Protocol

1. Project title:

LOPAC Trial: A randomised controlled trial to study the effectiveness of **L**aser ablation versus **O**bservation to **P**revent **A**nal **C**ancer in men with human immunodeficiency virus who have high-grade anal intraepithelial neoplasia

2. Planned investigation:

2.1. Research objectives

The incidence of anal cancer is rising in the general population, but the rise in incidence is alarmingly high in people with HIV, particularly in men who have sex with men (MSM) (70-100 per 100,000 person years). High-grade AIN (AIN 2/3), the likely precursor of anal cancer is widely prevalent amongst HIV-positive MSM patients and active treatment of AIN2/3 may prevent progression to anal cancer. The experimental arm of the study will treat biopsy proven AIN 2/3 lesions in HIV-positive MSM patients with laser ablation in an out-patient setting. In the control arm patients will undergo six-monthly observation using high resolution anoscopy but will not receive any active treatment for AIN 2/3 lesions.

Main Objective: To determine the long term (4/5 years) effectiveness of laser ablative treatment of AIN 2/3 in high-risk group of HIV-positive MSM in preventing anal cancer when compared to 6-monthly active observation.

Secondary Objectives: The secondary outcomes of the trial will include

- clearance rate of AIN 2/3
- clinical response (persistence, progression and regression) of AIN 2/3
- number of treatment sessions required to clear AIN 2/3 lesions
- the impact of HIV (CD4 count, viral load, duration of HAART, CD4 nadir and duration of HIV-positive status) on clinical response to treatment, its effectiveness, failure or progression to anal cancer.
- Determination of (a) whether laser ablative treatment acts against high-risk HPV infection and (b) whether in the untreated group type-specific HPV persistence is a 5-year prognostic marker of anal cancer development
- 5-year effect of laser ablative therapy on incidence of anal cancer, and the difference between those in the treated and the observation group of the study
- Cost-effectiveness of laser ablative treatment of AIN2/3 in preventing anal cancer in HIV-positive MSM when compared to observation alone.
- Outcomes of laser-based treatment of AIN2/3 on patients' quality of life (i.e. sexual behaviour, social relationships and anxiety levels), compared to QoL with observation.

2.2. Existing research

Significance of the problem: Anal cancer is a growing problem in the UK and elsewhere. The incidence may be low in the general population (2.0 women and 1.2 men per 100,000 persons of new cancers in 2009) [1] but is rising. In the UK, the number of newly diagnosed cases of anal cancer has increased from 385 women and 211 men in 2001, to 542 and 311 respectively in 2009 [1, 2]. Using a low estimate of completeness, 30% of anal cancer cases may not be registered by the cancer registries [3]. The incidence of anal cancer, however, is more alarming in people with HIV particularly in men who have sex with men (MSM). In HIV-negative MSM, the incidence of anal cancer is 35 per 100,000 person years [4] and is comparable to cervical cancer incidence in

women prior to the introduction of the cervical screening programme in 1988. The incidence in HIV-positive MSM is much higher at 70-100 per 100,000 person years [5]. It is assumed that 3% of the male population are MSM [6], and the Health Protection Agency (HPA) estimates that 35,050 MSM were living with HIV in the UK in 2009 [7]. A meta-analysis showed that the standardised incidence ratio for anal cancer in people with HIV is 28.75 (95% CI 21.6 - 38.3)[8], and some 86,500 people are estimated to be HIV positive in the UK [9]. The risk of anal cancer increases sharply with age [3], particularly for HIV - positive people as a consequence of increased longevity due to Highly Active Antiretroviral Therapy (HAART). Among HIV-positive men in the UK, anal cancer rates have increased from 35 per 100,000 men before the introduction of HAART to 92 per 100,000 men after 1996, and is still on the increase [10,11]. Hence, anal cancer has become a significant cause of morbidity and mortality among HIV-positive people. The economic burden of anal cancer, in terms of the direct medical costs (i.e.- hospitalisation and outpatient costs) and indirect costs (daily allowances charged by taxpayers) is considerable. The management costs of anal cancer is similar to that of cervical cancer, as is the case demonstrated in France, with 38 and 44 million Euros per year, respectively [12]. An effective outpatient based treatment to AIN would help.

