Full Title: Discovering the molecular signatures of cancer PROMotion to INform prevENTion (PROMINENT): Smoking Cessation Study

Short Title: PROMINENT Smoking Cessation Study

Protocol version number and date: V5.0, 19th January 2026

IRAS Number:	330336
SPONSORS Number:	NHS002126
FUNDERS Number:	*CGCATF-2021/100007*

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Date:
.....
......./...../.....

Name (please print):
.....

Position:
.....

Chief Investigator:

Date:19/01/2026

Signature: 

Name: (please print): Professor Phil Crosbie

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STUDY SUMMARY

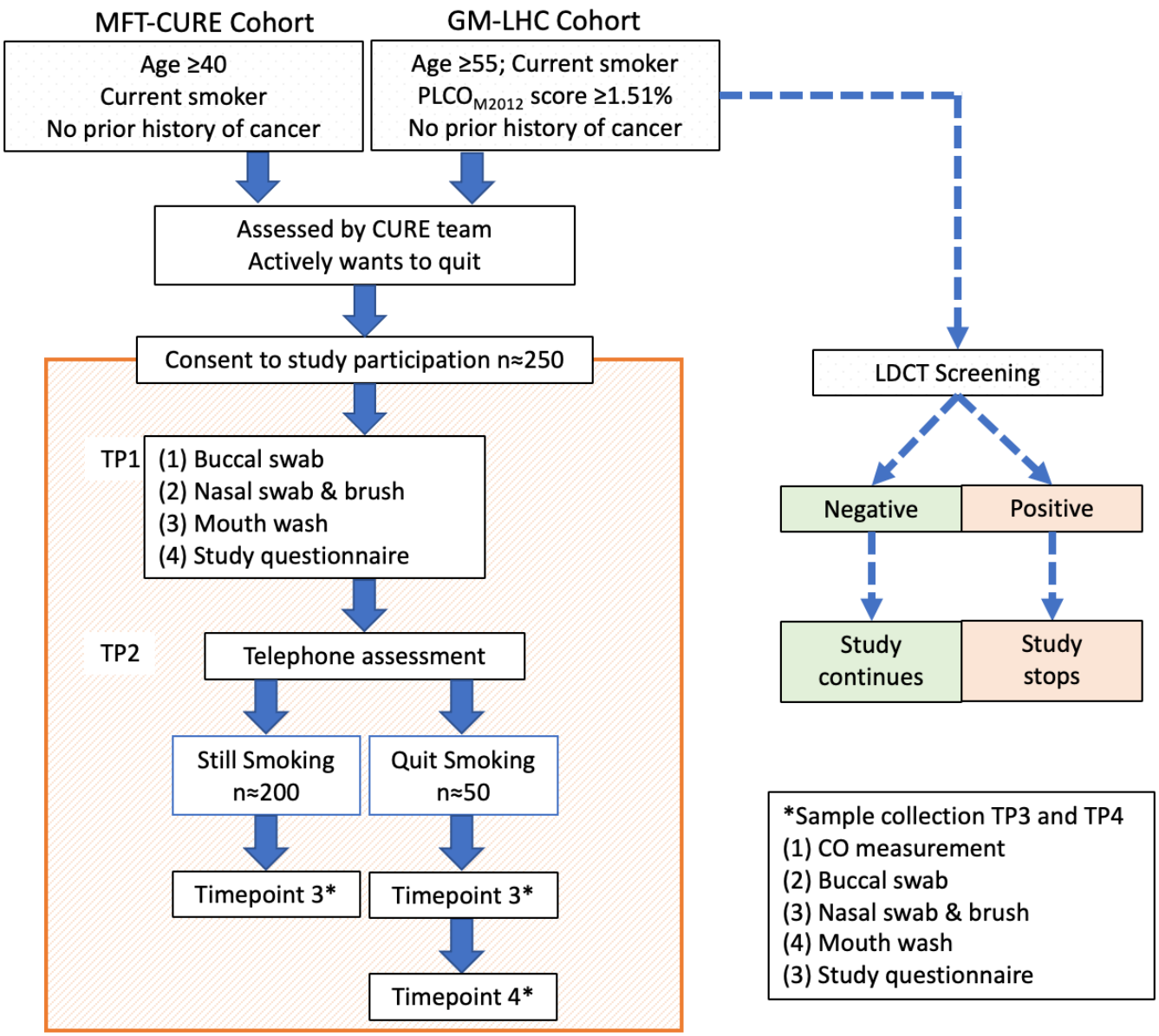
Study Title	Discovering the molecular signatures of cancer PROMotion to INform prevENTion (PROMINENT): Smoking Cessation Study
Internal ref. no. (or short title)	PROMINENT: Smoking Cessation Study (Sponsor Reference Number: NHS002126)
Study Design	Observational study
Study Participants	Current smokers, aged ≥55 years old, no prior history of cancer
Planned Size of Sample (if applicable)	150 - 250
Follow up duration (if applicable)	Between 3 and 12 months after recruitment
Planned Study Period	3rd June 2024 – 31st May 2027
Research Question/Aim(s)	Study the impact of smoking cessation on the molecular architecture of human buccal and nasal cells.

FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding ± support in kind for this study)	**FINANCIAL AND NON-FINANCIAL SUPPORT GIVEN**
Cancer Grand Challenge - Funded by Cancer Research UK (CRUK) and National Cancer Institute (NCI), project grant reference: CGCATF-2021/100007	Financial support awarded (Cancer Grand Challenge grant)

KEY WORDS: Lung cancer, smoking cessation, cancer prevention

STUDY FLOW CHART



STUDY PROTOCOL

Full Title: Discovering the molecular signatures of cancer PROMotion to INform prevENTion (PROMINENT): Smoking Cessation Study

Short Title: PROMINENT: Smoking Cessation Study

1. BACKGROUND & RATIONALE

Tobacco smoking is known to increase the risk of cancer in both humans and mouse models. The causal link between smoking and tumours of the lung (1), and up to 14 other cancer types (1, 2), has been well established. While there is compelling epidemiological evidence to link tobacco smoking with cancer risk, the underlying biological mechanisms are not well understood. Importantly, we do not understand how smoking impacts the phenotypes of normal tissues and how it promotes cancer development.

To determine the specific role of tobacco smoking in tumour promotion, we will conduct an observational study to investigate the effect of changes in smoking on tissue biology. Specifically, the PROMINENT Smoking Cessation Study will collect serial samples of buccal and nasal cells from volunteers undergoing a smoking cessation lifestyle modification. The underlying hypothesis is that through the comparison of samples obtained prior to the intervention and at subsequent time points thereafter, we will be able to observe differences in the molecular and clonal architecture of normal tissues. These changes will help us understand the mechanisms by which smoking promotes cancer development and could help identify molecular targets for prevention.

This key line of investigation will identify the changes that occur in human buccal and nasal cells upon smoking cessation, which may underlie the observed decreased risk of cancer development observed in people who cease smoking. A clear contribution of tobacco smoking to the mutation rate in certain tissues has been firmly established (3). However, whether it acts exclusively through a contribution to the somatic variation of tissues, or (in addition, or exclusively) as promoters of tumorigenesis is not well understood. In the case of smoking, there is clear evidence of its role in the development of mutations that are drivers of tumours in the lung and other organs. However, recent evidence suggests that quitting smoking promotes replenishment of the bronchial epithelium from mitotically quiescent cells that have avoided tobacco-induced mutagenesis (4).

In addition, there is growing interest in the effects of e-cigarettes on health. Although now widely used for smoking cessation, nicotine and other compounds contained in e-cigarettes can induce oxidative stress in human bronchial and lung epithelial cells resulting in inflammation, cytotoxicity and increased endothelial cell permeability (5). Therefore, it is of public health interest to understand the physiological impact of e-cigarettes. Within this study we will be able to assess the impact of smoking cessation on normal cells in those who go on to use e-cigarettes versus those who use other means. Buccal cells will be obtained through oral rinse which has also been shown to be an effective method for providing cells amenable to molecular profiling (6).

The nasal mucosa has a strong functional and immunological relationship with the lungs. The nasal mucosa may therefore act as a surrogate for the bronchial epithelium, without the need for invasive tests such as bronchoscopy or induced sputum. We will also include nasal sampling in this study.

1.1. Manchester Lung Health Check Programme

The study will recruit people who currently smoke and who attend the Greater Manchester Lung Health Check (GM-LHC) programme, which is a community-based lung cancer screening service located in highly deprived areas of Greater Manchester (https://mft.nhs.uk/lunghealthcheck/). People who have ever smoked, aged 55-74 years, and resident in areas covered by the service, are invited to have a Lung Health Check (LHC). The LHC includes an assessment of respiratory symptoms, measurement of lung function (spirometry), smoking cessation support, and an assessment of cardiovascular and lung cancer risk. Those at higher risk of lung cancer, as calculated by a risk prediction model e.g. $PLCO_{M2012}$ (threshold ≥1.51% six-year risk) or LLP_{v2} (threshold ≥2.5% five-year risk), are offered an immediate low dose CT (LDCT) scan in a co-located mobile CT scanner. Screening outcomes from the GM-LHC have previously been published (7, 8).

