	1
Withdrew from treatment (intolerance or patient's choice)	5	6
Incomplete record card	6	6
Number of treatment applications	17 (16–18)	16 (14–18)
During the 24-week treatment stage		
Patients with all four record cards completed*	50 (56%)	37 (41%)
Lost to follow-up	2	2
Withdrew from treatment (intolerance or patient's choice)	10	15
Clinician stopped treatment because of complications	1	0
Stopped early, progression	6	7
Stopped early, complete response	10	18
Incomplete record card	10	12
Number of treatment applications	67·5 (64–71)	63·0 (50–67)

Data are number of patients (%) or median (IQR). *Record cards completed at 6, 12, 18, and 24 weeks.

Table 3: **Treatment adherence and reasons for stopping treatment**

(defined by adapted RECIST). At the post-treatment assessment visit, we did the same tests as we did during the treatment stage. We also took two 4 mm biopsy specimens to assess histological response and for HPV testing, and we obtained blood samples for measurement of haemoglobin, white blood cell count, urea, and electrolytes. If more than one lesion was present, we took the biopsy specimen from the same lesion as the original diagnostic biopsy (and other lesions if judged clinically necessary). If no lesion was visible at the post-treatment assessment visit, we took a biopsy specimen from the same site as the original diagnostic biopsy.

We followed up patients who had a histological complete response at the post-treatment assessment visit every 6 months (at 6, 12, 18, and 24 months). We assessed toxic effects, did a clinical examination, and if a lesion was present, we took a biopsy specimen for histological analysis. We also took blood samples at baseline, at 6 weeks of treatment, and at the post-treatment assessment visit, and we banked these specimens for future translational research.

If a participant had a complete response or disease progression (defined by adapted RECIST) at any time during the 24-week treatment stage, we stopped treatment and did the post-treatment assessment visit 6 weeks later. Patients who had stable disease or disease progression (by adapted RECIST) at the post-treatment assessment visit, for whom invasive disease had been ruled out, were eligible for crossover to the alternate treatment for a maximum of 24 weeks. Subsequent management beyond the post-treatment assessment visit in patients without a complete response was at the discretion of the treating clinician.

Outcomes

The primary endpoint was a histologically confirmed complete response in baseline lesions at the post-treatment assessment visit (6 weeks after completion of treatment, a maximum of 30 weeks after the start of treatment). In this report, we present results from the point at which all patients had completed the primary endpoint. Secondary endpoints were adherence to the dosing regimen at 6 weeks and 24 weeks during the treatment stage (to assess feasibility), toxic effects during the 24-week treatment stage and at the post-treatment assessment visit (to assess safety), prediction of response according to HPV status, and the proportion of patients who had a recurrence (histologically confirmed vulval intraepithelial neoplasia grade 3) at 12 months.

Statistical analysis

The primary aim of the RT³VIN trial was to assess efficacy of cidofovir and imiquimod in terms of the primary endpoint. We used a Fleming's single-stage design in each treatment arm of the study to show efficacy for cidofovir and imiquimod separately; the study was not formally powered to compare the complete response at 30 weeks between arms. If fewer than 30% of patients had had a complete response at 30 weeks in either treatment arm, the treatment would be deemed insufficiently active to warrant further study; if, however, 45% or more patients had a complete response in either treatment arm, the treatment would be deemed worthy of further investigation. With Fleming's single-stage design, a p1 value of 0·30 and a p2 value of 0·45, an α of 0·05 (one-sided), and 90% power, we calculated that 87 participants needed to be assigned to each treatment group, giving a total of 174 participants. We analysed data with Stata 13.

For the primary endpoint, we analysed each treatment arm by both intention to treat (ie, including all patients randomly allocated to a group) and per protocol (ie, including all individuals who continued treatment until

complete response, disease progression, or 24 weeks). In the intention-to-treat analysis, we regarded all patients without a biopsy specimen after treatment as failures. We recorded the number of patients with a histologically confirmed complete response at the post-treatment assessment visit in each treatment group and calculated the corresponding proportion; we used the Clopper-Pearson exact binomial method to calculate the 90% CI for the proportion. To look for predictors of response in each treatment group, we did per-protocol subgroup analyses of the primary endpoint for several predefined variables, using either Pearson's χ^2 test or Fisher's exact method (if any cell included fewer than five patients).