Problems caused by high-grade AIN (AIN 2/3): The precursor to anal cancer is thought to be high-grade anal intraepithelial neoplasia (AIN). In HIV centres, over 50% of HIV-positive MSM and 20-30% of HIV-infected women will have abnormal anal pap smears (anal cytology) and require referral for specialist care [13]. However, specialist care is not available in many places in the UK. The psycho-social impact of HPV and AIN disease can be serious, ranging from depression, anger and shame, and negatively affects sexual enjoyment and activity [14]. Physical and emotional morbidities associated with progression of disease are significant, and the management of anal cancer is traumatic for patients, as it frequently leads to impairment of sphincter control and loss of sexual function. Overall survival of anal cancer in the UK (5-year survival) is currently 65% (95% CI 51-78%) [15], but falling to 22% in women and 10% in men in advanced stages of cancer [16].

Treatment of established high-grade AIN: Although several treatment modalities have been tried in the treatment of high-grade anal intraepithelial neoplasia (AIN 2/3), there is no consensus regarding the optimal treatment strategy. Currently, the choice of treatment method is often directed by the extent (low vs. high volume) and location (intra-anal vs. peri-anal) of disease, and local availability of skills and resources. In general, treatment for AIN falls into three broad categories: (a) - topical treatment (i.e. imiquimod [17], cidofovir [18], trichloroacetic acid [19], and 5-fluoroacetic acid [20]), (b) – ablative treatment (i.e. cryotherapy, electrocautery [21], infrared coagulation (IRC) [22, 23], laser [24, 25]; and surgical excision. Topical treatment is best suited for small lesions (< 1cm^2) but requires multiple treatment sessions and is associated with > 50% metachronous disease. With imiquimod, in one study, 26% (5/19 cases) had recurrent disease and 58% (11/19 cases) developed new disease after a mean follow-up period of 24.6 months [26]. In another randomised controlled trial, imiquimod cleared high-grade AIN in 14% (4/28) cases and in a further 28% cases down-graded their lesions after a median follow-up of 33 months [17]. In a retrospective study of 35 HIV-positive men with AIN 2/3 treated with 85% Trichloroacetic acid, 34% achieved disease clearance at 4 -8 months (4 applications at 1 to 2 month intervals) [19]. In a study using 5-fluorouracil for all grades of anal intraepithelial neoplasia (AIN1, AIN2 and AIN3) on 46 patients over 16 weeks, 18 patients (39%) cleared their lesions, and after 6 months, 9 patients had recurrence of disease [20]. Currently, there is expert agreement that ablative treatments are superior to topical methods of treatment for high-grade AIN. In a long term assessment of ablation using infrared coagulation, authors treated small area (extent) of AIN 2/3 disease on an outpatient-basis. Of 44 patients, 40 had recurrence at a mean follow-up of 17 months and no one developed anal carcinoma [27]. However, ablative treatment offered good individual lesion cure rate in both HIV-positive (67%) and HIV-negative individuals (77%).

Prevention of anal cancer: High-grade AIN is believed to be a precursor of anal cancer. Once established, AIN 2/3 rarely regresses. Furthermore, several studies have observed the progression of AIN 2/3 to invasive anal cancer with contemporary series reporting progression rates of 13 to 50% (cf. historical progression rates of ~ 6%) in immunocompromised patients managed expectantly [28]. Active treatment of high-grade AIN is

generally recommended and has the potential to prevent anal cancer and this premise has been investigated previously [29]. In a 10-year experience study, where patients with AIN 2/3 were treated with electrocautery or surgical excision under high resolution anoscopic guidance, 3 out of 246 (1.2%) patients developed anal cancer [21]. Applicants' own data, on 91 patients (56 HIV positive) with AIN 2/3 who were treated with laser ablation and followed up for a mean period of 69.9 months (range 36 – 180), showed no one developed anal cancer [submitted for publication; 30]. Furthermore, during the study period, two patients who declined treatment for AIN went on to develop invasive anal cancer [31, 32]. Currently, there are no completed prospective studies on treatment of high-grade anal intraepithelial neoplasia to prevent anal cancer.

Cost of AIN treatment to prevent anal cancer evidence. Currently, there is no existing data on the cost of treating high-grade AIN to prevent anal cancer. However, the morbidity and mortality associated with invasive anal cancer is significant. Furthermore, the economic burden of anal cancer, in terms of the direct medical costs (i.e.- hospitalisation and outpatient costs) and indirect costs (daily allowances charged by taxpayers) is considerable. The management costs of anal cancer is similar to that of cervical cancer, as is the case demonstrated in France, with 38 and 44 million Euros per year, respectively [12]. Consequently, there is an urgent need for a cost-effective, efficacious out-patient treatment modality to treat high-grade AIN in high risk patients to prevent anal cancer.