1.2. Manchester CURE (stop smoking) Project

Manchester has implemented a vanguard programme of hospital-based tobacco-dependency treatment services. The Manchester CURE project (*Conversation-Understand-Replace-Experts/Evidence-base*) has translated the published evidence base of the effectiveness of hospital-based tobacco dependency treatment (the Ottawa Model of Smoking Cessation and randomised controlled trial data) into a resilient, sustainable, and scalable real-world clinical service model (9-12). The CURE team approach every patient that smokes and offers a review of pharmacotherapy, specialist behaviour change support, and motivational interviewing. A key part of this specialist consultation is also offering and agreeing a treatment and support plan after discharge. The CURE team offer ongoing medications and support following discharge, through regular outpatient consultations (face to face or telephone) for 12 weeks or referral to community services for ongoing support. In a pilot of the CURE project the self-reported 12 week quit rate for current smokers was 22% (13). The CURE project has expanded its reach beyond the inpatient pathway to provide outpatient services and tobacco dependency treatment within the Greater Manchester Lung Health Check Programme. The same core principles are maintained within these programmes which include opt-out referral with immediate on the day specialist assessment to commence treatment and agree a further support package beyond the initial assessment. The clinical team will ask current smokers who wish to quit and are eligible for screening whether they would be interested taking part in the study. Those who express an interest will discuss the study with a member of the research team.

2. RESEARCH QUESTION/ AIM(S)

Main research question: What is the impact of smoking cessation on the molecular characteristics on nasal and buccal cells, and does exposure to e-cigarettes lead to specific molecular changes in these tissues?

2.1. Objectives

2.1.1. Primary

- Understand how smoking cessation affects normal nasal and buccal cells to modify tumorigenesis risk.
- Understand how e-cigarettes affects normal nasal and buccal cells compared to individuals who continue to smoke and individuals who have stopped smoking without the use of e-cigarettes.

2.1.2. Secondary

- Compare molecular changes with clinical characteristics from lung cancer screening.
- Assess the effectiveness of smoking cessation interventions in a screening cohort

2.2. Outcome measures

- Differences in molecular signals before and after smoking cessation
- Differences in molecular signals in those who do and do not use e-cigarettes

3. STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYIS

3.1. Study design

This study is an observational cohort design.

3.2. Data collection

A study questionnaire will be completed at baseline, following consent. This will include demographic information such as socioeconomic status, ethnicity, smoking behaviour, education and employment status in order to describe the sample. Further data collection will be undertaken at timepoints 2 and 3 where smoking status will be updated and information about quit attempts, use of nicotine replacement therapies and e-cigarette use recorded.

The questionnaire will be completed by the participant at the study visit or over the telephone, with a member of the research team recording the participant's response to questions. This may be done electronically (an electronic device will be available on site for this purpose) or on paper.

Consent will also be sought to access relevant data from the participant's medical records (primary or secondary care) which will be transferred to and securely stored on a research database at Manchester University NHS Foundation Trust (MFT). This will include information from the LHC and the outcome of screening (result of the LDCT scan). For those individuals diagnosed with cancer details of the type, stage and treatment will be recorded. Only authorised members of the research team at MFT will be allowed to access participants' medical records.

A personal data collection form will also be completed at the first visit. This will ask for details such as their name, NHS number, date of birth, gender and contact details. This form will be used to develop a research database which will be used to track participants' scan results, arrange telephone calls and the follow-up visit, confirm co-morbidities and record data collected from participants' medical records. This database will be stored on NHS Trust servers and will only be accessed by the research team based at MFT.

3.3. Data analysis

The clinical cohort will be stratified according to smoking status at timepoint 3 into a Quit Cohort (stopped smoking for 3 months or more at the time of sample collection) and a Smoking Cohort (smoked within 3 months of sample collection). Analysis of laboratory data will be stratified over time (baseline compared to timepoint three) and according to final cohort categorisation.

Machine learning algorithms will be developed to integrate across all of the features of the multi-omics analysis of normal tissues, to identify the combinations of parameters that contribute most to cancer risk. We will integrate salient features of the dynamics of tissue perturbation by tumour promoters including the clonal structure of tissues, protein and gene expression patterns, and differences in the pattern of infiltration of immune cell populations. In order to understand the association between these features and carcinogenesis. We will study whether they can be used to discriminate between groups of samples before or after intervention. We propose an analysis that is organized in logical steps with increasing degrees of complexity.