We assessed adherence to treatment at 6 weeks and 24 weeks in terms of the median (IQR) number of applications during each period for all patients completing treatment (and treatment diary cards) up to those timepoints. We recorded the number of toxic effects at baseline and in all patients who applied the assigned treatment at least once, in terms of the worst grade reported during treatment and at the post-treatment assessment visit, and we calculated proportions for each treatment group. We predefined seven expected adverse events—ie, either symptoms of vulvar intraepithelial neoplasia or known toxic effects of the drugs. These events were fatigue, pruritus, ulceration, pain in the vulva, headache, muscle pain, and proteinuria; we reported findings separately for these adverse events. We gathered data for other toxic effects but grouped them into categories according to CTCAE version 3.0, for analyses.

As an exploratory analysis, we compared toxicity outcomes between arms, in patients who had applied the treatment at least once. For example, we compared between treatment arms the proportions of expected adverse events and of all toxic effects of grade 2 or higher recorded during treatment, using Pearson's χ^2 test. Also, we assessed the difference in symptom scores from baseline to the post-treatment assessment visit for pruritus and vulval pain for each treatment arm, using McNemar's test (and Fisher's exact method if fewer than five patients were recorded in any cell). We also combined data for reported serious adverse events with toxic effects recorded on CRFs.

This trial is registered with Current Controlled Trials, ISRCTN 34420460.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation of data, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 21, 2009, and Jan 11, 2013, 180 patients were enrolled from 32 hospitals across the UK (appendix p 3); 89 were randomly allocated to receive cidofovir and 91 were assigned to receive imiquimod (figure). Median age of participants was 47 years (IQR 41–54) and 148 (82%) had a history of smoking. Vulval intraepithelial neoplasia was recurrent in 82 (46%) individuals. Biopsy specimens

	Cidofovir (n=84)			Imiquimod (n=84)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Expected adverse events						
Fatigue	38 (45%)	13 (15%)	0	42 (50%)	19 (23%)	3 (4%)
Pruritus	59 (70%)	11 (13%)	0	62 (74%)	10 (12%)	0
Ulceration	37 (44%)	4 (5%)	0	31 (37%)	5 (6%)	0
Pain in vulva	49 (58%)	16 (19%)	0	57 (68%)	12 (14%)	1 (1%)
Headache	34 (40%)	3 (4%)	0	45 (54%)	9 (11%)	1 (1%)
Muscle pain	25 (30%)	2 (2%)	0	45 (54%)	4 (5%)	0
Proteinuria	19 (23%)	0	0	31 (37%)	1 (1%)	0
Other adverse events*						
Constitutional symptoms	7 (8%)	0	0	4 (5%)	1 (1%)	0
Dermatological or skin event	14 (17%)	2 (2%)	0	13 (15%)	1 (1%)	0
Gastrointestinal event	6 (7%)	0	0	12 (14%)	0	0
Neurological event	4 (5%)	1 (1%)	0	12 (14%)	1 (1%)	0
Pain	22 (26%)	4 (5%)	2 (2%)	21 (25%)	1 (1%)	0
Influenza-like symptoms	2 (2%)	2 (2%)	0	12 (14%)	1 (1%)	0

Data are number of patients (%). Adverse events were recorded in patients who applied the treatment at least once. Five (6%) of 89 patients assigned cidofovir and seven (8%) of 91 patients allocated imiquimod were lost to follow-up before the first assessment for toxic effects and were excluded from this analysis. Adverse events were defined by Common Terminology Criteria of Adverse Events, version 3. PTAV=post-treatment assessment visit. *Included if at least one patient had an event of grade 3 or higher, or if grade 1–2 toxic effects in more than 10% of the population were present in any column.