Need for multi-centre RCT: There is compelling evidence to suggest that high-grade AIN (AIN 2/3) progresses to invasive anal cancer in an untreated setting, particularly in high-risk population. This risk seems to be highest amongst HIV-positive men who have sex with men. The more contemporary literature estimates the rate of progression of high-grade AIN to invasive anal cancer to be much higher than the historically quoted progression rate of 6% [28]. Retrospective studies involving small number of patients report a reduction in progression rates when high-grade AIN are treated with ablative therapy. Our own series of 91-patients treated with laser ablation showed no patients progressed to develop invasive cancer during a mean follow-up of 69.9 months. In the absence of definitive data on progression rates of AIN 2/3 to anal cancer (natural history studies) and on the effectiveness of treatment of AIN 2/3 in preventing anal cancer development, we urgently need a randomised controlled trial that could answer both these questions. By involving multiple centres, we envisage a more robust estimation of the risks and effectiveness of intervention for the selected population (HIV-positive men who have sex with men).

2.3. Rationale for the proposed study

Anal cancer rates have increased over time, and are still increasing. There are no large scale prospective studies showing that treatment of high-grade anal intraepithelial neoplasia is successful in preventing anal cancer development. Preliminary data (from small prospective studies) suggests that AIN 2/3 may lead to anal cancer. The available retrospective data either show a smaller breakthrough rates of anal cancer with electrocautery [21] or no anal cancer in selected group of patients with low volume (small extent) disease with infrared coagulation [27]. Our own retrospective data over a mean follow-up of 69.9 months in high volume AIN 2/3 disease show no break-through cancers in 91 AIN 2/3 cases, when treated with laser ablation as an outpatient procedure under local anaesthesia [30]. We now need a prospective randomised study to prove that treatment of AIN 2/3 can and do prevent cancer.

Results of retrospective study (The Homerton Hospital & Barts Health NHS Trust): A retrospective analysis of 91 patients with AIN who underwent laser ablative treatment and who had a minimum of 36 months follow-up was undertaken [30]. Eighty-two (90.1%) were men and 56 (61.5%) were HIV-positive. Of the HIV-positive patients, 25 (45%) were positive for 15-years or more at their last follow-up. Thirty-seven cases (68%) had a CD4 nadir of 200 cells/μL or less. The mean follow-up for the group was 69.9 months (total 530 person years). No patient treated in this group developed invasive anal carcinoma. The effectiveness of laser treatment and regular follow-up when further investigated using a Markov model also suggested that treatment may have prevented lesion progression in a proportion of patients. This study from our institution using both descriptive

and model-based analyses concluded that laser ablative treatment is an effective treatment for high-grade AIN and that it may prevent anal cancer. Moreover, patients tolerated the out-patient laser treatment well and there were no major complications.

2.4. Research methods

The proposed study is a UK based multi-centre (n ~ 5) two-armed randomised controlled trial involving a total of 660 patients, randomised to receive the intervention (laser ablative treatment) or control (6 monthly observation): 330 in each arm. Eligible patients will be identified from a multi-centre cohort of 12,563 HIV-positive men who have sex with men, attending 5 HIV clinics of the participating centres. Published data suggest the prevalence of high-grade anal neoplasia in these populations to be 30 - 35% [33, 34]. All patients will follow a pre-recruitment screening, recruitment, and randomisation, laser ablative treatment in the treatment arm and observation in the observation arm. All patients will undergo six monthly follow-up, up to a maximum of 5 years (some up to 3 years). The primary outcome will be determined at 72 months.

	Screening	Baseline	6 Wk	6 Mo	12 Mo	24 Mo	30 Mo	36 Mo	42 Mo	48 Mo	54 Mo	60 Mo	66 Mo	72 Mo
Eligibility assessment	√													
Patient information sheet	√	√												
Informed consent		√												
Allocation		√												
Demographics + clinical data (CRF 1)		√												
Questionnaire (QoL, EQ-5D (CRF 2))		√		√		√						√		
Clinical examination (DRE, HRA)	√		√	√	√	√	√	√	√	√	√	√	√	√
Anal cytology (Pap smear)			√											√
HPV test			√											√
Biopsy for histopathology[¥]	√													√
Laser ablation (treatment arm only)[#]			√											
Record medication/adverse events				√	√	√	√	√	√	√	√	√	√	√
Record outcome data (CRF 3)				√	√	√	√	√	√	√	√	√	√	√

Table 2: Events at each visit

CRF – case report form; [#]Laser ablation – up to 4 treatment sessions during the whole study period; [¥]Biopsy – Any new/ persistent lesions will be biopsied at each follow-up visits

Study centre eligibility and clinician education: The involved study centres are large HIV centres in London that care for HIV infected populations. We have identified 2 centres for the feasibility study (Barts Health NHS Trust and Mortimer Market Centre, University College London) and 3 further centres (Chelsea & Westminster Hospital, Royal Free Hospital and St Mary's Hospital) will participate in the full study. We will screen from a 4,058 patient cohort for the feasibility study and from a 12,563 patient cohort for the full trial. We intend to train one more clinician in laser ablative technique during the trial, and there are trained clinicians, potentially, in all recruitment sites for high resolution anoscopy (HRA) technique, used for screening patients for case finding. In addition, the trial group in association with British Association for Sexual Health and HIV (BASHH) is planning to run a national education course in high resolution anoscopy and management of anal intraepithelial neoplasia (AIN 2/3) early next year.