First, we will conduct univariate group comparison analysis to assert any outstanding correlations feature-wise. But the high-dimensionality of the feature space will require a meaningful low-rank feature representation. For some features a domain-specific dimensionality reduction method can be applied, e.g., gene expression features may be encoded as eigengenes using gene co-expression network analysis. More general linear dimensionality reduction methods like principal component analysis (PCA, unsupervised) or linear discriminant analysis (LDA, supervised) will complete the study of the feature space structure. From this analysis we will derive a compendium of features that convey the most information about the difference in the risk of tumour development across exposures linked to cancer or with lifestyle changing interventions.

We will then use supervised classification procedures (e.g., logistic regression and/or the more sensitive gradient boosting models) to combine these features into explanatory models able to weigh the relative contribution of each feature to the carcinogenic process. The aim of this part of the analysis is to further include putative additive effects as well as non-trivial feature interactions in relation to carcinogenesis. The labels of the training and test instances will be assigned based on the carcinogenesis endpoints specified above. Models able to discriminate between normal tissue before and after intervention will be built and evaluated. Cross-validation and other statistical information criteria (e.g., AIC) will be employed for model evaluation. In the case of gradient boosting models, local feature explanations (e.g., Shapley Additive Explanations or SHAP) will be derived to render the models interpretable.

The outcome of this statistical analysis will serve as a tool for data integration to learn how combinations of different features predict risk and exposure to promoters. Although we do not intend to translate these models to clinical predictive tools, some of the outcomes of this key line of investigation may well lead into some applicable knowledge for risk prediction. Given that the main goal of these models is to help us learn the molecular features of tumour promotion, we will put special emphasis in developing models that are interpretable, using approaches such as SHAP values that we have used before.

4. STUDY SETTING

This is a single centre study. Participants will be recruited from:

- **GM-LHC attendees**
  - People attending the Greater Manchester Lung Health Check programme.

The LHC is a community-based service using mobile trucks often (but not exclusively) located in supermarket car parks in areas around Greater Manchester. There are rooms within the mobile trucks dedicated to the CURE team (smoking cessation) and the research team. It is anticipated that the baseline sample collection will take place on the mobile truck. We plan to collect follow up samples on the screening truck, however, alternative locations will be considered if more convenient. This might include hospital sites within Manchester University NHS Foundation Trust or the participant's home.

- **MFT-CURE attendees**
  - People attending the CURE programme within Manchester University NHS Foundation Trust

The CURE team (smoking cessation) will assess current smokers attending MFT and those willing to participate in the study will be consented and have samples collected in hospital sites.

5. SAMPLE AND RECRUITMENT

5.1. Eligibility Criteria

5.1.1. Inclusion criteria

- **All**
    - Self-reported current cigarette smoker
    - Carbon Monoxide (CO) reading ≥5 ppb
    - Intends to quit smoking / undertake a smoking quit attempt within 3 months.
    - No prior history of cancer
    - Able to provide informed consent
- **Lung Health Check attendees**
    - Aged ≥55 years
    - $PLCO_{M2012}$ score ≥1.51% (or eligible for screening if alternative risk model is used)
- **MFT-CURE attendees**
    - Aged ≥40 years

5.1.2. Exclusion criteria

- **All**
    - Never or former smoker (has not smoked within the last 2 weeks)
    - Carbon Monoxide (CO) reading <5 ppb
    - Prior history of cancer
- **Lung Health Check attendees**
    - Aged <55 years
- **MFT-CURE attendees**
    - Aged <40 years

5.2. Sampling

5.2.1. Size of sample

The study is exploratory; the primary aim is to analyse paired sequential samples from current smokers who successfully quit smoking. Based on our data, approximately 20% of current smokers are able to quit therefore to ensure a minimum of 50 paired samples, up to 250 current smokers will be recruited at baseline. We also aim to recruit paired samples from participants who are current smokers at baseline and at timepoint 3.

5.2.2. Sampling technique

We will recruit consecutive individuals attending the Greater Manchester LHC service or MFT-CURE service who meet the study's eligibility criteria. It is anticipated that recruitment will be completed over a 30-month period. Follow up will commence any time after the first telephone assessment and continue for a further period of 3 months after the last participant has been recruited.

5.3. Recruitment

5.3.1. Sample identification

All attendees of the Greater Manchester LHC service or MFT-CURE service who currently smoke are reviewed by a smoking cessation practitioner as part of the CURE team. At their clinical appointment, individuals who wish to stop smoking will be informed of the study by their clinical care team and if interested in taking part in research, will then be directed to discuss the study with a member of the research team who will be based on site. This will either be at the same visit or at another time depending on the preference of the individual.