Table 4: **Adverse events during the 24-week treatment stage and at the PTAV**

	Cidofovir (n=72)	Imiquimod (n=69)
Response at PTAV*		
Complete response	41 (57%) [90% CI 47–67]	42 (61%)[90% CI 50–71]
Partial response	14 (19%)	10 (14%)
Stable disease	8 (11%)	6 (9%)
Disease progression	8 (11%)	10 (14%)
No RECIST measurement	1 (1%)	1 (1%)
Type of VIN at PTAV		
VIN not present	41 (57%)	42 (61%)
VIN1	0	2 (3%)
VIN2	2 (3%)	3 (4%)
VIN3	25 (35%)	22 (32%)
Inconclusive	4 (6%)	0
Number of treatment applications in the first 6 weeks		
In women with a complete response	17 (16–18)	17 (15–18)
In non-responders	17 (15–17)	16 (13–17)
Complete responders reaching 12-month follow-up	23/41 (56%)	32/42 (76%)
Complete response maintained at 12 months	20/23 (87%)	25/32 (78%)

Data are number of patients (%) or median (IQR), unless otherwise stated. 90% CI calculated with the Clopper-Pearson method. Per-protocol population includes all participants who continued treatment until complete response, disease progression, or 24 weeks. PTAV=post-treatment assessment visit. RECIST=Response Evaluation Criteria for Solid Tumors. VIN=vulval intraepithelial neoplasia. *Response assessed with adapted RECIST (appendix pp 1–2).

Table 5: **Response of patients at the PTAV and characteristics of complete responders (per-protocol population)**

	Cidofovir (n=72)			Imiquimod (n=69)		
	Complete responders (n)	Non-responders (n)	p	Complete responders (n)	Non-responders (n)	p
HPV status*						
HPV DNA positive	31	28	..	32	25	..
HPV DNA negative	6	2	0·281†	6	1	0·225†
HPV 16 E6 DNA positive‡	27	25	..	26	23	..
HPV 16 E6 DNA positive‡	4	3	1·000†	6	2	0·444†
Disease focality						
Unifocal	24	13	..	20	13	..
Multifocal	17	18	0·163	22	14	0·966
Disease recurrence						
First presentation	22	17	..	24	13	..
Recurrent disease	19	14	0·921	18	14	0·465
Smoking status						
Current smoker	24	15	..	23	16	..
Not current smoker	17	16	0·392	19	11	0·713
Immune status						
Immunocompromised	1	1	..	2	4	..
Not immunocompromised	40	30	1·000†	40	23	0·201†

Data are number of patients. Per-protocol population includes all participants who continued treatment until complete response, disease progression, or 24 weeks. HPV=human papillomavirus. VIN=vulval intraepithelial neoplasia. *Data missing for five patients in each treatment group. †Exact; when fewer than five patients were analysed, p values were calculated with Fisher's exact method. ‡In patients who were HPV DNA positive.

Table 6: **Subgroup analyses of complete responders versus non-responders (per-protocol population)**

for HPV testing were obtained at baseline from 166 (92%) patients. High-risk HPV DNA was present in 151 (91%) of 166 baseline biopsy specimens, of which 135 (89%) were HPV type 16. Baseline demographics, disease variables, and HPV characteristics were balanced between treatment groups (table 1), and adverse events at baseline (defined by CTCAE version 3) were also similar (table 2).

Five (6%) of 89 patients allocated cidofovir and seven (8%) of 91 assigned imiquimod either withdrew or were lost to follow-up before the first 6-week assessment and were regarded as non-compliant with the protocol. Adherence to treatment was similar in each group at both 6 and 24 weeks, and the median number of treatment applications by patients did not differ at these timepoints for either cidofovir or imiquimod (table 3). A further two patients in each group were lost to follow-up before 24 weeks and were regarded as non-compliant with the protocol. Treatment was stopped early because of intolerance in ten (11%; 95% CI 5·7–20·1) of 87 patients allocated cidofovir and 15 (17%; 9·8–26·3) of 89 assigned imiquimod. Ten (11%; 2·7–20·1) of 87 patients assigned cidofovir had a complete response by 18 weeks and stopped treatment early, compared with 18 (20%; 12·4–30·1) of 89 individuals allocated imiquimod.