Participant recruitment: HIV-positive patients attending five large HIV treatment centres in London (2 centres (Barts Health NHS Trust and Mortimer Market Centre, UCL) for feasibility part of study) with a total patient cohort of 12,563 patients will be screened for potential participants. All patients attending the clinic will be given patient information sheet explaining the study. The lead investigator and a nominated local investigator from each of the 5 participating centres will screen potential patients for the trial. Once eligibility on the basis of defined inclusion and exclusion criteria is determined, subjects will be provided with further explanation of the aims, methods, anticipated benefits and hazards of the study and a patient information sheet will be sent

along with the results of the original screening biopsy. Patients will be allowed ample time to consider their participation in the study (in accord with GCP guidelines). A member of the local team (trained in informed consent) under the guidance of the local investigator or the local investigator will administer the written informed consent at a second visit after answering any outstanding questions. Once informed consent is signed the local investigator or a nominated team member will record base-line data including relevant questionnaires (QoL and EQ-5D).

Randomisation and allocation: Participants will be randomised, at a ratio of 1:1 once the informed consent has been signed. The next available sequential number will be allocated by the study manager, which then forms the unique identifier for the trial patient. This number will identify all the CRF's for the patient in the study.

Data collection and blinding: Hard copies of baseline documentation will be filed under patients' unique identifier in accordance with DPA agreed practice. The files will not include the patient's personal data or their allocation, which will be stored separately together with the patient's unique id. In all locations, any hard copy patient data will be stored in locked, filing cabinets with access restricted to pre-specified essential users.

2.5. Planned Interventions

The study assesses the effectiveness of ablative treatment for high-grade anal intra-epithelial neoplasia (AIN 2/3), using a diode laser, for prevention of anal cancer. After randomisation, those in the intervention arm, will be invited to attend for treatment within 6 weeks of randomisation. All treatment cases will be invited to apply EMLA cream (topical analgesia) prior to examination as an outpatient. A proctoscope is introduced and high-resolution anoscopic examination (HRA) is conducted to identify AIN 2/3 disease with 5% acetic acid applications. Local anaesthetic (3% citanest with octapressin) is injected submucosally to the AIN 2/3 areas. Using a diode laser delivered through a fibreoptic fibre, under HRA guidance, all areas of AIN 2/3 will be ablated. Any external AIN 2/3 will be similarly ablated under HRA guidance. Patient returns home with oral analgesics, topical analgesics, and lactulose and reviewed at their 6 month visit. If the disease is extensive, they may need admission as day case for treatment under general anaesthesia.

Control arm: In the control arm, patients do not receive any treatment. They have six monthly observation using high resolution anoscopic examination (HRA). A disposable plastic proctoscope is introduced and 5% acetic acid applied to all of the anal transformation zone and external perianal skin. Using HRA, all areas are inspected for the presence of AIN 2/3. A photographic documentation of the lesions are made. If a suspicious area of AIN 2/3 is detected, a submucosal injection of a local anaesthetic (3% citanest with octapressin) is given. A punch biopsy using a Tischler or Eppendorff biopsy forceps is completed. Ferric subsulphate is applied for haemostasis and visit is completed.

Treatment arm: In the experimental arm, during laser treatment, patients are examined using a microscope (similar to colposcope) in the lithotomy position. A proctoscope is introduced into the anal canal to visualize the entire transformation zone, including the squamocolumnar junction, and 5% acetic acid is applied on moistened swabs and visualized through the microscope to identify all disease. Local anaesthetic (3% citanest with octapressin) is injected submucosally to areas of high-grade AIN (AIN 2/3). A 980nm wavelength diode laser delivered through a flat tip 1,000-micron fibre optic fibre is applied at constant energy (7 Watts) and in a continuous wave mode to effect ablation. Treatment takes 10 - 90 minutes, depending on the extent of disease. Patients leave the outpatient clinic after treatment and receive oral analgesics, topical anaesthesia and lactulose to help ease discomfort. Pain typically lasts between 2 to 14 days. If anal fissures develop, pain may be present for 4 to 6 weeks, while opening bowels. Any anal fissures will be treated using chemical sphincterotomy (2% Diltiazem cream, 1 application two times daily, perianally for 6-8 weeks). Patients generally resume normal activity in one to 4 weeks' time after treatment.