5.3.2. Screening

Patients will be screened by a member of the research team, prior to consent, to ensure that they are eligible for the study. This will be done via an eligibility check of the inclusion and exclusion criteria. An eligibility screening proforma will be used for this purpose. This document will collect data such as name, date of birth and whether the patient meets the inclusion criteria. It will be stored in the site file and serve as source document should there be any questions around eligibility to participate. This will be explained verbally to the patient by a member of the research team.

5.3.3. Consent

A written participant information sheet (PIS) will be provided. This will give information about the purpose of the study, what participation entails and will highlight the benefits and risks of participation. It will be clearly stated that taking part is voluntary and whether they participate or not this will not impact the medical care they receive. The study will also be explained verbally by a member of the research team. Participants will be given adequate time to review the information and have the opportunity to ask any questions they may have about the study.

Fully informed written consent is required prior to participation in the study. This will take place on the day of the study visit and will involve the participant signing a paper consent form. Consent will be taken by an appropriately trained member of the research team, who will also sign the consent form and give the participant a copy to take home. Participants are free to withdraw from the study at any time, without needing to give a reason. If a participant would like to leave the study the research team will ask for all data and samples collected up to the time of withdrawal to be kept; however, participants have the right to have data removed from the research database and samples collected as part of the study destroyed.

5.4. Biological sample collection

All samples will be collected at the time of the study visit, following Standard Operating Procedures. Buccal swabs, nasal swabs, nasal brushes and blood samples will be collected by a suitably trained member of the research team. The mouthwash sample will be collected by the participant, under the guidance of a member of the research team. The breath carbon monoxide measurement will be performed by the clinical care team or a member of the research team.

5.4.1. Timepoint 1 (TP1)

Samples collected at baseline include the following

- Buccal swab
- Mouthwash sample
- Breath carbon monoxide measurement
- Personal data collection form
- Study questionnaire
- Nasal swab (optional)
- Nasal brush (optional)
- Blood sample (optional) – 50mL volume maximum

If it is not possible to take the nasal sample (brush or swab) for whatever reason the individual may continue in the study. If the buccal sample is not collected, then the individual will be withdrawn from the study.

5.4.2. Timepoint 2 (TP2)

Study participants will be contacted by a member of the research team to ascertain smoking status. This will be undertaken over the telephone and will involve the completion of a study questionnaire. It is anticipated that phone calls will be made every 3 months to ascertain smoking status. TP3 will be arranged if the participant has not smoked a cigarette for at least three consecutive months prior to the proposed date of TP3. If by 3-12 months after baseline a participant remains a current smoker (smoked within three months of the telephone assessment) they will then have TP3 arranged.

5.4.3. Timepoint 3 (TP3)

Samples collected at TP3 include the following

- Buccal swab
- Mouthwash sample
- Breath carbon monoxide measurement
- Study questionnaire
- Nasal swab (optional)
- Nasal brush (optional)
- Blood sample (optional) - 50mL volume maximum

TP3 participants will be classified as either **a former smoker** (last cigarette over three months ago i.e. a last cigarette 3 months plus 1 day ago = former smoker) **or current smoker** (smoked a cigarette within the last three months).

5.4.4. Timepoint 4 (TP4)

Participants who attend TP3 who are classified as a former smoker will be invited to attend a further appointment 3 to 6 months after TP3 (TP4). Samples collected at TP4 include the following

- Buccal swab
- Mouthwash sample
- Breath carbon monoxide measurement
- Nasal swab (optional)
- Nasal brush (optional)
- Blood sample (optional) – 50mL volume maximum

Study questionnaires will be completed by the participant, at all timepoints, either at the study visit or over the telephone (with a member of the research team recording the participant's response to questions). This can be completed electronically (an electronic device will be available on site for this purpose) or on paper.

5.5. Sample processing and storage

The procurement, handling and storage of biological samples will be undertaken by NHS research staff based at MFT and personnel who have received training in the risks associated and safety requirements. Standard operating procedures (SOPs) will be used. Samples will be labelled with the unique study ID number, study title and time point. To provide an ethical framework for samples and pseudonymised data to be transferred to collaborators across the globe, this will be included in the consent form. The sponsor (University of Manchester) will serve as custodian of all samples taken for this study. Sample usage will be under the control and jurisdiction of the Chief Investigator.