Most adverse events recorded during the 24-week treatment stage or in the 6 weeks after treatment ended were grade 2 (table 4). Adverse events of grade 2 or higher were less frequent in patients assigned cidofovir compared with individuals allocated imiquimod (61 [73%] of 84 patients *vs* 73 [87%] of 84 patients; p=0·021). The main differences between treatments with respect to toxic effects of grade 2 or higher were fatigue, pruritus, and headache, which were all at least 10% more frequent in the imiquimod arm (table 4). Toxic effects of grade 3 or higher were similar between groups (31 [37%] of 84 patients allocated cidofovir *vs* 39 [46%] of 84 patients assigned imiquimod; p=0·211). By per-protocol analysis, the proportion of patients with pruritus grade 2 or higher fell significantly between baseline and the post-treatment assessment in both treatment groups (cidofovir, 14 [19%] of 72 patients *vs* five [7%] of 72 patients; p=0·013; imiquimod, 15 [22%] of 69 patients *vs* five [7%] of 69 patients; p=0·008). No difference in the proportion of patients with grade 2 or higher vulval pain was seen between baseline and the post-treatment assessment (cidofovir, nine [13%] of 72 patients *vs* three [4%] of 72 patients; p=0·109; imiquimod, four [6%] of 69 patients *vs* four [6%] of 69 patients; p=1·000). No deaths were reported.

By intention-to-treat analysis, at the 6-week post-treatment assessment visit, 41 (46%, 90% CI 37·0–55·3) of 89 patients allocated cidofovir had a complete response compared with 42 (46%, 37·2–55·3) of 91 patients allocated imiquimod. This result was replicated by per-protocol analysis (table 5). New lesions arose during the 24-week treatment stage in 19 (21%) of 87 patients assigned cidofovir and 11 (12%) of 91 patients allocated imiquimod; however, we do not know whether any of these new lesions arose within the treatment area or not.

Table 6 presents the per-protocol analysis of predictors of treatment response. The presence of high-risk HPV DNA or HPV type 16 DNA did not predict response, nor did disease focality, recurrence of disease, or smoking status. In the per-protocol population, two patients allocated cidofovir had previously been immunocompromised: one responded to treatment and one did not. Six individuals assigned imiquimod had also been immunocompromised, of whom two responded and four did not.

23 patients crossed over to the alternative treatment (figure), of whom 16 had a post-treatment biopsy sample taken. Three (43%) of seven patients who received cidofovir after crossover had a complete response compared with four (44%) of nine individuals who were given imiquimod after crossover.

Follow-up data for the 83 patients who had a complete response is not yet mature (median follow-up 24·9 months [IQR 13·5–31·2]), but findings currently suggest that complete response (ie, absence of vulval intraepithelial neoplasia) is maintained at 12 months. At the time of this analysis, 20 (87%) of 23 patients assigned cidofovir still had a complete response at 12-month follow-up, as did 25 (78%) of 32 individuals allocated imiquimod.

Discussion

The findings of the RT³VIN trial show that both imiquimod and cidofovir are safe, active, and feasible for treatment of vulval intraepithelial neoplasia grade 3. Both treatments met the predefined criteria for efficacy, with complete responses in more than 45% of patients recorded by intention to treat. Adverse events were similar between treatments, although events of grade 2 or higher were more frequent in patients assigned imiquimod compared with those allocated cidofovir. Biomarkers related to demographics, disease characteristics, or viral status were not predictive of treatment response. As far as we know, our study is the largest to date to assess topical treatments for vulval intraepithelial neoplasia, with patients recruited from both teaching and general hospitals, supporting the relevance of these findings to general clinical practice (panel). Although cidofovir had slightly fewer reported toxic effects, imiquimod is available for use and is an effective alternative to surgery that can be offered to women with vulval intraepithelial neoplasia after exclusion of occult invasive disease.