Planned inclusion/exclusion criteria

Inclusion criteria:

1) HIV-positive men who have sex with men (MSM) over 18 years of age

2) Have CD4 count of over 350; if less, been on highly active antiretroviral treatment for at least 3 months

3) Histologically proven high-grade AIN (AIN 2/3)

Exclusion criteria:

1) Previous laser or other ablative treatment for AIN 2/3 disease (previous topical treatment is not excluded)

2) Any treatment for AIN 2/3 in the previous six months

3) Previous or current diagnosis of anal cancer

2.6. Ethical arrangements

We plan to submit ethics application for ethics approval for the study in November 2014. We plan to start recruitment to the study in February 2015.

The main ethical issue is that half of the study population will not receive treatment for high-grade anal neoplasia (AIN 2/3). However, to-date, there is no proven intervention that prevents the development of anal cancer in cases of AIN 2/3. This study is justified as those in the observation arm will be closely monitored. If cancer developed they will be fast-tracked into care at an earlier stage than currently possible in routine NHS care. Further, all participants will be offered laser ablative treatment, if this intervention proved to be effective in preventing cancer development. The data monitoring committee meets annually to address this issue. If there is sufficient evidence at any time during the trial, then the study will be stopped and those in the observation arm will be prioritised for receiving laser treatment. Many in the field of HIV-care are of the opinion that not doing anything may not be an option, regarding anal cancer in the high-risk populations. Through this research, the entire at-risk population will benefit in the future.

2.7. Risk and anticipated benefits

Success criteria:

1) Retention of 75% of those randomised to the observation arm

2) Completion of 4 / 5 year follow-up for 75% of the primary cohort of the study

Barriers for the trial:

1) Patients in the observation arm may clamour for treatment

2) They may resort to treatment elsewhere, though availability of such treatment is still very limited

3) If patients in the observation arm availed of treatment elsewhere, the study may need to be extended over time and more number of participants needed to establish the benefit of treatment of AIN 2/3 using laser ablation.

2.8. Informing potential trial participants

The study will be advertised in relevant media, such as GAYDAR website, gay magazines, in addition to the planned leaflet campaign in HIV clinics held in London. We will also sign-post them to a website (e.g.:

AINUK) for further information. At screening visit, all potential participants will be provided with a detailed patient information sheet including the details of the trial. This information will be re-sent to the patients along with the letter giving the results of their tests including the biopsy report. Patients will have further opportunity to discuss the study and their participation with senior clinician members of the trial team and the trial co-ordinator.

2.9. Obtaining informed consent

At the screening visit, all patients will be given a detailed patient information sheet that includes the details of the trial and potential to participate in the trial. Once the biopsy results are known from the screening visit, patients will be written to with the test results. A leaflet explaining the study and recruitment details will be sent to the patient (in accord with GCP guidelines) along with the test results. The written informed consent will be sought at a subsequent visit by a senior member of the team who is trained in obtaining consent for clinical trial participation. The patients' GP will be informed by a letter of their patient's inclusion in the study. Copies of the informed consent will be stored in patient's clinical records.

2.10. Proposed time period for retention of documents

Trial documents will be retained for a 10 year period from final publication.

2.11. Proposed sample size

The trial is to be run in three phases: phase I is to pilot recruitment – we aim to recruit 75 patients in the first 6 months; phase II is to pilot follow-up –additionally, by the end of 12 months, we aim to have recruited a total of 165 patients. In phase III we aim to accelerate recruitment by adding more sites if necessary and to have recruited 400 by the end of year 2 and 660 by the end of year 3. The feasibility of these numbers will be shown in phases I and II.

The total of 660 has been chosen so as to provide 80%-90% power with follow-up until 6 years after the first patient is randomised under a number of scenarios consistent with published progression rates in untreated and minimally treated patients with AIN2/3 (about 11% after a median of 5 years) and published results of treated patients. In a 10-year experience study, patients with mostly extensive AIN 2/3 (81.2%) were treated with electrocautery and 3 (1.2%) developed anal cancer, but in our cohort no patient had anal cancer during 5 years (median) follow-up (0%).

The table below shows the power to detect a difference between the randomised arms at the 0.05=level using the two-sided log-rank test under a variety of scenarios after a maximum of 6 years follow-up. Throughout we assume that 10% of patients will be lost to follow-up in year 1, and 5% of those still in the trial will be lost in each subsequent year. These rates represent those who will stop coming to clinic. We anticipate close to 100% passive follow-up for anal cancer though flagging with the English Cancer Registry. We do not explicitly allow for surveillance-only patients receiving treatment, but we believe that the more modest hazard ratios (for intent to treat) would allow for a certain amount of treatment in the surveillance only arm. (Calculations were done in Stata using the command "artsurv").

We consider incidence rates in the surveillance only arm of 1.0%/year, 2.0%/ year and 2.5%/year and treatment effects of 90% (corresponding to a hazard ratio of 0.1), 80%, 65% and 50%. For instance, with 2.0%/year in observation arm and 0.4%/year in treatment arm (hazard ratio 0.2) we have 95% power, whereas with 2.5%/year vs. 0.875%/year (hazard ratio 0.35) we have 87% power, and with 1.0% vs. 0.1%/year (hazard ratio 0.1), we have 85% power. Note that under many of these scenarios, there is adequate power even with only 500 patients recruited.

No. Randomised	Base rate/year	0.1	0.2	0.35	0.5
660					
	1.0%	85.2	72.3	49.7	29.5
	2.0%	98.9	95.0	78.6	51.6
	2.5%	99.7	98.1	86.7	60.7
500					
	1.0%	74.4	60.3	39.7	23.5
	2.0%	95.9	88.1	66.8	41.4
	2.5%	98.5	93.9	76.2	49.3

We have also considered the additional power that would come from extended follow-up if the event rate is lower than expected. With 660 patients randomised, an event rate of 1.5%/year in untreated patients and a hazard ratio of 0.35 associated with treatment, the power after 8 years follow-up would be 80.0%.

2.12. Statistical analysis

The proposed study seeks to measure the long-term (5-6 year) clinical effect and cost-effectiveness of laser ablative therapy of AIN 2/3 on incidence of anal cancer in HIV-positive MSM. Participants will be examined 6-12 monthly for up to 6 years. In addition to active follow-up during the study, there will be further follow-up, passively, for 5 more years upon completion of the study. For this, participants will be asked to consent to flagging in the national cancer registry.

The trial is divided into three phases so that substantial resources are not committed in the unlikely event that we are unable to recruit or follow-up patients. Thus the primary outcome of phase I is the numbers recruited in the first six months, and the primary outcomes of phase II are the compliance with the 6 month follow-up visit and the continued recruitment in the second six months.

PRIMARY OUTCOME MEASURE:

1. Incidence of anal cancer in HIV-positive MSM after treatment with laser, compared to incidence of anal cancer in an observed group [to be analysed at the end of year six using the log-rank test].

SECONDARY OUTCOME MEASURES:

2. Proportion of patients with clearance of AIN 2/3 lesions in the treated group, compared to proportion in the untreated group, and to measure this difference for low- (1-2 quadrants) and high-volume (3-4 quadrants) AIN 2/3 disease [Time frame: 1, 3 and 5 years after randomisation].

3. Number of treatment attempts necessary to clear AIN 2/3 lesions, and the difference between low- and high-volume disease [Time frame: 1, 3 and 5 years after first treatment].

4. Incidence of metachronous (new) lesions and the rate of recurrence of AIN 2/3 disease after clearance [[Time frame: 1, 3 and 5 years after randomisation].

5. Adherence and drop-out rates of participants undergoing this treatment regime, compared to those undergoing observation [Time frame: through 6 years].

6. Correlation of viral loads, CD4 cell count and lesion size with a) clinical response (progression, persistence, regression) of AIN 2/3 and b) treatment effectiveness or failure.

7. Correlation of high-risk HPV types with a) clearance of AIN 2/3 after treatment, and b) progression of disease to anal cancer.

8. Quality of life in HIV-positive MSM with AIN 2/3 undergoing treatment with laser therapy, compared to quality of life with a watch and wait approach (observation).

9. Differences in psychological outcomes (anxiety, depression, sexual function, cancer fear), between the treatment and surveillance arms.

10. Intervention costs of laser ablative therapy in HIV-positive MSM with AIN 2/3 lesions to prevent anal cancer.

11. NHS treatment costs related to anal neoplasia and cancer in both intervention and control arms.

The primary outcome will be assessed using the log-rank test based on an intention to treat analysis of all randomised patients. Assuming passive follow-up is complete; patients will be censored at death or emigration but not otherwise. Analysis of active follow-up will be censored at the last visit of each patient. If some patients in the surveillance only arm receive excisional or ablative treatment for their AIN (prior to the development of cancer) we will also use methods appropriate for the estimation of the treatment effect in randomised controlled trials with non-compliance.

The data will be held in a secure database. A detailed statistical analysis plan will be written and independently reviewed before the statistician is given access to any un-blinded data.

An annual statistical report will be written based on an agreed format for the data monitoring committee. Two versions will be made: one for the independent members of the Data Monitoring Committee and a redacted version for the non-independent members of the committee.

A formal interim analysis has not been planned because the power to observe a significant result with alpha of 0.002 (two-sided) after four years is low even under extremes of what is reasonable in the two arms. Thus for instance, with 2.5%/year in the surveillance arm and 0.25%/year in the treated arm, there would be 69% power. Were one to obtain a p-value of less than 0.002 at year 4, it would be up to the data monitoring committee to make a recommendation regarding early stopping and the offering of treatment to all patients in the surveillance arm.

2.13. Proposed outcome measures

Primary outcome measure is the incidence of anal cancer in the treatment arm versus observation arm.

Secondary outcome measures:

1) Clearance of AIN 2/3 in the treated versus untreated group (observation arm).

2) Evaluation of clinical response (persistence, progression, and regression) of AIN 2/3 in treatment group v observation group.

3) Determination of number of treatment attempts needed to clear AIN 2/3 lesions.

4) To determine the impact of HIV (CD4 counts, viral load, duration of HAART, CD4 nadir and duration of HIV+ status) on clinical response of treatment effectiveness, failure or progression to anal cancer.

5) Clearance or persistence of high-risk HPV types in the treated v observation groups.

6) HPV persistence in relation to incidence of cancer.

7) Ten year effect of laser ablative therapy on incidence of anal cancer, in comparison to the observation group of the study.

9) Cost-effectiveness of laser ablative treatment of AIN 2/3, compared to observation, in preventing anal cancer in HIV-positive MSM.

10) Quality of life outcomes of laser-based treatment of AIN 2/3 (ie- sexual behaviour, social relationships and anxiety levels), compared to Quality of Life in the observation group.

Details of Health economic analysis

The cost-effectiveness of laser therapy to prevent anal cancer will be assessed by two separate methods: 1) an economic evaluation alongside the clinical trial to directly estimate the incremental cost of each case of anal cancer prevented, and 2) use of data on anal neoplasia grading to parameterize a model of AIN progression, regression and clearance as a result of treatment. This will provide a separate estimate of the effect of treatment on anal cancer prevention. The advantage of the second approach is that it allows to validate the first approach, to increase the statistical power of the data (since intermediate endpoints such as AIN will be used in addition to anal cancer), to elucidate the natural history of AIN, and to compare our findings to other natural history studies of AIN. For this approach, we expand an existing model of anal neoplasia recently developed by the applicants [32]. The economic evaluation will be conducted based on assumptions for the NICE reference case [35]. Costs will be assessed from the perspective of the NHS, a lifetime time horizon will be used, and outcomes will be calculated in terms of cost per quality adjusted life year (QALY) gained. Cost per clinical outcome, e.g. anal cancer case prevented will be estimated, and one-way and probabilistic sensitivity analyses will be conducted to show the uncertainty around cost-effectiveness estimates. The cost of clinic interventions will be estimated by observing staff time (clinician, nurse, admin) taken for a sample of study visits, using standard sources to cost staff-time [36]. The cost of hospital care for anal cancer will be estimated by updating an analysis on the cost of cancer treatment conducted at the HPA, using the most recent data from Hospital Episode Statistics and the Office for National Statistics [37].

Data Collection Plan

To measure the impact of treatment on Quality of Life (QoL), patients in both arms are asked to complete questionnaires at baseline and a month after the 6, 24 and 60 month clinic visits. These will include validated measures [38-42], and new items on AIN and anal cancer are assessed with the 'think aloud' technique for clarity and acceptability [43]. The EuroQol EQ-5D is included to enable economic evaluation conforming to the NICE reference case. Patients receiving treatment are asked to report their experience of each treatment episode and post-treatment symptoms using measures from the TOMBOLA trial [44] and from studies of anal microsurgery [45].

2.14. Research governance

Homerton University Foundation Trust Hospitals will act as sponsor for the study. The project will be under the auspices of the Chief Investigator and the Cancer Prevention Trials Unit (CPTU), QMUL. The project will be overseen by a Trial Steering Committee (TSC).

Trial Steering Committee: The TSC will meet at 3 months and either meet or teleconference every 6 months thereafter throughout the lifetime of the project. The role of the TSC is to provide overall supervision for the trial on behalf of the Trial Sponsor and Trial Funder and to ensure that the trial is conducted to the rigorous standards set out in the Medical Research Council's (MRC) Guidelines for Good Clinical Practice. Specifically, the TSC will ensure:

1) that views of users and carers are always taken into consideration;

2) the scientific rigour of the study and adherence to protocol;
3) that project milestones are met;
4) expertise / advice are provided to the Trial Management Group (TMG).

Membership of the TSC will be in accordance with HTA guidance: The TSC will have an independent chair. The other membership will include the CI (Dr Mayura Nathan), Professor Peter Sasieni (CPTU- Director), grant holders, external independent members, patient representatives and public involvement representatives . Representatives of the Trial Sponsor and the Trial Funder will be invited to all TSC meetings.

Trial Management Group (TMG): TMG will be responsible for day to day project delivery in each participating centre. It will meet/teleconference every six-months and include the local lead, and relevant research staff. This group will answer to the TSC.

Data monitoring & ethics committee (DMEC): A DMEC will be appointed to monitor un-blinded comparative data and make recommendations to the TSC. The DMEC will meet together with the TSC for an initial meeting and subsequently two weeks prior to the TSC to enable any findings / recommendations to be fed to the TSC to whom they report. The DMEC will comprise an independent lead, a further appointed statistician and one other independent health / social scientist. A DMEC charter will be adopted, and the project team should provide the DMEC with a comprehensive report, the content of which should be agreed in advance by the Chair of the DMEC and follow guidelines set out in the charter.

3. **Project timetable and milestones**

We plan to start recruitment to the study in February 2015. We will advertise and recruit a trial co-ordinator and advertise the study in the relevant media. We will set up the screening and recruitment clinics at Barts Health NHS Trust and at University College London/ Royal Free hospital. First patient randomisation will start by 2nd February 2015. Recruitment will continue to reach the 1st target of 75 patients by 1st November 2015. The first funding decision point is reached on 2nd November 2015. Recruitment continued till the second decision point on 1st May 2016, to recruit a minimum of 165 patients into the trial. Thereafter, on agreement, recruitment continued, till 1st February 2017, when 400 patients are randomised into the study. After 1st May 2016, trial screening and case detection for the trial extended to 3 other centres where large HIV positive patient cohorts attend, so as to recruit a total of 400 cases into the study. 1st February 2017 will mark the completion of recruitment of primary cohort for the study. We will continue screening and recruitment till 1st February 2018 to recruit a total of 660 participants (a further 260 patients) to the trial. Participants attend every six months since recruitment, and the study ends on 31st January 2021. Statistical analysis, research evaluation, and publication of results will occur over the following three months. The project milestones and Gantt chart of the project are shown below.

		Start date	Finish date
Contract start date		1/10/2014	
Trial set-up			
Trial co-ordinator recruitment		30/6/2014	1/10/2014
Trial document development and IRAS		25/8/2014	3/10/2014

form completion			
Database development and testing		3/10/2014	1/1/2015
Ethics application		6/11/2014	24/12/2014
NHS approval		29/12/2014	30/1/2015
Site agreements		29/12/2014	30/1/2014
Feasibility study			
1st patient recruitment	23/2/2015		
Phase I	0 -9 months (allowing lead-in period)	24/2/2015	23/11/2015
Target for recruitment	75		
Phase II	9 – 15 months	24/11/2015	24/5/2016
Target for recruitment	165 (inclusive of Phase I recruitment)		
Main Trial			
Phase III	15 – 24 months	25/5/2016	23/2/2017
Target for recruitment	400 (inclusive of phase I and II recruitment)		
Phase IV	24 – 36 months	24/2/2017	23/2/2018
Target for recruitment	660 (inclusive of Phase I, II, III recruitment)		
Last patient recruitment	23/2/2018		
Last patient's last visit	25/2/2021		

4. Service users

A patient advisory group has been formed for this trial following consultation meetings held last year, prior to outline proposal. This group consists of patients, voluntary organisations, and interested media representatives. The patient advisory group has formally met up on the 5th July 2012 and will be advising on the trial application. The group is made of Terence Higgins Trust (THT) representatives, Gay Men Fighting Aids (GMFA) representatives, and people who have had experience of HIV and anal neoplasia.

Members will bring to the trial their views of patient priorities and a patient perspective in design and recruitment to the trial. They will bring a balanced view of the current interventions available against the need for scientific verification of their effectiveness. Formal meetings are envisaged to take place, every six to twelve months throughout the study. Furthermore, during the early trial application period, regular e-mail correspondence will occur. Many of the members of the group have actively supported other research projects in the past.

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