Pseudonymized biological samples and data will be transferred either to the University of Manchester or to the International Agency for Research on Cancer (IARC)-WHO following laboratories for storage and analysis. The transfer will be done under the right Material Transfer Agreements/ contracts. IARC will operate a centralized sample processing facility and will ensure a smooth flow of biological samples (according to each specific analysis related to the different key lines of investigation). Samples and data collected within this project may also be transferred to other research laboratories for further analyses. Other institutions that the research data/ samples may be sent to for analysis include, but are not limited to, Imperial College London, Institute for Research in Biomedicine (Barcelona, Spain), Science for Life Laboratory at the Royal Institute of Technology (Stockholm, Sweden), Stanford University, USA and National Institutes of Health/ National Cancer Institute, USA

The link to participants' personal data will only be held at MFT and accessed (as appropriate) by authorised research team members. This link will never be transferred to other institutions/ collaborators.

Any samples left over at the end of the study will be kept in storage by the research team pending ethical approval for use in another project.

5.6. End of study

The end of the study is the point at which all the study data and samples have been analysed; and queries resolved. This includes the long-term remote follow-up.

6. ETHICAL AND REGULATORY CONSIDERATIONS

6.1. Adverse Events

It is not anticipated that participants in this study will suffer any adverse events. In the unlikely event this does occur a reporting mechanism is described below. If an adverse event (AE) occurs because of study participation this will be reported in accordance with Good Clinical Practice and Sponsor requirements.

By extension, a Serious Adverse Event (SAE) will be any AE that:

• Results in death

• Is life-threatening* (subject at immediate risk of death)

• Requires in-patient hospitalisation or prolongation of existing hospitalisation**

• Results in persistent or significant disability or incapacity

• Other important medical events***

*'Life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, including elective procedures that have not worsened, do not constitute an SAE.

***Other important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event/experience when, based upon appropriate medical judgment, they may jeopardise the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Any untoward event that occurs as a consequence of the LDCT screening or further investigations in the cancer clinic are due to the screening service and not the research study and will therefore not be considered an AE.

All SAEs noted by a member of staff within the chest clinic, or self-reported to members of staff by participants during their time in the community or immediately after, should be reported to the NM MRD research team within 24 hours of becoming aware of the event.

Reported SAEs will be reviewed within 24 hours of being received. This clinical review for causality will be completed by the Chief Investigator and clinical lead of the service. Information on AEs and SAEs will be summarised for trial oversight, funder and other progress reports, as required. SAE's will also be reported to the Sponsor office on a quarterly basis.

6.2. Research Ethics Committee (REC) and other Regulatory review & reports

This study protocol will be submitted to HRA, and a Research Ethics Committee (REC) for review and approval. The HRA letter of approval and a favourable ethical opinion must be obtained from the REC before commencement of any trial procedures (including approaching patients) occur. In accordance with HRA guidance, all correspondence with the REC will be retained, an annual progress report will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given and annually until the study is declared ended. The Chief Investigator will notify the REC of the end of

the study, including the reason if the study is terminated prematurely. The Chief Investigator will submit a final report with study results including any publications/abstracts within one year of study end.

6.3. Study amendments

Any substantial amendments to the study protocol will be submitted to the REC for consideration. The Chief Investigator will be responsible for the decision to amend the protocol and will decide whether any amendment is substantial or non-substantial following discussion with the sponsor. A full amendment history will be tracked in the protocol appendix.

6.4. Monitoring arrangements

Monitoring will be the responsibility of the CI and sponsor (UoM). To ensure quality of data, study integrity, and compliance with the protocol and the various applicable regulations and guidelines, the "Sponsor" may conduct site visits to participating institutions. The investigator agrees to co-operate fully with any quality assurance visit undertaken by third parties, including representatives from the "Sponsor", or other authorised regulatory authorities or company supplying the product under investigation, as well as to allow direct access to documentation pertaining to the study (including source documents, hospital patient charts and other study files) to these authorised individuals. The investigator will inform the "Sponsor" immediately in case a regulatory authority inspection would be scheduled.

6.5. Peer review

The PROMINENT study has been through extremely rigorous peer review process by multiple international experts.

6.6. Protocol compliance

Accidental protocol deviations will be documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately. Recurrent deviations from the protocol require immediate action.

6.7. Risks

Blood samples: The risks involved in donating a blood sample are the same as for routine blood tests. There may be discomfort or pain in the skin and tissue around the vein where the blood is taken. There may be bruising over the vein after the procedure. Blood will be taken by trained professionals who are experienced in the procedure.

Nasal brush: Participants may experience some irritation and discomfort during the collection of the nasal brush sample. The sample will be taken by trained professionals who are experienced in the procedure.