The International Society for the Study of Vulval Disease uses morphological criteria to classify vulval intraepithelial neoplasia into usual-type (HPV-related) or differentiated (non-HPV-related). This classification was debated[3] and, initially, not applied widely in the UK. In the RT³VIN trial, we wanted to use a definition that was familiar to pathologists in the UK, provided a clear threshold for entry into the study, and minimised intraobserver variability. Therefore, we used the previous classification of vulval intraepithelial neoplasia grades 1–3, and we chose grade 3 disease for our entry criteria. This system does not attribute disease origin by histological appearances; instead, HPV testing was undertaken on all samples to establish the cause of disease. An alternative approach would have been to record clinical evidence of vulval dermatoses such as lichen sclerosus. The primary endpoint of the RT³VIN trial was a complete response—ie, no evidence of any vulval intraepithelial neoplasia on histological analysis, which also minimised intraobserver variability. We considered central histological review, but that would have caused substantial logistical difficulties in an already challenging trial.

Table 3 shows that the primary reason for exclusion from the per-protocol analysis was withdrawal from treatment because of intolerance or by patient's choice. All exclusions were regarded as treatment failures in the intention-to-treat analysis; therefore, our results represent a worst-case scenario. Data for adherence to treatment tailed off after the first 6 weeks of the study; however, before this timepoint, data collection was good (around 86%) and response to treatment did not seem to be related to adherence. Although the RT³VIN trial was randomised, the Fleming's design is not aimed primarily at direct comparison between arms; thus, the study was not powered to detect a difference in efficacy between cidofovir and imiquimod. More adverse events were noted with imiquimod than with cidofovir, but most were grade 2. Although this comparison was not a primary endpoint, the difference was significant. No validated method for measurement of quality of life in patients with vulval intraepithelial neoplasia was available at the start of the RT³VIN trial; therefore, we did not gather these data, other than for symptoms and adverse events. Such a method is now in development,[25] and future trials should include quality-of-life data, along with a health-economic analysis.

In view of the complexity of setting up trials in uncommon disorders such as vulval intraepithelial neoplasia, future studies should allow new topical treatments to be assessed alongside existing ones in phase 3 assessments. Moreover, drug concentrations and dosing regimens for cidofovir and imiquimod could be optimised further. In our trial, cidofovir seemed to be better tolerated than imiquimod; therefore, in future studies, we might be able to augment complete responses

Panel: Research in context

Systematic review

Current options for the management of vulval intraepithelial neoplasia are conservative, surgery, or an unlicensed alternative. As most women with vulval intraepithelial neoplasia have symptoms, conservative management is typically not appropriate. Many women wish to avoid surgery, which has substantial—generally longstanding—morbidity. A Cochrane systematic review of medical treatments for vulval intraepithelial neoplasia was done in 2011, which comprised three randomised controlled trials of imiquimod treatment for vulval intraepithelial neoplasia, but no published studies were included for cidofovir or other topical treatments.[18] Findings of the three randomised trials showed a combined complete response in 36 (58%) of 62 patients assigned imiquimod compared with none of 42 allocated a placebo.[19–21] Also, two retrospective studies including outcomes after treatment with imiquimod have been published, in which complete responses were reported in 47 (76%) of 62 patients and ten (31%) of 32 patients.[22,23] To date, no further randomised trials of topical treatment for vulval intraepithelial neoplasia have been completed. In addition to our cidofovir pilot study, in which a complete response was seen in four (40%) of ten women completing treatment,[10] one further trial of topical treatment with cidofovir for patients with vulval intraepithelial neoplasia has been completed—a phase 2a study in men and women with high-grade anal intraepithelial neoplasia and vulval intraepithelial neoplasia, all of whom were HIV-positive.[24] Treatment was for 5 days, with a 9-day treatment gap, repeated for a total of six cycles. By intention-to-treat, a complete response was noted in five (15%) of 33 patients and a partial response was recorded in 12 (36%). An update of the Cochrane review awaits publication of data from the RT³VIN trial.

Interpretation

Allowing for differences in treatment regimens, the results of previous studies accord with those of the RT³VIN trial. Our study provides—to the best of our knowledge—the largest evidence-base so far from which an alternative to surgery can be offered: either topical imiquimod or cidofovir. The RT³VIN trial was not powered to establish whether cidofovir or imiquimod was the most effective treatment. However, complete responses in more than 45% of patients accord with available published work. Although more adverse events were noted with imiquimod, most were grade 2, and imiquimod is already licensed for use on the vulva, is readily available for prescription within hospitals as a topical treatment, and is much cheaper than cidofovir. Therefore, cidofovir could be reserved for patients who do not respond to imiquimod, although the numbers treated in this way within the RT³VIN trial were very small.

by increasing the dose of cidofovir in patients who do not respond initially. Alternatively, imiquimod could be tested at a lower dose to begin with, then raised if no response is recorded. Also, predictive markers for treatment response are needed urgently, because up to now, researchers have been unable to identify patients who are likely to respond to topical treatment. A comprehensive analysis of viral characteristics in the RT³VIN cohort is ongoing and might suggest potential predictive biomarkers. Comparing either cidofovir or imiquimod with excisional surgery will be fraught with difficulty: the outcomes that are the most important to women are not always easy to measure: methods to assess quality of life will emphasise symptoms that could be associated with one treatment or another. Complete regression is much more likely with surgery in the short term, and recurrence might depend on factors other than the treatment. Comparison of topical treatment with laser ablation should also be considered. HPV vaccination is expected to prevent most cases of vulval intraepithelial neoplasia in the future; therefore, obtaining funding for future trials could be challenging. In the meantime, a pragmatic approach will most likely have to be taken, whereby we explain to patients the potential benefits and risks of the available treatment options and take their priorities for treatment into account in this difficult management decision.

Contributors

AT, AF, GG, CNH, SM, and NP designed and developed the study and wrote and reviewed the protocol. AT, CNH, TM, AF, and GG played a part in day-to-day running of the study. NP, SJ, and SH did HPV testing. CNH and PD did statistical analyses. AT, CNH, and GG wrote the report. AT, RN, and AJN reviewed serious adverse events during the study. AT and AJN were principal investigators at centres recruiting more than 10% of patients. All authors have seen and approved the final report.

Declaration of interests

GG has received educational grants from Pfizer for investigator-initiated clinical trials and has acted as a statistical consultant to Pfizer. All other authors declare no competing interests.

Acknowledgments

The RT³VIN trial was funded by Cancer Research UK (CRUK/06/024) and CRUK core funding to the Wales Clinical Trial Unit (WCTU) at Cardiff University. The trial was run independently at WCTU. Gilead Sciences supported the study by provision of cidofovir at a discounted price, which was funded by a central subvention from the Department of Health (England) and the National Institute for Social Care and Health Research (Wales). We thank current and former staff of Cardiff University and Cardiff & Vale NHS Trust for supporting the development and running of this trial; Kim Smith, the patients' representative on the trial management group; and members of the independent data monitoring committee and trial steering committee. Finally, we thank all patients who participated in the trial and the principal investigators (appendix p 3) and their colleagues for recruitment and treatment of patients.

References

1 Judson PL, Habermann EB, Baxter NN, Durham SB, Virnig BA. Trends in the incidence of invasive and in situ vulvar carcinoma. *Obstet Gynecol* 2006; **107:** 1018–22.

2 Baandrup L, Varbo A, Munk C, Johansen C, Frisch M, Kjaer SK. In situ and invasive squamous cell carcinoma of the vulva in Denmark 1978–2007: a nationwide population-based study. *Gynecol Oncol* 2011; **122:** 45–49.

3 de Sanjose S, Alemany L, Ordi J, et al. Worldwide human papillomavirus genotype attribution in over 2000 cases of intraepithelial and invasive lesions of the vulva. *Eur J Cancer* 2013; **49:** 3450–61.

4 McCluggage WG. Recent developments in vulvovaginal pathology. *Histopathology* 2009; **54:** 156–73.

5 Shylasree TS, Karanjgaokar V, Tristram A, Wilkes AR, MacLean AB, Flander AN. Contribution of demographic, psychological and disease-related factors to quality of life in women with high-grade vulval intraepithelial neoplasia. *Gynecol Oncol* 2008; **110:** 185–89.

6 van Seters M, van Beurden M, de Craen AJ. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. *Gynecol Oncol* 2005; **97:** 645–51.

7 Likes WM, Stegbauer C, Tillmanns T, Pruett J. Pilot study of sexual function and quality of life after excision for vulvar intraepithelial neoplasia. *J Reprod Med* 2007; **52:** 23–27.

8 Fehr MK, Baumann M, Mueller M, et al. Disease progression and recurrence in women treated for vulvovaginal intraepithelial neoplasia. *J Gynecol Oncol* 2013; **24:** 236–41.

9 Snoeck R, Noel JC, Muller C, De Clercq E, Bossens M. Cidofovir, a new approach for the treatment of cervix intraepithelial neoplasia grade III (CIN III). *J Med Virol* 2000; **60:** 205–09.

10 Tristram A, Fiander A. Clinical responses to cidofovir applied topically to women with high grade vulval intraepithelial neoplasia. *Gynecol Oncol* 2005; **99:** 652–55.

11 Davis G, Wentworth J, Richard J. Self-administered topical imiquimod treatment of vulvar intraepithelial neoplasia: a report of four cases. *J Reprod Med* 2000; **45:** 619–23.

12 Jayne CJ, Kaufman RH. Treatment of vulvar intraepithelial neoplasia 2/3 with imiquimod. *J Reprod Med* 2002; **47:** 395–98.

13 Todd RW, Etherington LJ, Luesley DM. The effects of 5% imiquimod cream on high-grade vulval intraepithelial neoplasia. *Gynecol Oncol* 2002; **85:** 67–70.

14 van Seters M, Fons G, van Beurden M. Imiquimod in the treatment of multifocal vulvar intraepithelial neoplasia 2/3: results of a pilot study. *J Reprod Med* 2002; **47:** 701–05.

15 Marchitelli C, Secco G, Perrotta M, Lugones L, Pesce R, Testa R. Treatment of bowenoid and basaloid vulvar intraepithelial neoplasia 2/3 with irniquimod 5% cream. *J Reprod Med* 2004; **49:** 876–82.

16 Wendling J, Saiag P, Berville-Levy S, Bourgault-Villada I, Clerici T, Moyal-Barracco M. Treatment of undifferentiated vulvar intraepithelial neoplasia with 5% imiquimod cream: a prospective study of 12 cases. *Arch Dermatol* 2004; **140:** 1220–24.

17 Collins SI, Constandinou-Williams C, Wen K, et al. Disruption of the *E2* gene is a common and early event in the natural history of cervical human papillomavirus infection: a longitudinal cohort study. *Cancer Res* 2009; **69:** 3828–32.

18 Lockhart J, Gray NM, Cruickshank ME. The development and evaluation of a questionnaire to assess the impact of vulval intraepithelial neoplasia: a questionnaire study. *BJOG* 2013; **120:** 1133–43.

19 Pepas L, Kaushik S, Bryant A, Nordin A, Dickinson HO. Medical interventions for high grade vulval intraepithelial neoplasia. *Cochrane Database Syst Rev* 2011; **4:** CD007924.

20 Sterling JC, Smith, NA, Loo WJ, et al. Randomized, double blind, placebo controlled trial for treatment of high grade vulval intraepithelial neoplasia with imiquimod. *J Eur Acad Dermatol Venereol* 2005; **19** (suppl s2)**:** 1–58 (abstr FC06.1).

21 Mathiesen O, Buus SK, Cramers M. Topical irniquimod can reverse vulvar intraepithelial neoplasia: a randornised, double-blinded study. *Gynecol Oncol* 2007; **107:** 219–22.

22 van Seters M, van Beurden M, ten Kate FJW, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod. *N Engl J Med* 2008; **358:** 1465–73.

23 Westermann C, Fischer A, Clad A. Treatment of vulvar intraepithelial neoplasia with topical 5% imiquimod cream. *Int J Gynecol Obstet* 2013; **120:** 266–70.

24 Frega A, Sesti F, Sopracordevole F, et al. Imiquimod 5% cream versus cold knife excision for treatment of VIN 2/3: a five-year follow-up. *Eur Rev Med Pharmacol Sci* 2013; **17:** 936–40.

25 Stier EA, Goldstone SE, Einstein MH, et al. Safety and efficacy of topical cidofovir to treat high-grade perianal and vulvar intraepithelial neoplasia in HIV-positive men and women. *AIDS* 2013; **27:** 545–51.