Monitoring of Cervical lesions at Colposcopy using a novel non-invasive technique for monitoring of CIN2 lesions (a pilot/ feasibility study)

Ethics Ref: IRAS 272534

CUH R&D: A095370

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Sponsor:	Cambridge University Hospitals NHS foundation Trust and University of Cambridge

AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	**2**	**2nd April 2020**	**Richard Skells**	**Additional clarification on approach and consent in response to ethics committee comments**
2	**3**	**26/03/2021**	**Dr. Shiraz & Tulay Gulsen**	**Additional Inclusion Criteria and changes related to use of different colposcopists at subsequent visits.**
3	**4**	**11/01/2022**	**Tulay Gulsen**	**Additional clarification on recruitment end date and trial end date. Extension of trial until end of December 2022.**

List details of all protocol amendments here whenever a new version of the protocol is produced.

1. SYNOPSIS

Study Title	Monitoring of Cervical lesions at Colposcopy using a novel non-invasive technique for monitoring of CIN2 lesions (a pilot/ feasibility study)
Internal ref. no.	R&D
Study Design	The is a single-centre, non-randomised, cohort interventional & lab based study
Study Participants	Women with abnormal cytology attending the colposcopy clinic that have single quadrant disease that is histologically CIN2. The women will be identified from the larger Cervical Cell Lift study (17/SC/0203) or from the pre-existing standard of care conservative management pathway.
Planned Sample Size	10
Follow-up duration	From 6 months to 1 year (3 monthly intervals)
Planned Study Period	12 to 18 months
Primary Objective	To non-invasively monitor women with CIN2 using a novel non-invasive approach in order to identify biomarker patterns that are consistent with lesion regression/ progression
Secondary Objectives	None
Primary Endpoint	As this is a feasibility study, the outcome is considered to be the ability to identify biomarker patterns consistent with lesion regression/ progression
Secondary Endpoints	-
Intervention (s)	• If enrolled to the study after CIN2 diagnosis: 1) Primary visit for Colposcopy – CIN2 identified,acetowhite photograph and Patch taken followed by biopsy 2) 3-month visit for Colposcopy – Patch, acetowhite photograph taken and colposcopy done to ensure no change in lesion and comparison with previous photograph of acetowhite change 3) 6-month visit for Colposcopy - Patch, acetowhite photograph taken and colposcopy done to ensure no change in lesion and comparison with previous photograph of acetowhite change 4) 9-month visit for Colposcopy - Patch, acetowhite photograph taken and colposcopy done to ensure no change in lesion and comparison with previous photograph of acetowhite change 5) 1-year visit for Colposcopy – Patch, acetowhite photograph taken and either further biopsy or excisional treatment as per colposcopist impression • If enrolled to the study from the pre-existing clinical pathway, participants will start the study at either the 3 month visit (-/+ 2 week) or the 6 month routine clinical visit (-/+ 2 week) following CIN2 diagnosis.

BACKGROUND AND RATIONALE

Outline the scientific justification for the research. Give an outline of the background to the study, with references to literature and other relevant research.

Give an outline of the main research questions. Give a brief outline of the intervention (if applicable) and summary of findings from previous studies (if relevant) that potentially have clinical significance.

Provide summary of the known and potential risks and benefits of any of the study procedures (where applicable)

Describe the population to be studied.

The purpose of this study is to non-invasively monitor women with histologically identified CIN2 lesions. CIN2 lesions are pre-cancer lesions that are caused by HPV. These lesions can in a small subset of patients progress onto CIN3 lesions and eventually cervical cancer. The duration of this progression is usually over 1 year (for CIN3 and longer for cervical cancer) and indeed the majority of these lesions would actually regress (especially in <30 year olds) due to immune control and HPV clearance(1–5). This understanding of CIN2 progression has led to changes in the management of CIN2 lesions, where in the past all such patients would be offered excisional treatment, we have now moved towards adopting a watch and wait policy (with usually 6 monthly colposcopy follow-ups)(6).

Our current ongoing study (17/SC/0203 - Collection of Cervical Cells at Colposcopy using a novel technique for analysis of high risk vs. low risk lesions) has demonstrated that using our non-invasive approach we can identify high-grade (CIN2+) and low-grade (CIN1/ negative) lesions using a biomarker panel. This approach has yielded numerous advantages and in particular the ability to identify lesions in their entirety together with their location on the cervix (Fig 1). This approach is now being validated on a larger cohort of patients and is being assessed to become the test of choice for HPV triage. The biomarkers we have settled on are p16/MCM (markers of CIN2+) and E4 (marker of CIN1). These markers have been independently verified by our group and others as being sensitive markers to clinically relevant disease and indeed this panel of markers, by improving the objectiveness of diagnosis, improves the sensitivity of subjective approaches such as histology/ cytology to CIN2+ lesions(7).

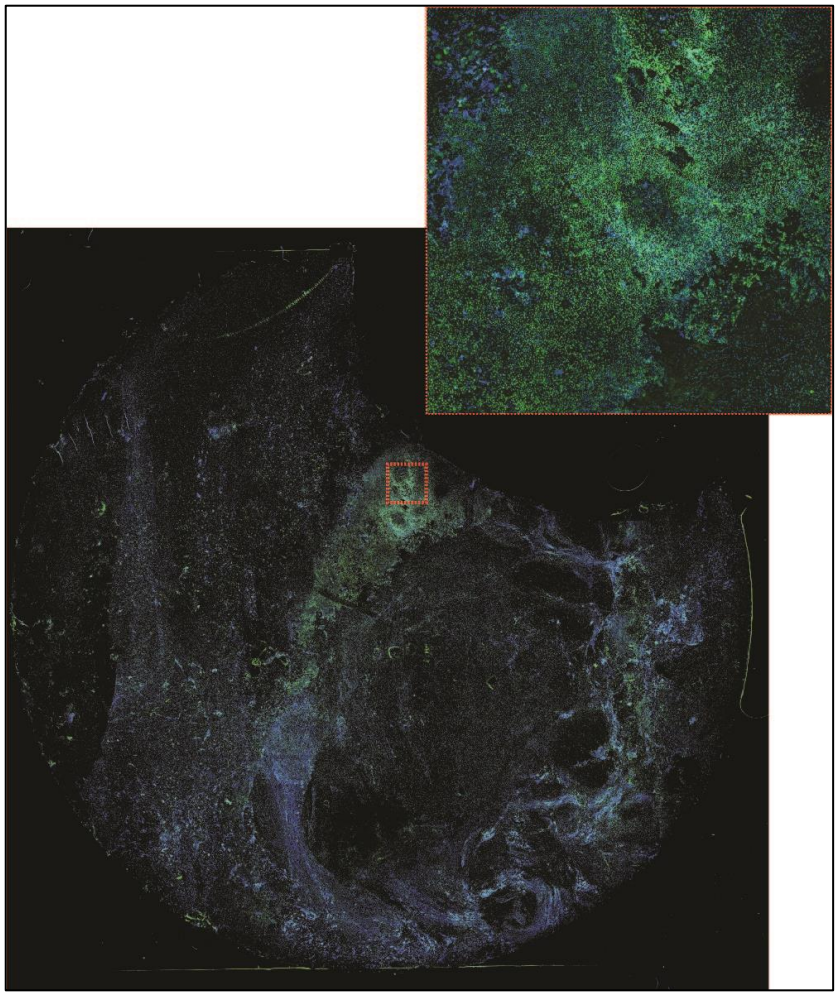


Fig 1. Patch stained with MCM demonstrating a CIN3 lesion in its entirety. Inset demonstrates lesion magnified

Our current approach involves the use of a disk of nitrocellulose to lift the cells from the surface of the cervix. Prior to the disc being applied a photograph of the cervix is taken and once the disc is removed (application time of <15 seconds) a photograph of the cervix treated with acetic acid is taken (as per routine colposcopy practice). The disc is then probed in the lab with our marker panel and then analysed for lesions. In our pilot study of 50 patients we have demonstrated a sensitivity of 88% to CIN2+ lesions with a corresponding positive predictive value of 84% (in this enriched population). These results are better than cytology triage of HPV positive women and in terms of detecting clinically relevant disease superior p16 as well (Fig 2). We are now expanding this approach to a larger cohort over multiple sites.

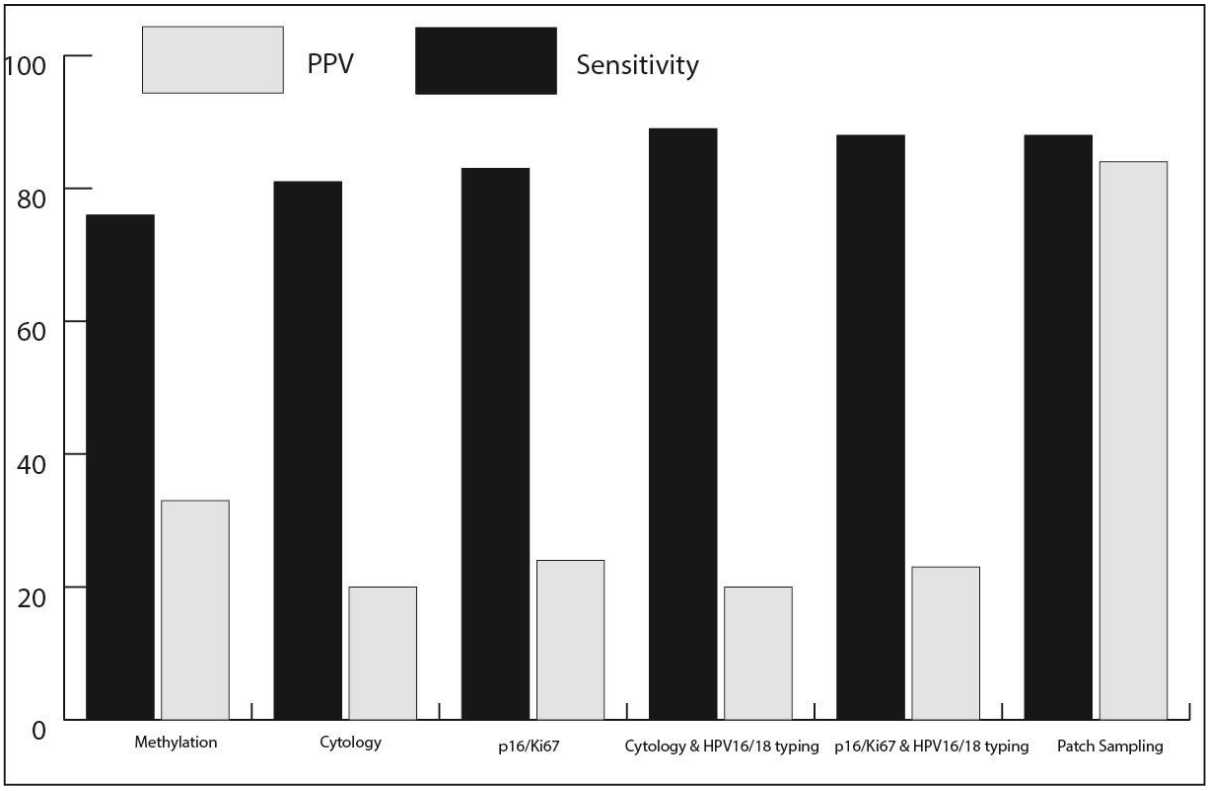


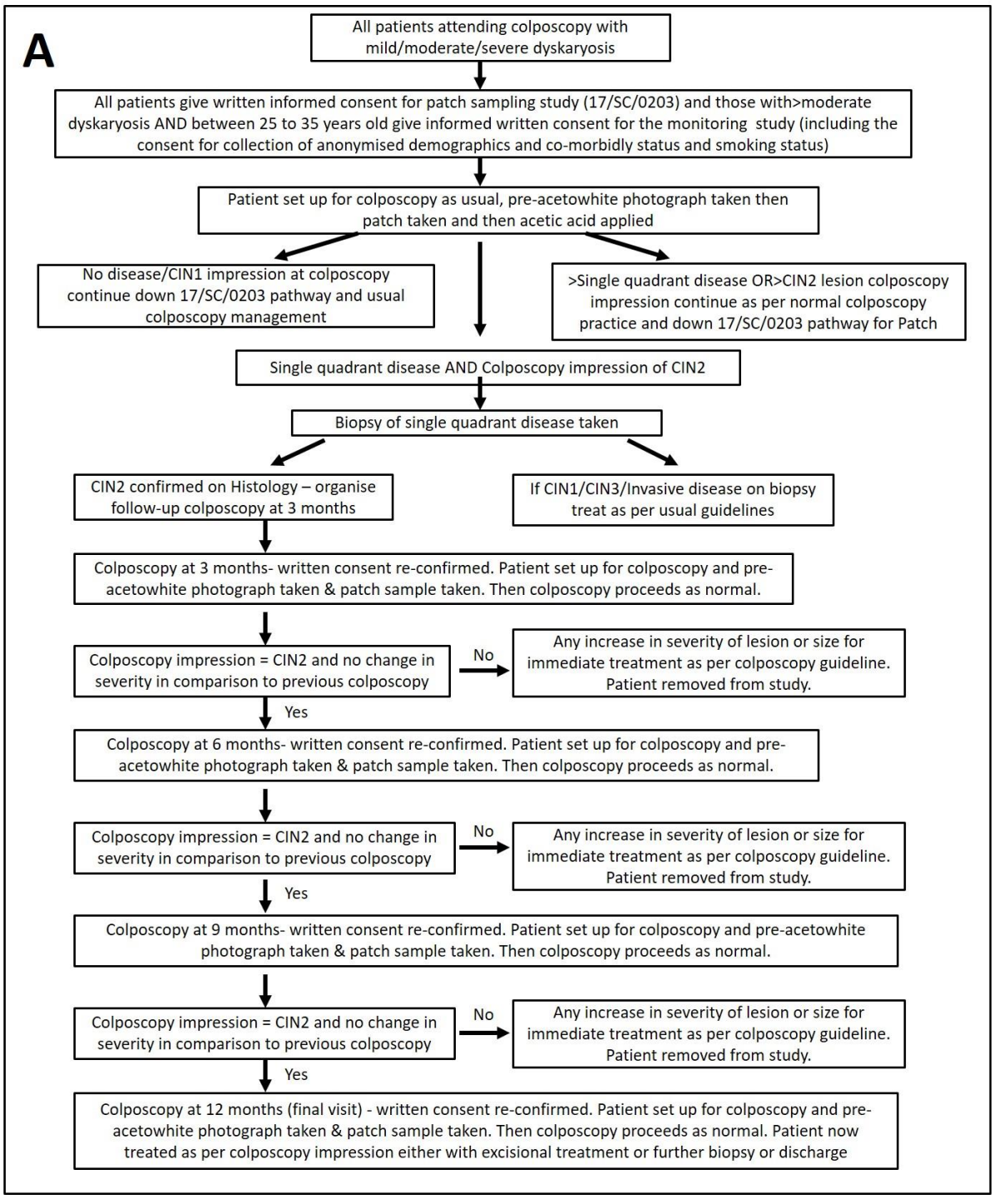
Fig 2. Comparison of Patch sampling against current triage methodologies

Concurrent to this we would now like to use this approach to monitor disease in a subset of patients (n=10) who have CIN2 lesions on histology. The use of a non-invasive sampling process enables diagnosis of patients without the need for a biopsy, the ability to monitor patients in the community and finally, due to the low positive-predictive value (14%) of HPV screening, the ability to triage patients with confidence in the community thus improving the overall patient diagnostic pathway. Moreover, the treatment for CIN2 lesions historically has been excisional in nature (Loop excisions), which we now know increases the risk of pre-term labour. This is significant as the majority of women requiring treatment are of child-bearing age, thus the ability to offer these women a non-invasive molecular monitoring method, in an era of personalized treatment, is warranted.

The study will involve monitoring patients with our non-invasive technique in conjunction with colposcopy (Fig 3) to assess the feasibility of this method. The main risk to any patient is the risk of progression to CIN3 and worse cancer. However, as the majority of CIN2 patients across the UK are now offered conservative management and as many recent papers have demonstrated lesion regression in >50% of CIN2 cases this risk (over the duration of the study) is extremely low(1–6). Moreover, in order to further minimize this risk, we will follow-up these patients more intensively than the norm (usually conservative management of CIN2 involves 6-month follow-up colposcopy) with 3 monthly colposcopy visits for up to 1 year with the same named colposcopist (to reduce any inter-observer variability) where possible. If a different colposcopist is used, we will aim to mitigate

any increased variability by using and comparing colposcopic photographs taken during each colposcopy visit. This will allow us to ensure that variations in diagnosis are kept to a minimum.

Finally, as we are using biomarkers to identify lesions on the patch, any marker pattern that is suggestive of higher-grade disease will result in the patient having a colposcopic visit together with adequate counselling and biopsies/ treatment as required. We feel that this approach enables us to minimize the already low risk of progression even further thus allowing women to give informed consent in an ethical manner. There are no operceivable risks involved.



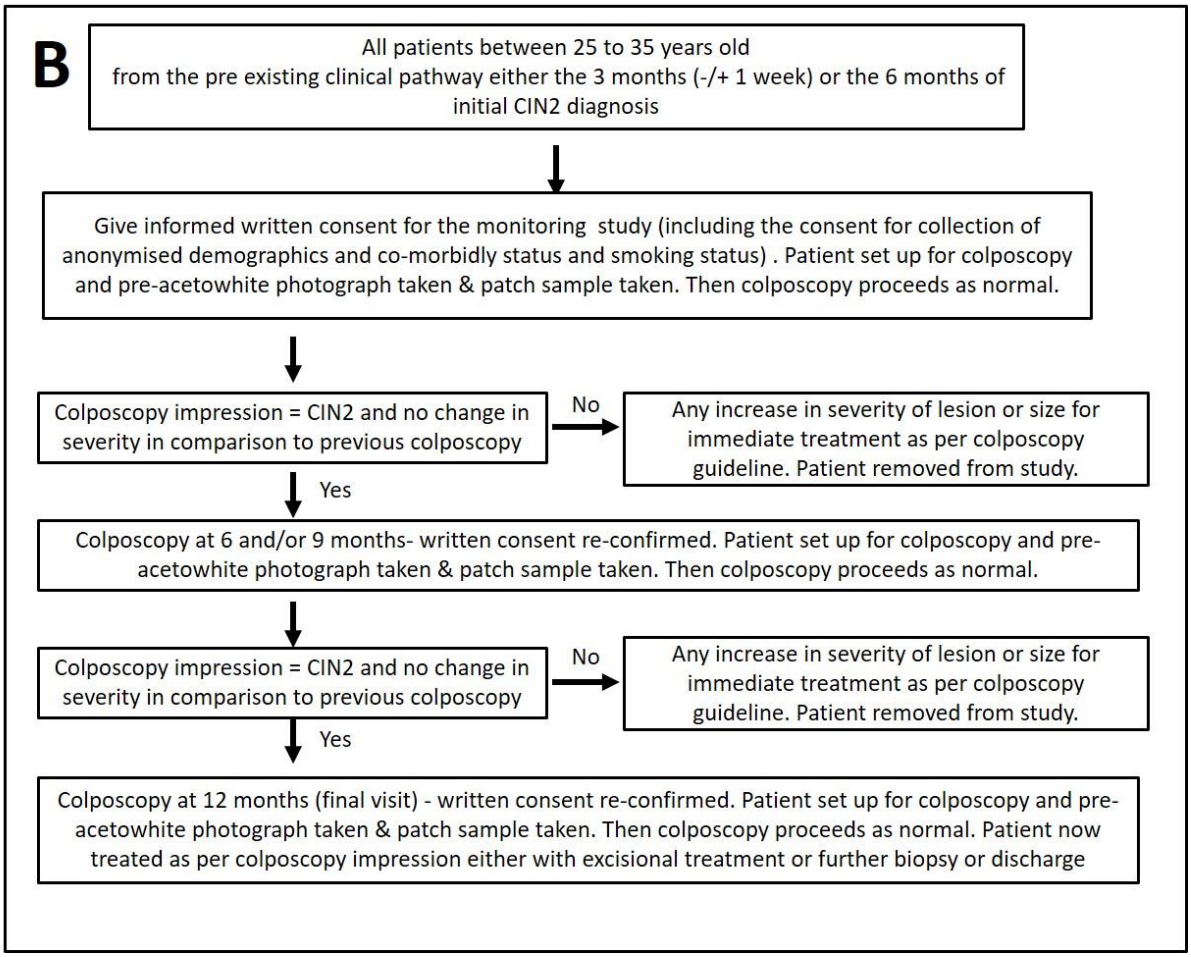


Fig 3. Study scheme demonstrating entire patient pathway including patient withdrawal points. Colposcopic photographs will be taken during each colposcopy visit and all histology will be reviewed/compared by histopathologists. A) Pathway for patients who recruited after diagnosis with CIN2, B)) Pathway for patients who recruited from the pre-existing clinical pathway either at 3 month visit (-/+ 2 week) or at the 6 month routine clinical visit (-/+ 2 week) .

2. OBJECTIVES

There is usually only one primary objective, the rest are secondary objectives.

The wording of the objectives should be clear, unambiguous and as specific as possible.

3.1 Primary Objective

The aim of the project is to assess the feasibility of this non-invasive sampling method to monitor CIN2 lesions in order to identify biomarker patterns that are consistent with lesion progression/ regression.

3.2 Secondary Objectives: None

3. STUDY DESIGN

4.1 Summary of Study Design

This is a single-centre, non-randomised, cohort interventional and lab-based study

Duration: 12 to 18 months

4.2 Primary and Secondary Endpoints/Outcome Measures

As this is a feasibility study, the primary measure is that of demonstrating initial utility of this approach to non-invasively monitor women with CIN2 lesions and demonstrate biomarker patterns that are consistent with lesion regression or progression.

4.3 Study Participants

4.3.1 Overall Description of Study Participants

Female patients with >moderate dyskaryosis who are between 25 and 35 years of age with histologically confirmed CIN2 in a single quadrant of the cervix

4.3.2 Inclusion Criteria

To enter the study all of the following criteria must be satisfied:

- Participant is willing and able to give informed consent for participation in the study.
- Female, aged between 25 to 35 years
- (1) Diagnosed with CIN2 and have been co-enrolled into the cervical Cell Lifts Study OR
  (2) Diagnosed with CIN2 and have been in a conservative management pathway for up to 6 months

4.3.3 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- Participant is unable to give consent.
- Pregnant
- HIV/Systemic Immuno-suppression
- Previous contraindication or allergy nail varnish/ nitrocellulose

3.4 Study Procedures

1. Written informed consent will be given prior to the patient's initial colposcopy
2. During the colposcopy examination, a digital image of the cervix will be taken according to standard practice.
3. A filter disk of nitrocellulose (of up to 50mm diameter and marked to indicate the 12 O'clock position) will then be gently pressed onto the surface of the cervix for 10 to 15 seconds prior to it being peeled off and removed for storage. The filter with adherent exfoliated cells will then be fixed (in acetone/methanol, ethanol, or 5% formaldehyde) prior to analysis at the level of cytology or immunostaining or DNA/ RNA analysis as part of the research collaboration between the University of Cambridge and Addenbrookes Hospital.
4. The remainder of the colposcopy examination will then follow its standard course, with identification of single quadrant disease and impression of CIN2
5. Biopsy of single quadrant disease (to confirm CIN2)
6. Up to 4 further f/u visits follow the same format with the notable exception of the absence of a further biopsy until the 12-month final visit

4.4.1 **Informed Consent**

Informed consent will be given prior to the patient's first colposcopy visit associated with this study. When they attend the clinic, the research team will discuss the study merits and limitations and written consent will be given prior to the participant seeing the colposcopist. Consent will be re-confirmed

verbally at each subsequent follow-up visit (and documented in the patient's notes). Concurrent to this study all patients would also be approached for informed written consent for the larger patch sampling study as currently occurs.

4.4.2 Sample Analysis

For this feasibility study, we envisage that 10 patients will be adequate.

These will be triple stained with a pan E4 antibody to identify areas of virus infection (LSIL), along with p16/MCM to identify cells that are abnormally progressing through the cell cycle (HSIL). Images of the stained cells will be captured using a digital imaging system prior to H+E staining or staining with the Pap stain. The immunostaining and histology images will be compared against the images taken during the colposcopy examination and with the results of the biopsy analysis. For all patients, we will also request sections from the biopsy material in order to better understand how biomarker expression at the epithelial surface (in the cell-lifts) relates to expression of the same biomarkers in the underlying disease and Patch approach.

4.5 Definition of End of Study

The recruitment of new participants will stop when 10 evaluable patients have been recruited to the study or by the end of 2022, whichever is sooner. Evaluability is determined by having at least 3 full datasets (3 different time points). The study will end when the last patient has completed her last follow-up visit. Any participants recruited after the 10th evaluable participant who are still active in the study will be followed -up and included in the final analysis. The total number of participants recruited to the study may therefore be more than 10. All data capture will stop at the end of 2022, regardless of whether the full set of 10 evaluable participants has been accrued.

4. INTERVENTIONS

1 baseline and up to 4 further colposcopic examinations during the course of the year. This is 1-2 additional follow-up examinations in comparison to the normal 6 monthly follow-up appointments for patients that are currently having conservative management for CIN2

5. STATISITICS

6.1 The Number of Participants

We have identified 10 patients as a suitable number for this study (as it is a pilot). This will enable a robust analysis of the merits of non-invasive sampling and CIN2 progression. There is a risk of patients dropping out/ withdrawing consent during the study time period. Thus, we will only count patients that have completed the full study duration.

6.2 Analysis of Endpoints

Each patient will have their samples probed for evidence of lesions. During the follow-up period we will be observing if these lesions progress or regress with concurrent marker pattern changes i.e. if lesion is of low progression potential, we expect high E4 expression and low p16/MCM expression and vice versa. The final endpoint analysis will look for correlation of these biomarker patterns to the final histology or colposcopy outcome. We expect high E4 expression lesions to regress and vice versa.

6. ETHICS

The main ethical consideration is the intensive follow-up for these patients over the course of one year i.e. up to 4 visits vs. 2. However, we feel this is justified as this work could enable us to better understand marker patterns that correlate with regression/ progression and will also enable us to assess the feasibility of non-invasively sampling these patients which may lead to a better patient

experience in the future. A further consideration is that of only focusing of the CIN2 patients rather than the CIN1 patients. This stems from the current understanding that CIN1 patients would have a very low risk of progression and indeed do not require treatment and thus this patient group is no exposed to the consequences of excisional treatment. In contrast the CIN2 patients would be offered treatment or conservative management and therefore this work has the potential to better inform us on which patients to offer conservative or excisional treatment.

7.1 Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant's ID number on the CRF and any password protected electronic database that will be stored on secure NHS computers. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

7.2 Other Ethical Considerations

Only participants able to understand the study will be involved

7. DATA HANDLING AND RECORD KEEPING

All study data will be entered on a password protected MS Excel spreadsheet by the research nurse. This database will only be accessible by those directly involved in this study. The participants will be identified by a study specific participants number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file.

8. FINANCING AND INSURANCE

Finance:

There will be no cost incurred by Addenbrookes Hospital. The additional costs for up to 2 additional colposcopy visits (3 & 6 months) will be borne by the research team through their BSCCP Jordan-Singer award. The cost of staining and handling of the templates will be borne by the programme grant from Professor John Doorbar (University of Cambridge). Incidental expenses required at Addenbrookes will be borne by ACT (Gynaecological Cancer Fund)

Insurance: Cambridge University Hospitals NHS Foundation Trust

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