6.8. Expenses and Benefits

We will offer participants who donate at least two sets of samples a £20 shopping voucher as a token of appreciation for their participation in the study. Those who attend the third visit will receive an additional £20 shopping voucher. We will also offer to pay carpark tickets or reasonable travel expenses for participants.

6.9. Data protection and patient confidentiality

All information that is collected during the research will be kept strictly confidential. Data for study participants will be collected at Manchester University NHS Foundation Trust, on Trust-owned mobile trucks or over the phone.

The consent form and personal data collection form will be completed at MFT/ mobile trucks. Forms containing patient identifiable information will be kept securely at MFT (Wythenshawe Hospital). The data from these forms will then be entered by the hospital research team into the study database held at Wythenshawe Hospital on a secure password protected network. Paper copies of forms will be kept in a locked filing cabinet in the hospital and will be stored separately, depending on whether they contain personal data (i.e. consent form and personal collection form) or pseudonymised research data (i.e. questionnaires). CT images and reports will be held securely on NHS Trust servers as is standard practice.

Personal data will be held for 10 years after the study has ended. The study is looking at the biology of smoking cessation. Smoking is linked with the development of cancer. We would like the opportunity to assess which patients develop cancer over the long term and link that with the biological findings from the lab science. Participants will have had a CT scan showing no evidence of cancer; therefore we need long term follow up because it can take years to go from no cancer to symptomatic presentation of cancer.

Staff with responsibility for data entry / data handling will be made familiar with both the sources of data and the database; they will be trained appropriately. All participants will be assigned a unique study ID number. Data in the study database will be pseudonymised. The link between individuals' identifiable data and the unique study ID will be securely stored in a different location. Data from remote medical case record review (for a period of 10 years after recruitment, to allow for remote monitoring to see if participants go on to develop cancer in the future and link with findings from the study) will also be recorded on the study database.

The University of Manchester, as Data Controller for this project, takes responsibility for the protection of the personal information that this study is collecting. In order to comply with the legal obligations to protect personal data the University has safeguards in place such as policies and procedures. All researchers are appropriately trained and data will be looked after in the following way: only the research team based at MFT will have access to this information. Research data is kept separate from the main health record and is stored in a locked office and in a password-protected database in MFT.

6.9.1. Access to Data

Access to personal identifiable information during and after the study will only be restricted to the research team based in MFT, and this will be at the authorisation of the Chief Investigators. Only those directly involved with the study and care of the individual will be able to view this data; this will require a username and password. The sponsor will be able to access data from consented patients for monitoring purposes, in line with the consent form. Pseudonymised study data/images/samples may be securely

sent to other sites in the UK or around the globe for analysis. A formal data sharing agreement will be made with commercial or academic partners who undertake biomarker / data / imaging analysis, to ensure data security. Only pseudonymised data will be released to third parties. We will ask participants' permission to allow the release of data globally under the appropriate ethical framework.

6.9.2. Data Security

All study staff will understand the principles of confidentiality and will be encouraged to hold current Good Clinical Practice (GCP) certificates. Any personal information collected and stored will be number coded and anonymised to protect the individuals involved within the study. Questionnaires and data collection forms will only contain this unique patient ID. A document linking ID number with the relevant participant will be stored securely on a password protected computer at MFT, where the Chief Investigator is based. This document will be stored in a separate location to any study data and will only be accessed by authorised members of the research team.

6.9.3. Data Confidentiality

All data will be treated confidentially in accordance with NHS guidelines. Participant identifiable data will be stored on secure password-protected networks based in MFT. Pseudonymised data will be held on University of Manchester password-protected secure networks.

6.9.4. Minimisation of missing data

We will attempt to minimise the quantity of missing data. When asking a person for consent, we will request access to their primary and secondary care health records so that any inconsistencies or missing data identified can be cross checked. In the event that consenting participants leave the Manchester area or we become aware that they are seeking treatment from other hospitals or private providers we will attempt to follow-up their data, where possible.

6.9.5. Withdrawal of data or consent

Consented individuals wishing to withdraw from the study will be able to do so at any time. If a participant asks to withdraw from the study, researchers will ask if the participant is willing to share their reason for withdrawing, although it will be made clear that they are not required to share this information if they do not wish. If the participant still wishes to withdraw from further trial activity a member of the research team will request that the participant allows us to retain any data that has been collected and stored up to the point of withdrawal, including any qualitative data where relevant. Follow-up will continue unless the patient explicitly also withdraws consent for follow-up. If the participant explicitly states they do not wish to contribute further data to the study, and desires that their existing data should not be retained, their preference will be recorded and action taken within the database to ensure this request. This does not affect participant data protection rights.

6.9.6. Mental Capacity

A person may lose mental capacity from the point of their initial consent to the study. It is therefore proposed that any patient confirmed as now lacking capacity, be withdrawn from further intervention. The Chief Investigator will be responsible for assessing continued capacity.

6.10. Indemnity

The University of Manchester will arrange insurance for research involving human subjects that provides cover for legal liabilities arising from its actions or those of its staff or supervised students, subject to policy terms and conditions.

NHS indemnity (for negligent harm) will cover MFT employees, both substantive and honorary, who are working in the course of their NHS employment and in respect of conducting research projects which must have received HRA Permission.

7. DISSEMINIATION POLICY

7.1. Dissemination policy

The findings will be communicated and disseminated to the research community and also to potentially interested wider audiences. The conclusions from this project will also be disseminated by publications in peer-reviewed scientific journals and presentations in scientific meetings and conferences. The findings will be shared in line with the consent provided by the research participants that will incorporate information and consent on data sharing.

The requirements for manuscripts submitted to biomedical journals (http://www.icmje.org/) will be respected. All clinicians and researchers will be acknowledged where they have been involved in written papers. With the assistance of our collaborators and patient representatives we will disseminate the trial findings to a wide NHS and general audience.

All data collected in the study will be pseudonymised at the point of collection. To guarantee the reproducibility of results published, we also aim to publish all code needed to reproduce the analyses. To that end we will create a public bitbucket or github repository.

7.2. Sharing data outside the consortium

We adhere to the principles of FAIR data sharing, that is, we commit to providing Findable, Accessible, Interoperable and Reusable data for all the scientific community. Data produced from this study will be regularly deposited in public repositories throughout the duration of the project.

Publicly shared data will be kept under embargo for a limited period of time, to be agreed among researchers of the consortium, in order to guarantee a period of data use by the PROMINENT team. This will not be longer than 12 months.

7.3. Authorship eligibility guidelines and any intended use of professional writers

All publications and presentations relating to the trial will be authorised by the Chief Investigator. We will publish main study results in peer-reviewed journals and present at national and international scientific meetings. The requirements for manuscripts submitted to biomedical journals (http://www.icmje.org/) will be respected. All clinicians and researchers will be acknowledged where they have been involved in written papers. With the assistance of our collaborators and patient representatives we will disseminate the trial findings to a wide NHS and general audience.

7.4. Archiving

All study documents will be retained for a period of 10 years following the conclusion of the study. Following the submission of the end of study report, the Sponsor will arrange for archiving of the Trial Master File in accordance with the Sponsor's process. Following the end of the retention period, the Sponsor will notify the Chief Investigator in writing that the required retention period has completed and that documents will now be destroyed. A copy of the instruction to the archivist to destroy the records will be requested.

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9. APPENDICIES

9.1. Appendix 1 – Schedule of Procedures

Procedures	Visits		
	Visit 1 (TP1 Baseline)	Visit 2 (TP3 Follow-up)	Visit 3 (TP4 Follow-up)
Screening	X		
Informed consent	X		
Personal data collection form	X		
Study questionnaire	X	X	X
Buccal cell collection	X	X	X
Nasal swab collection (optional)	X	X	X
Nasal swab collection (optional)	X	X	X
Blood sample (optional)	X	X	X
Mouthwash collection	X	X	X
Carbon monoxide measurement	X	X	X

9.1.1. Collection of biological samples

The collection, processing and storage of all biological samples will be detailed in the study laboratory manual.

9.2. Appendix 2 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	3.0	04/10/2024	Chinenye Amadi	• Extension of the duration of recruitment to 24 months • Exclusion criteria (for Lung Health Check attendees) adjusted to recognise that some participants may have their LDCT after enrolment into the study • Addition of remuneration for participants who complete both study visits to complete questionnaires and donate samples
2	4.0	14/05/2025	Prof Philip Crosbie, Dr Laure Dossus, Chinenye Amadi	• Reduction of the sample size to 250 max. • Revision of recruitment period to 18 months • Adjustment of follow-up window to 3 – 12 months from baseline. • Addition of extra visit for participants who quit smoking by TP3 follow-up visit. • Additional remuneration for participants who attend the extra visit. We will also offer to pay car parking charges to facilitate this visit. • Minor change to TP3 Study Questionnaire
3	5.0	19/01/2026	Chinenye Amadi	• Extension of both recruitment and study end date, by 12 months, to 31st January 2027 and 30th April 2027 respectively.

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

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC.