Observational Study Protocol - Amendment

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Study amendment:
HANSE - Holistic implementation study Assessing a Northern German interdisciplinary lung cancer Screening Effort

Additional follow-up and implementation of newly available blood biomarkers to strengthen the results and refine the diagnostic workup of the prospective, randomized, and comparator-controlled population-based screening study.

Sponsor: Medizinische Hochschule Hannover

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
CAT	COPD assessment test
CBC	Complete blood-cell count
CMP	Comprehensive molecular profiling
LC	Lung cancer
LDCT	Low-dose computed tomography
LungRADS	Lung CT Screening Reporting & Data System
MDT	Multidisciplinary tumor board
mMRC	Modified medical research council
OR	Odds ratio
PLCO	Prostate, lung, colorectal, and ovarian cancer screening
PPV	Positive predictive value

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PROTOCOL SYNOPSIS

Study amendment:

HANSE - Holistic implementation study Assessing a Northern German interdisciplinary lung cancer Screening Effort

Additional follow-up and implementation of newly available blood biomarkers to strengthen the results and refine the diagnostic workup of the prospective, randomized, and comparator-controlled population-based screening study.

Background/Rationale:

HANSE is an ongoing pilot lung cancer (LC) screening study in three specialized LC centers in northern Germany (NCT04913155). It was intended to provide evidence that a holistic and effective LC screening program can be implemented in Germany and that such a screening program can be integrated in the current infrastructure of certified lung cancer centers. For details, please find the initial study protocol in section 9 (Attachments).

Participants between 55 and 79 years of age with a smoking history were eligible for low-dose computed tomography (LDCT) given that they met the inclusion criteria of the NELSON trial or had a $PLCO_{M2012}$ risk score ≥1.58 % (6 year risk).[1,2] The primary objective of this study, which was recently met.[4] , is to compare the efficiency of the $PLCO_{M2012}$ and the NELSON inclusion criteria in identifying patients with LC[3] by comparison of the positive predictive value (PPV) for LC detection with the two different inclusion methods (NELSON vs. $PLCO_{M2012}$) after 2 rounds of LDCT screening (at baseline and after 1 year follow-up) Secondary endpoints include the proportion of individuals selected for screening, proportion of LC cases detected within the different cohorts after a 5-year follow-up, sensitivity and specificity after 5-year follow-up, rate of initiation of cardiovascular treatments, efficiency of nodule management algorithms, success and quality of the screening program, success of smoking cessation counselling, evaluation of blood-based biomarkers in positive LDCT cases, as well as analysis of the cost effectiveness.

HANSE included 13,016 participants which were assessed for their individual lung cancer risk, of which 5,191 met the risk criteria of NELSON and/or $PLCO_{M2012}$ and received a baseline LDCT and 4,356 participants received a 2nd round LDCT. In total, 111 LC cases were detected in both screening rounds (64 in round 1 and 47 in round 2), of which 108 LC cases were detected by the $PLCO_{M2012}$ ≥1.58 % risk score and 85 were detected by the NELSON criteria, showing a significantly higher LC detection rate of 97.3 % in $PLCO_{M2012}$-selected when compared to 76.6 % in NELSON-selected participants ($p<0.0001$). Positive predictive values (PPV) were 2.59 % ($PLCO_{M2012}$) and 2.17 % (NELSON), respectively.[4]

However, longer follow-up is needed to confirm these very promising results and to proof their validity considering also slowly growing lung cancer which might be overseen within only two LDCT rounds with an interval of 12 months and which could have an influence on the detection rate of the two risk scores. In the NELSON trial, the highest LC detection rate was found 3 years after the initial baseline LDCT. Therefore, this amendment is intended to include a third LDCT round in participants of the HANSE study fulfilling the NELSON and/or $PLCO_{M2012}$ ≥1.58 % risk criteria after a follow-up of approximately 4 years after the baseline screening round to further support the primary objective.

Furthermore, recently novel blood biomarkers have been tested with promising performances in early-stage lung cancer detection.[5–7] It is yet to be proven in how far such biomarkers can expand and refine the established risk criteria and/or the diagnostic workup within LDCT-based LC screening programs to increase LC detection and to reduce the rate of false positive interventions.

Integration of a certified smoking cessation program was already part of the initial HANSE protocol. Since intensified cessation programs comprising a combination of behavioural counselling and pharmacotherapy were shown to produce the highest success rates in smoking cessation[8], effectiveness and safety of an intensified smoking cessation program is to be studied in comparison to the short smoking cessation counselling following the WHO recommendations. Participation is fully voluntary.

Objectives and Hypotheses:

The primary objective of the HANSE study remains unchanged and can be found in section 2.1 of the initial study protocol (for details, see section 9. Attachments). However, the primary endpoint will be extended by a third LDCT screening round after follow-up of ~4 years to further strengthen the positive results of the HANSE study.

In addition, this amendment is intended to include three additional secondary objectives:

- To proof the real-world practicability of the $PLCO_{M2012}$ and NELSON risk scores by re-evaluating HANSE participants with an initially low risk score. Participants changing from initially low to high risk due to their increased age by ~4 years or by other changed variables will be invited to receive a baseline LDCT scan.
- To study the effectiveness of a guideline-based and intensified smoking cessation program in comparison to the already included short smoking cessation counselling with respect to the smoking cessation rates (participants being nicotine free).
- To proof if novel LC blood biomarkers can:
    - expand the risk assessment of LC screening. For this, HANSE participants with a low risk profile (after $PLCO_{M2012}$ and NELSON risk re-evaluation) will be invited for blood sampling, and participants with positive LC biomarker findings will be invited to receive a LDCT scan.

- improve the diagnostic workup of positive LDCT findings within Multidisciplinary Tumor Boards (MDT). Therefore, participants will be randomized 1:1 and for half of the participants with positive LDCT findings, MDT discussions will be supplemented by blood biomarker results (biomarker reporting arm) whereas the other half will be discussed without these results (control arm). This randomization does not affect the initial study randomization (for details, see section 6.1.2).

All participants with a positive LC blood biomarker test will receive a 2nd LDCT about 6 months after the baseline CT of the HANSE 3rd round or baseline round respectively to confirm the negative diagnosis according to the mod. Lung RADS 1.1 score used in the HANSE study, except for participants with a positive lung cancer diagnosis on histology.

All other objectives and endpoints of the HANSE study remain unchanged (for details, see the initial study protocol in section 9. Attachments).

Methods:

The principal methodology of the HANSE study remains unchanged. For better overview, only changes to the initial study protocol will be described in this amendment.

Study design:

Unchanged

Data Source(s):

In addition to the initially defined data sources, blood samples will be taken from all participants of the HANSE study (high risk and low risk participants) and blood biomarkers for early LC detection will be analyzed by a metabolomic blood test of 9 metabolites (BioMark Diagnostics, Richmond, Canada).

Study Population:

The general HANSE study population remains unchanged. However, the high risk population will be extended by initially low risk participants who become high risk by $PLCO_{M2012}$ and NELSON re-evaluation.

Exposure(s):

Participants in the initial high risk group will undergo a third LDCT screening round after approximately 4 years after the baseline screening round. Participants either switching from the low to high risk group by $PLCO_{M2012}$ and NELSON re-evaluation or who are tested positively for metabolic LC biomarkers will undergo a first LDCT screening round. To analyze the rate of false-positives, participants with positive LC biomarkers but without LC findings after the first LDCT will undergo a follow-up LDCT 6 months after baseline.

Outcome(s):

Primary endpoint:

The primary endpoint will be extended by a third LDCT screening round after follow-up of ~4 years to proof and validate the positive results of the HANSE study considering slowly growing lung cancer.

Additional secondary endpoints:

- LC detection rates (PPV) will be assessed for participants initially rated as low risk who become high risk after $PLCO_{M2012}$ and NELSON re-evaluation after ~4 years of follow-up.
- Success of a guideline-based and intensified smoking cessation program will be analysed in comparison to the short smoking cessation counselling by:
    - Assessing the proportion of participants being nicotine-free (i.e. absolute abstinence from taking nicotine from any source) after 6 months (determination of cotinine in urine).
    - Assessing the proportion of participants being nicotine-free (i.e. absolute abstinence from taking nicotine from any source) after 1, 3, and 12 months (determination of cotinine in urine).
    - Assessing the proportion of participants being abstinent from combustible cigarettes, after 1, 3, 6, and 12 months
    - Survey of the Fagerström test, survey after craving, absence/sick days of the previous year as well as QoL survey
    - Assessment of the adverse events of the products as well as the withdrawal symptoms (no nausea, difficulty falling asleep and staying asleep, dry mouth, etc.)
    - Assessment of respiratory symptom burden (cough, perceived shortness of breath via mMRC or CAT)
    - Optional: Assessment / improvement of the function of the small airways or lung function
    - Optional: Assessment / improvement of central blood pressure measurements and arterial vascular stiffness

- LC detection rates (PPV) will be assessed after LDCT for low risk participants (after $PLCO_{M2012}$ and NELSON re-evaluation) with positive LC biomarker findings (metabolomic blood test, BioMark Diagnostics, Richmond, Canada).

- LC detection rates (PPV) and application rates of invasive procedures will be assessed for positive LDCT findings which are discussed within MDT with respect to supplementation with blood biomarker results.

Sample Size Estimations:

Sample size estimation for the primary endpoint remains unchanged. However, expected LC cases in initially low risk participants who will receive baseline LDCT screening within this study amendment either by switching to high risk after $PLCO_{M2012}$ and NELSON re-evaluation or by positive blood biomarker results for early LC detection will be estimated in section 5.4 of this protocol amendment for LDCT justification. In addition, sample size estimation for the intensified smoking cessation program and power calculation of the new secondary endpoints can be found in section 5.4 of this protocol.

Statistical Analysis:

No major changes to initial protocol (for details, see section 9. Attachments). Only details concerning the new secondary endpoint to study the effectiveness of a guideline-based and intensified smoking cessation program in comparison to the already included standard smoking cessation counselling are included in section 5.1.2.

AMENDMENT HISTORY

Date	Section of study protocol	Amendment or update	Reason
15.09.2020		N/A	Initial Protocol Version 1.0
23.11.2020	Study objectives and power calculation of primary endpoint	Amendment	Protocol Version 2.0
09.02.2021	Administrative changes: Recruitment methods, potential sampling bias and biomarkers	Amendment	Protocol Version 2.1
13.12.2024	Addition of 3rd LDCT screening round (primary endpoint) and extension of secondary objectives	Amendment	Protocol Version 3.0

MILESTONES

Milestone	Planned date
August 2020	Final study protocol
August 2020	Contract Astra Zeneca
August 2020	Contract CRO
August 2020	Contract Coreline
August 2020	Contract truck/CT
September 2020	Ethics approval
September 2020	BfS application
March 2021	Study fully set-up
July 2021	Start of enrolment: First patient in
August 2023	Date of last data entry in database
September 2023	Date of Database Lock (Clean Database)
December 2023	Publication and Final report
October 2024	Final protocol amendment
October 2024	Contract AstraZeneca (amendment)
December 2024	Ethics submission (amendment)
December 2024	Final study setup (amendment)
May 2025	BfS approval (amendment)
September 2025	Start blood sampling low risk participants
November 2025	LDCT screening start (amendment)
January 2027	Date of last data entry in database
February 2027	Date of Database Lock (Clean Database)
July 2027	Publication and Final report

1. BACKGROUND AND RATIONALE

1.1 Background

HANSE is an ongoing pilot lung cancer (LC) screening study in three specialized LC centers in northern Germany (NCT04913155). It was intended to provide evidence that a holistic and effective LC screening program can be implemented in Germany and that such a screening program can be integrated in the current infrastructure of certified lung cancer centers. For details, please find the initial study protocol in section 9 (Attachments).

Participants between 55 and 79 years of age who were current or former smokers and who met the inclusion criteria of the NELSON trial (smoking quit time ≤10 years, >15 cigarettes/day for >25 years or >10 cigarettes/day for >30 years) or a $PLCO_{M2012}$ risk score of at least 1.58 % within 6 years were recruited from 3 certified lung cancer sites (Hannover, Großhansdorf, and Lübeck).[1,2] Such high risk participants received two consecutive LDCT screening rounds: at baseline and after 12 months follow-up.[3] The primary objective of this study, which was recently met.[4] , is to compare the efficiency of the $PLCO_{M2012}$ and the NELSON inclusion criteria in identifying patients with LC by a comparison of the positive predictive value (PPV) for LC detection with the two different risk selection methods (NELSON vs. $PLCO_{M2012}$) after two rounds of LDCT screening For testing the null hypothesis of equal PPVs for LC detected in $PLCO_{M2012}$-selected versus NELSON-selected individuals, the weighted generalized score statistic by Kosinski was used. Secondary endpoints include the proportion of individuals selected for screening, proportion of LC cases detected within the different cohorts after a 5-year follow-up, sensitivity and specificity after 5-year follow-up, rate of initiation of cardiovascular treatments, efficiency of nodule management algorithms, success and quality of the screening program, success of smoking cessation counselling, evaluation of blood-based biomarkers in positive LDCT cases (LungRADS 4A PET, 4B, 4X), as well as analysis of the cost effectiveness.

HANSE included 13,016 participants which were assessed for their individual lung cancer risk, of which 5,191 met either one or both of the two risk criteria and received a baseline LDCT scan, and 4,356 participants received a 2nd round LDCT. In total, 111 LC cases were detected in both screening rounds (64 in round 1 and 47 in round 2), of which 108 LC cases were detected by the $PLCO_{M2012}$ ≥1.58 % risk score and 85 were detected by the NELSON criteria, showing a significantly higher cancer detection rate of 97.3 % by $PLCO_{M2012}$ in comparison to 76.6 % by the NELSON criteria ($p<0.0001$, see Figure 1). When the sample was supplemented with the low risk population, who did not meet the high risk criteria and consented to participate in the HANSE study (n=7,463), the calculated lung cancer detection rates (sensitivity) of the $PLCO_{M2012}$ and the NELSON criteria were 77.1 % and 60.7 %, respectively. Accordingly, the HANSE study showed a 19.4 % relative increase of the PPV for LC detection in the $PLCO_{M2012}$-selected group (PPV=108/4,167 [2.59 %] compared to the NELSON-selected group (PPV=85/3,916 [2.17 %], p=0.004). Hence, $PLCO_{M2012}$ is reliable and more efficient than the NELSON criteria for selecting individuals to be enrolled into a LC screening program and should be used for identifying high risk individuals based on the results of the HANSE study.

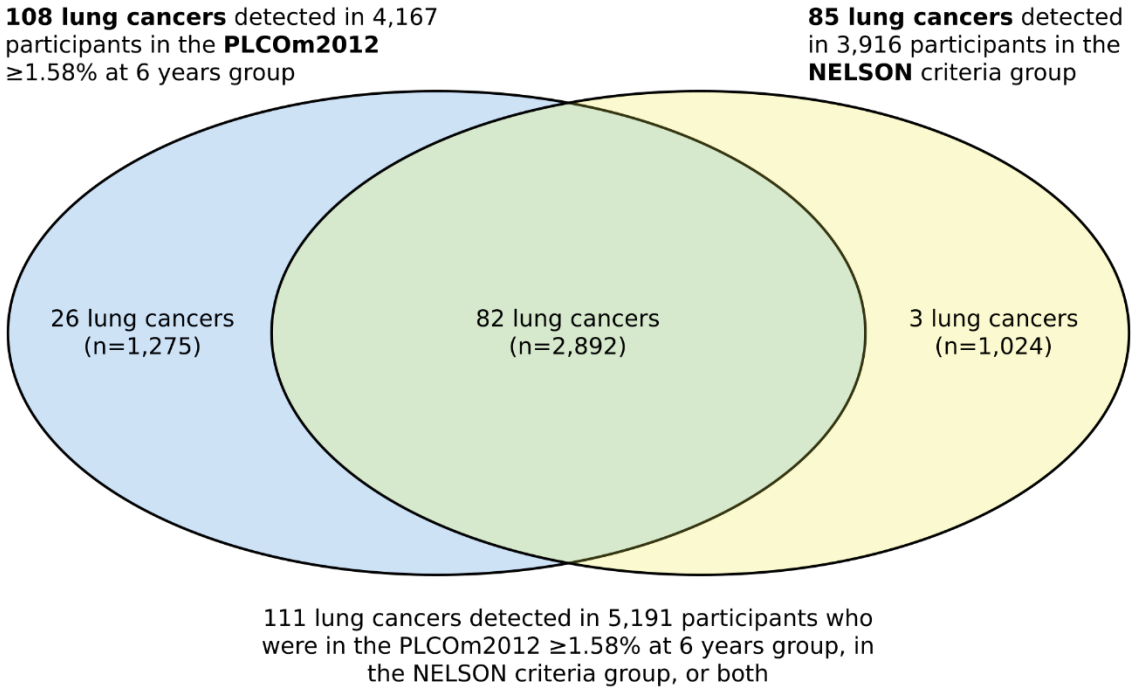

Figure 1 Lung cancer detection within two LDCT screening rounds (baseline & after 12 months follow-up) of $PLCO_{M2012}$- and NELSON-selected participants.

1.2 Rationale

Despite the positive findings of a significant difference of LC detection between $PLCO_{M2012}$ and NELSON criteria within HANSE, longer follow-up is needed to confirm these results and to proof their validity considering slowly growing lung cancer which might be overseen within only two LDCT rounds with an interval of 12 months and which could have an influence on the detection rate of the two risk scores. For example, in the NELSON trial the highest LC detection rate was found 3 years after the initial baseline LDCT scan.[1] Therefore, this amendment is intended to include a third LDCT round for high risk participants of the HANSE study fulfilling the NELSON and/or $PLCO_{M2012}$ ≥1.58 % risk criteria after a follow-up of approximately 4 years to further support the primary objective.

Furthermore, recently novel blood biomarkers have been tested with promising performances in early-stage lung cancer detection.[5–7] It is yet to be proven in how far such biomarkers can expand and refine the established risk criteria and/or the diagnostic workup within LDCT-based LC screening programs to increase LC detection and to reduce the rate of false positive interventions.

Integration of a certified smoking cessation program was already part of the initial HANSE protocol, according to the requirements defined by the Joint Statement of the German Radiological Society. For this, participants were informed about and encouraged to participate

in smoking cessation programs comprising professionally guided group courses within short counselling sessions by trained staff. However, numerous studies have shown that a combination of behavioural counselling and pharmacotherapy produces the highest success rates in smoking cessation leading to implementation of respective treatment recommendations into the German S3 smoking cessation guidelines.[8] Therefore, within this amendment effectiveness and safety of an intensified smoking cessation program consisting of behavioural counselling and pharmacotherapy is going to be studied in comparison to the short smoking cessation counselling following the WHO recommendations of 5 R's and 5 A's.

2. OBJECTIVES AND HYPOTHESES

Details of the initial study protocol can be found in section 9. Attachments.

2.1 Primary Objective(s) & Hypothesis(es)

The primary objective of the HANSE study is to compare the efficiency of the $PLCO_{M2012}$ ≥1.58 % (6 year risk) risk score and the NELSON inclusion criteria in identifying participants with lung cancer in the age group 55-79 years, which remains unchanged from the initial protocol. However, the primary endpoint will be extended by a third LDCT screening round after follow-up of ~4 years to potentially further strengthen the positive results of the HANSE study.

2.2 Secondary Objective(s) & Hypothesis(es) (Optional)

Secondary objectives will be extended by the following three objectives:

- To proof the real-world practicability of the $PLCO_{M2012}$ risk score by re-evaluating HANSE participants with an initially low risk score who have not yet received a LDCT. Since the $PLCO_{M2012}$ risk score incorporates a person's age, longer follow-up will lead to an increased risk score crossing the threshold of 1.58% (6 year risk) for some participants. In addition, also other personal variables and the continued or changed smoking habits relevant for $PLCO_{M2012}$ and NELSON risk calculation (i.e. smoking behaviour, BMI, comorbidities) can change over time and consequently can change a person's risk score. From the HANSE data, approximately 800 participants are estimated to change from low to high risk. After proofing their actual $PLCO_{M2012}$ and Nelson risk scores on site, these participants will receive a baseline LDCT scan.
- To study the effectiveness of a guideline-based and intensified smoking cessation program in comparison to the already included standard smoking cessation counselling with respect to the smoking cessation rates (participants being nicotine free). For this, participants who change from low to high risk $PLCO_{M2012}$ risk score will be randomized 1:1 to receive an intensified or a short smoking cessation counselling. The participation in smoking cessation programs is voluntary. This randomization does not affect the initial study randomization (for details, see section 6.1.2).
- To proof if novel LC blood biomarkers can:

- expand the risk assessment of LC screening. For this, HANSE participants with a low risk profile (not fulfilling the high risk criteria of NELSON and/or $PLCO_{M2012}$) will be invited for blood sampling. Participants with positive LC biomarker findings will be invited to receive a LDCT scan, and the PPV will be compared to the NELSON and $PLCO_{M2012}$ risk criteria.

- improve the diagnostic workup of positive LDCT findings within Multidisciplinary Tumor Boards (MDT) in terms of detected LC cases and application of invasive procedures (biopsies or resections). Therefore, participants will be randomized 1:1 and for half of the participants with positive LDCT findings, MDT discussions will be supplemented by blood biomarker results (reporting arm) whereas the other half will be discussed without these results (control arm).

All participants with a positive LC blood biomarker test will receive a 2nd LDCT about 6 months after the baseline CT of the HANSE 3rd round or baseline round respectively to confirm the negative diagnosis according to the mod. Lung RADS 1.1 score used in the HANSE study, except for participants with a positive lung cancer diagnosis on histology.

All high risk participants (by $PLCO_{M2012}$, NELSON, and/or blood biomarker) receiving an LDCT with a positive LC blood test will be informed personally about their positive biomarker results by trained local staff after the date of their (potential) MDT, except for

- low risk participants theoretically switching from low to high risk by an increase of their age by four years, who are then rated as low risk at the on-site physician $PLCO_{M2012}$/NELSON risk confirmation and who are tested positively for LC blood biomarkers afterwards. Such patients will get a second LDCT screening invitation due to their biomarker test results and therefore can conclude that they are biomarker-positive. Consequently, such participants are excluded from the randomization for MDT reporting since blinding cannot be maintained. However, they will also be informed personally about their positive results by trained local staff after the date of their (potential) MDT.

All (high and low risk) participants with negative LC blood test will be informed by mail about their negative blood test results also after the date of their (potential) MDT.

All other objectives and endpoints of the HANSE study remain unchanged (for details, see the initial study protocol in section 9. Attachments).

2.3 Exploratory Objective(s) & Hypothesis(es) (Optional)

N/A

3. METHODOLOGY

The principal methodology of the HANSE study remains unchanged. For better overview, only changes to the initial study protocol will be described in this amendment.

3.1 Study Design – General Aspects

No changes to initial protocol (for details, see section 9. Attachments).

3.1.1 Data Source(s)

In addition to the initially defined data sources, blood samples will be taken voluntarily from all participants of the HANSE study (high risk and low risk participants) and blood biomarkers for early LC detection will be analyzed by a metabolomic blood test of 9 metabolites (BioMark Diagnostics, Richmond, Canada).

In addition, urine sampling and questionnaire completion is planned for participants of the intensified and standard smoking cessation programs within additional study visits at 1, 3, 6, and 12 months after the initial LDCT scan.

3.2 Study Population

The general HANSE study population remains unchanged. However, the high risk population will be extended by initially low risk participants who become high risk by $PLCO_{M2012}$ or NELSON risk criteria re-evaluation.

3.3 Inclusion Criteria

No changes to initial protocol (for details, see section 9. Attachments).

3.4 Exclusion Criteria

No changes to initial protocol (for details, see section 9. Attachments).

3.5 Participant Follow-up (Optional)

In the initial protocol, a regular follow-up of 1 year per patient, resembling two LDCT screening rounds, was planned with an additional follow-up of 5 years to assess LC development and of 10 years to assess cardiovascular and all-cause mortality. Within this amendment, the regular follow-up is to be expanded by a third LDCT screening round at approximately 4 years after the baseline LDCT screening.

For confirmation of a false positive blood test, all participants with a positive LC blood biomarker test and negative LC diagnosis will receive a 2nd LDCT about 6 months after the

baseline CT of the HANSE 3rd round or baseline round respectively to confirm the negative diagnosis according to the mod. Lung RADS 1.1 score used in the HANSE study, except for participants with a positive lung cancer diagnosis on histology. If the LDCT report of this 6 months LDCT is Lung RADS, 3, 4A, 4B or 4X they will be discussed in the MDT.

For analysis of an intensified smoking cessation program, voluntary participants of the two smoking cessation programs will be invited to additional visits at 1, 3, 6, and 12 months after their LDCT scan for determination of cotinine in urine and for completion of questionnaires.

4. VARIABLES AND EPIDEMIOLOGICAL MEASUREMENTS

4.1 Exposures

In the initial study protocol, it was defined that recruited participants in the high risk group undergo 2 low-dose CT screening rounds (baseline and 1 year follow up).

This amendment adds a third LDCT screening round to participants in the initial high risk group at ~4 years follow-up. In addition, participants of the initial low risk group can become high risk by $PLCO_{M2012}$ and NELSON re-evaluation (i.e. due to an increase of their age by ~4 years or changes in personal or behavioural variables). Such newly rated high risk participants will undergo a baseline LDCT screening round. Furthermore, all participants will be asked to provide a blood sample which will be analysed for biomarkers of early LC detection (metabolomic blood test, BioMark Diagnostics, Richmond, Canada). In addition to initially high risk participants and participants switching from low to high risk by $PLCO_{M2012}$ and NELSON reassessment, also blood biomarker-positive participants will be eligible for baseline LDCT screening. MDT discussions of positive LDCT scans will be supplemented with the respective biomarker findings in half of the participants (1:1 randomization, for details, see section 6.1.2). To study the rate of false-positives, blood biomarker-positive low risk participants without detection of lung cancer will undergo a follow-up LDCT after approximately 6 months.

Table 1 Blood biomarkers to be used in HANSE after study amendment.

Biomarker	Biomarker Type	# of Biomarkers	Sensitivity	Specificity	Area under the curve
Metabolomic Blood Test (BioMark Diagnostics)	Metabolites	9	0.93	0.93	0.93

4.1.1 Definition of Primary Drug Exposure (Optional)

Participants who are current smokers and who change from low to high risk by $PLCO_{M2012}$ or NELSON re-evaluation or by a positive blood test will be randomized 1:1 to receive an intensified smoking cessation program or a short standard smoking cessation counselling,

when they show-up for their baseline LDCT exam. The intensified smoking cessation program comprises behavioural counselling and pharmacotherapy with a partial agonist of nicotinic acetylcholine receptors in accordance with the guideline recommendations.[8] The counselling intervention allows for an intensive examination of personal motives and barriers to smoking, while the medication support alleviates withdrawal symptoms and reduces the craving for nicotine. This dual approach significantly increases the chances of permanent smoking cessation. A partial agonist of nicotinic acetylcholine receptors, for example cytisine, will be given for 25 days, and dosage will be tapered over 12 weeks. Behavioural counselling is based on group-based tobacco cessation according to the 4+2 scheme in the sense of behavioural therapy.

4.1.2 Definition of Comparison Drug Exposure (Optional)

N/A

4.2 Outcomes

Primary endpoint:

The primary endpoint will be extended by a third LDCT screening round after follow-up of ~ 4 years to proof and validate the positive results of the HANSE study considering slowly growing lung cancer.

Additional secondary endpoints:

- LC detection rates (PPV) will be assessed for participants initially rated as low risk who become high risk after $PLCO_{M2012}$ and NELSON re-evaluation after ~4 years of follow-up. These LC detection rates will be compared with the rates of the initial high risk participants (NELSON and/or $PLCO_{M2012}$) after the baseline LDCT screening round.

- Success of a guideline-based and intensified smoking cessation counselling will be analyzed in comparison to the standard smoking cessation counselling by:

    - Assessing the proportion of participants being nicotine-free (i.e. absolute abstinence from taking nicotine from any source) after 1, 3, 6, and 12 months (determination of cotinine in urine).

    - Assessing the proportion of participants being abstinent from combustible cigarettes, after 1, 3, 6, and 12 months

    - Survey of the Fagerström test, survey after craving, absence/sick days of the previous year as well as QoL survey

- Assessment of the adverse events of the products as well as the withdrawal symptoms (no nausea, difficulty falling asleep and staying asleep, dry mouth, etc.)
- Assessment of respiratory symptom burden (cough, perceived shortness of breath via mMRC or CAT)
- Optional: Assessment / improvement of the function of the small airways or lung function
- Optional: Assessment / improvement of central blood pressure measurements and arterial vascular stiffness

For this, participants who change from low to high risk by risk re-evaluation and who report to be current smokers will be randomized 1:1 to receive an intensified smoking cessation program or a short standard smoking cessation counselling.

- LC detection rates (PPV) will be assessed after LDCT for low risk participants (after $PLCO_{M2012}$ and NELSON re-evaluation) with positive LC biomarker findings (metabolomic blood test, BioMark Diagnostics, Richmond, Canada) and compared to detection rates of the NELSON and $PLCO_{M2012}$ risk criteria.
- LC detection rates (PPV) and application rates of invasive procedures will be assessed for positive LDCT findings which are discussed within MDT with respect to supplementation with blood biomarker results (metabolomic blood test, BioMark Diagnostics). Therefore, high risk participants who receive an additional LDCT (initial and by risk re-evaluation) will be randomized 1:1 to a biomarker-reporting arm, in which MDT discussions of positive LDCT findings will be supplemented with blood biomarker results, and a control arm without reporting of biomarker results to the MDT.

4.3 Other Variables and Covariates

No changes to initial protocol (for details, see section 9. Attachments).

5. STATISTICAL ANALYSIS PLAN

5.1 Statistical Methods – General Aspects

No major changes to initial protocol (for details, see section 9. Attachments). Only details concerning the new secondary endpoint to study the effectiveness of a guideline-based and

intensified smoking cessation program in comparison to the already included standard smoking cessation counselling are included in section 5.1.2.

5.1.1 Primary Objective(s): Calculation of Epidemiological Measure(s) of Interest (e.g. descriptive statistics, hazard ratios, incidence rates, test/retest reliability)

No changes to initial protocol (for details, see section 9. Attachments).

5.1.2 Secondary Objective(s): Calculation of Epidemiological Measure(s) of Interest (e.g. hazard ratios, incidence rates, test/retest reliability)

Secondary objectives:

- to integrate blood biomarkers prospectively in a LDCT screening program to reduce the number of false positive invasive procedures (resections or biopsies).

Hypotheses: Null hypothesis (H0): The positive predictive value (PPV) for the invasive procedures in the HANSE study in the MDT group with integrated blood biomarker (BioMark Diagnostics, Richmond, Canada) and for the MDT group without integrated blood biomarker (BioMark Diagnostics, Richmond, Canada) are equal.

Alternative hypothesis (H1): The positive predictive value (PPV) for the invasive procedures in the HANSE study in the MDT group with integrated blood biomarker (BioMark Diagnostics, Richmond, Canada) and for the MDT group without integrated blood biomarker (BioMark Diagnostics, Richmond, Canada) are different.

Statistical procedure: Kosinski test (weighted generalized score statistic)

- to report the test performance of the blood test (BioMark Diagnostics, Richmond, Canada) in the low risk and the high risk populations

Statistical procedure: ROC curve analysis, AUC values, sensitivity, specificity.

Statistical significance of the effects of integrating blood biomarker tests into the MDT is determined using the p-values, at which Bonferroni correction for multiple testing will be applied to keep the overall alpha level < 0.05. If the p-value is below the limit yielded by the Bonferroni correction, the null hypothesis can be rejected and the alternative hypothesis that the PPV for invasive procedures is increased by integration of blood tests into the MDT can be accepted.

The study compares an intensified smoking cessation program (including group therapy together with medication and non-medication support) with a standard smoking cessation counselling in terms of the success of nicotine abstinence. The main endpoint is the number of patients who are nicotine-free after 6 months.

Hypotheses: Null hypothesis (H0): The success rates (nicotine abstinence) between the brief intervention and the group therapy are equal.

Alternative hypothesis (H1): The success rates between the brief intervention and the group therapy differ.

Statistical procedure: As this is a binary dependent variable (nicotine abstinence: yes/no), a logistic regression or a chi-square test is suitable. In this case, logistic regression is recommended, as additional covariates can be included in the model.

Procedure for the statistical analysis:

In the first step, the data are cleaned and variables are created which, among other things, code nicotine abstinence after 6 months (1 = nicotine-free, 0 = not nicotine-free) and the intervention groups (0 = standard smoking cessation, 1 = intensified smoking cessation).

The distribution of the characteristics (age, gender, nicotine abstinence, previous lung diseases, CT findings) is calculated in the two groups.

In the third step of the analysis, a logistic regression is performed to evaluate the effect of intensified smoking cessation compared to standard smoking cessation, with the intervention group included as a predictor variable and other variables as covariates to control for. The estimated coefficients, odds ratios (ORs) and confidence intervals (CIs) are documented as results.

In the fourth step, the statistical significance of the effects of the intervention group is determined using the p-values, at which Bonferroni correction for multiple testing will be applied to keep the overall alpha level < 0.05. If the p-value is below the limit yielded by the Bonferroni correction, the null hypothesis can be rejected and the alternative hypothesis that the group therapy is significantly more effective than the brief intervention can be accepted.

In the fifth step, a sensitivity analysis is carried out to check the robustness of the results. The possible influencing factors (age, gender, etc.) are taken into account and interaction effects are examined. Secondary parameters will be analysed by similar analysis models if possible. Safety parameters will be analysed by descriptive statistics only.

The results are interpreted in the context of the efficiency of the forms of intervention and possible limitations of the study are discussed.

5.1.3 Exploratory Objective(s): Calculation of Epidemiological Measure(s) of Interest (e.g. hazard ratios, incidence rates, test/retest reliability)

No changes to initial protocol (for details, see section 9. Attachments).

5.2 Bias

5.2.1 Methods to Minimize Bias

No changes to initial protocol (for details, see section 9. Attachments).

5.2.2 Adjustment for Multiple Comparisons

Bonferroni correction for multiple testing will be applied to keep the family wise error rate of the secondary endpoints < 0.05 when testing for statistical significance of the effects of integrating blood biomarker tests into the MDT as well as of the effect of an intensified smoking cessation program, respectively. If the p-values are below the limit yielded by the Bonferroni correction, the null hypotheses can be rejected and the alternative hypotheses can be accepted.

5.2.3 Strengths and Limitations

The general strengths and limitations of the HANSE study remain unchanged (for details, see section 9. Attachments). However, this protocol amendment possesses the great potential of reducing two important limitations of the initial study protocol:

I) LC detection rates within the low risk cohort, which were up to now only estimated on the basis of the respective $PLCO_{M2012}$ score, can now be proven - at least for participants changing from low to high risk by $PLCO_{M2012}$ and NELSON re-evaluation or by positive blood biomarker results.

II) The relatively short time horizon of the two screening rounds within the initial study protocol will be broadened to ~4 years, including a third screening round. This will allow for confirmation of the significant difference in terms of LC detection between the $PLCO_{M2012}$ ≥1.58 % (6 years) risk score and the NELSON criteria, also considering slowly growing LC cases which might affect LC detection rates.

5.3 Interim Analyses (Optional)

Unchanged from initial study protocol

5.4 Sample Size and Power Calculations

Expected LC cases in initially low risk participants who will receive LDCT screening within this study amendment either by switching to high risk after risk re-evaluation or by positive blood biomarker results for early LC detection will be estimated for LDCT justification.

Low → high risk participants by $PLCO_{M2012}$ re-evaluation
Approximately 800 initially low risk participants are estimated to become high risk by $PLCO_{M2012}$ re-evaluation (≥1.58 % after 6 years). However, correct high risk-status will be

assessed before LDCT performance by qualified medical staff. During the baseline LDCT screening of the HANSE study within initially high risk participants, the median $PLCO_{M2012}$ score was 2.47 % and 64 LC cases were detected within 5.191 LDCT scans. Conservatively assuming a median $PLCO_{M2012}$ score of 1.60 % for participants changing from low to high risk, a total of 6 LC cases can be expected to be found within this population (X = 64 LC cases / 2.47 % / 5.191 LDCT scans * 800 LDCT scans * 1.60 % = 6 LC cases).

Low risk participants with positive blood biomarker findings
7,464 participants initially did not meet the high risk criteria and consented to participate in the HANSE study. Their median $PLCO_{M2012}$ was 0.45 %, wherefore 17 LC cases can be deduced to be detectable within LDCT screening (X = 64 LC cases / 2.47 % / 5.191 LDCT scans * 7,464 LDCT scans * 0.45 % = 17 LC cases). Minus ~800 participants and 6 LC cases changing to high risk after $PLCO_{M2012}$ re-evaluation, approximately 6,600 low risk participants will be eligible for blood sampling, and 11 LC cases can be estimated to be detectable by LDCT within these participants. Taking a sensitivity and specificity of 0.93 each as a basis for the metabolomic blood test (BioMark Diagnostics, Richmond, Canada) used for testing the low risk group (for details, see Table 1 in section 4.1), it can be estimated that approximately 461 low risk participants will be tested positively for LC blood biomarkers and will receive a LDCT scan.

	Lung cancer	No lung cancer
Biomarker-positive	True positive (A)	False positive (B)
Biomarker-negative	False negative (C)	True negative (D)

- Sensitivity = 0.93 = A / (A+C)
- Specificity = 0.93 = D / (B + D)
- A + B + C + D = 6,600 participants
- A = 11 detectable LC cases
- C = A / 0.93 – A = 11 / 0.93 – 11 = 1 case
- D = 0.93 * (B + D) = 0.93 * (6,600 – 11 – 1) = 6,127 cases
- B = 6,600 – 11 – 1 – 6,127 = 461 cases

Supplementation of MDT discussions with blood biomarker results
During LDCT rounds one and two, 236/9,547 (2.47 %) LDCT scans revealed a positive result (LungRADS 4A PET, 4B, or 4X) and were discussed within MDT. Within these 236 cases, 93 LC cases were found, revealing a PPV of 39.4 % (93/236). Subsequently, 184 invasive procedures (biopsies or resections) were performed in which 115 malignancies were found, revealing a PPV of 62.5 % and a rate of false positive invasive procedures of 37.5 %. Taking the rate of 2.47 % positive LDCT results per screening round as a basis and assuming approximately 5,261 LDCT scans to be performed in high risk participants within this amendment (~4,000 initially high risk + ~800 becoming high risk after $PLCO_{M2012}$ and NELSON re-evaluation + ~461 blood biomarker-positive participants), approximately 130

positive LDCT results and 51 LC cases can be estimated to be detected within this screening round. 1:1 randomization of the high risk participants aims at equal distribution of these cases to MDT blood biomarker supplementation (biomarker reporting group, n = 65) and normal MDT discussion (control group, n = 65). If one estimates, that the PPV can be increased from 62.5% to 90 % by the MDT supplementation with blood biomarker results, a total of 128 positive LDCT cases randomized in two groups of 64 are sufficient to achieve a power of 80% and an alpha level of 0.025 if the PPV of the blood biomarker is 86%.

Intensified smoking cessation program
To calculate the number of cases comparing two types of intervention – standard smoking cessation (group B) and intensified smoking cessation (group A) – the following steps were carried out:

Selection of statistical assumptions:

- Effect size (odds ratio, OR): For group A versus group B, the OR is 1.77.
- Effect size (odds ratio, OR): Within group A with or without drug support with an (odds ratio, OR): For the group with drug support versus group B, the OR is 3..5.
- Two-sided significance threshold (alpha): Typically 0.025.
- Power (1 - beta): Typically 0.8 (80%).
- Ratio of group sizes: Assume that the group sizes are equal.

Baseline frequency of the event:

The baseline frequency of the event (nicotine abstinence) in Group B was estimated from the literature in order to accurately perform the case number calculation. For a hypothetical baseline frequency, 6% is used for group B.[8]
For pooled group therapy, a pooled odds ratio is calculated to capture the benefit of total group therapy (with or without medication) over standard smoking cessation.
A pooled odds ratio that takes into account the effect of group therapy (both with and without medication support) is used to calculate the number of cases. To do this, we consider the combined effect of group therapy. Taking into account a dropout rate of 25%, this results in a required total number of participants of 260, with 130 participants per group (brief intervention versus total group therapy).

6. STUDY CONDUCT AND REGULATORY DETAILS

6.1 Study Conduct

6.1.1 Study Flow Chart and Plan

No changes to initial protocol (for details, see section 9. Attachments).

6.1.2 Procedures

General details of the initial protocol remain unchanged and only details differing from the initial protocol will be described (for details, see section 9. Attachments). A flow chart of the planned procedures within this amendment is shown in Figure 2.

Only participants who already gave their informed consent to participate in the HANSE study will be considered to take part in this study amendment. For this, participants will be informed via letter and/or email about the content and purpose of the study amendment and invited to participate. Before participants will be contacted, the $PLCO_{M2012}$ risk score will be re-evaluated for low-risk participants by increasing their initial age by 4 years. Participants revealing a risk score ≥1.58 % (6 years) will be handled as high-risk participants and invited for LDCT screening.

Low risk participants not crossing the risk score ≥1.58 % (6 years) after age-corrected $PLCO_{M2012}$ re-evaluation will be informed and invited for blood sampling. On site, $PLCO_{M2012}$ and NELSON will be re-assessed and participants revealing a $PLCO_{M2012}$ risk score ≥1.58 % (6 years) or fulfilling the NELSON criteria will be handled as high-risk participants and invited for LDCT screening. Blood sampling will take part during two months prior to the start of the third LDCT round, and blood samples will be shipped to BioMark Diagnostics (Richmond, Canada) for early LC detection via the metabolic blood test. Participants with positive test results will be invited for LDCT screening, together with the total high-risk cohort (initially high-risk + high-risk by age-corrected $PLCO_{M2012}$ re-evaluation and by on-site $PLCO_{M2012}$ and NELSON re-assessment). All participants with positive LDCT results scheduled for MDT conference will be randomized 1:1 to a biomarker reporting group or a control group. MDT discussions of positive LDCT findings within the reporting group will be supplemented with the respective biomarker findings, whereas MDT discussions of the control group will take place without biomarker results.

All participants with a positive LC blood biomarker test will receive a 2nd LDCT about 6 months after the baseline CT of the HANSE 3rd round or baseline round respectively to confirm the negative diagnosis according to the mod. Lung RADS 1.1 score used in the HANSE study, except for participants with a positive lung cancer diagnosis on histology.

All high-risk participants receiving an LDCT with a positive LC blood test will be informed personally about their positive results by trained local staff after the date of their (potential) MDT, except for

- low risk participants theoretically switching from low to high risk by an increase of their age by four years, who are then rated as low risk at the on-site physician $PLCO_{M2012}$/NELSON risk confirmation and who are tested positively for LC blood biomarkers afterwards. Such patients will get a second LDCT screening invitation due to their biomarker test results and therefore can conclude that they are biomarker-positive. Consequently, such participants are excluded from the randomization for MDT reporting since blinding can not be maintained. However, they

will also be informed personally about their positive results by trained local staff after the date of their (potential) MDT.

All (high- and low-risk) participants with negative LC blood test will be informed by mail about their negative blood test results also after the date of their (potential) MDT.

Participants eligible for LDCT (low risk participants with positive biomarker test results as well as the total high-risk cohort) will be invited for LDCT screening. All study participants are required to sign informed consent. All participants except for the original high-risk cohort, which received at least one baseline MDCT (n=5191), will be assessed for their final LDCT eligibility (check for the initially defined inclusion and exclusion criteria, for details, see sections 3.3 and 3.4 within the initial study protocol which can be found on section 9. Attachments) at the study sites by qualified medical staff prior to the LDCT scan. Participants of the total high-risk cohort will answer standardized questionnaires as previously used for the baseline and one year LDCT screening rounds. In addition, these participants will be asked to provide blood samples for biomarker analyses of early LC detection (metabolomic blood test, BioMark Diagnostics, Richmond, Canada) and will be randomized 1:1 to a biomarker reporting group or a control group, if they have a positive LDCT exam (LungRADS 4A PET, 4B or 4X). MDT discussions of positive LDCT findings within the reporting group will be supplemented with the respective biomarker findings, whereas MDT discussions of the control group will take place without biomarker results.

LDCT performance tests and workup will be conducted as described within the initial study protocol, except for MDT discussions of positive LDCT findings, which will be supplemented with the biomarker results in half of the participants.

In addition, participants who are current smokers and who change from low to high risk by $PLCO_{M2012}$ or NELSON re-evaluation or by a positive blood test will be randomized 1:1 to receive an intensified smoking cessation program or a standard smoking cessation counselling following the WHO recommendations of 5 R’s and 5 A’s, when they show-up for their baseline LDCT exam. The intensified smoking cessation program comprises behavioural counselling and pharmacotherapy with a partial agonist of nicotinic acetylcholine receptors in accordance with the guideline recommendations.[8] During the standard smoking cessation counselling, participants are informed about and encouraged to participate in smoking cessation programs comprising professionally guided group courses within short counselling sessions by trained staff. Participation is fully voluntary.

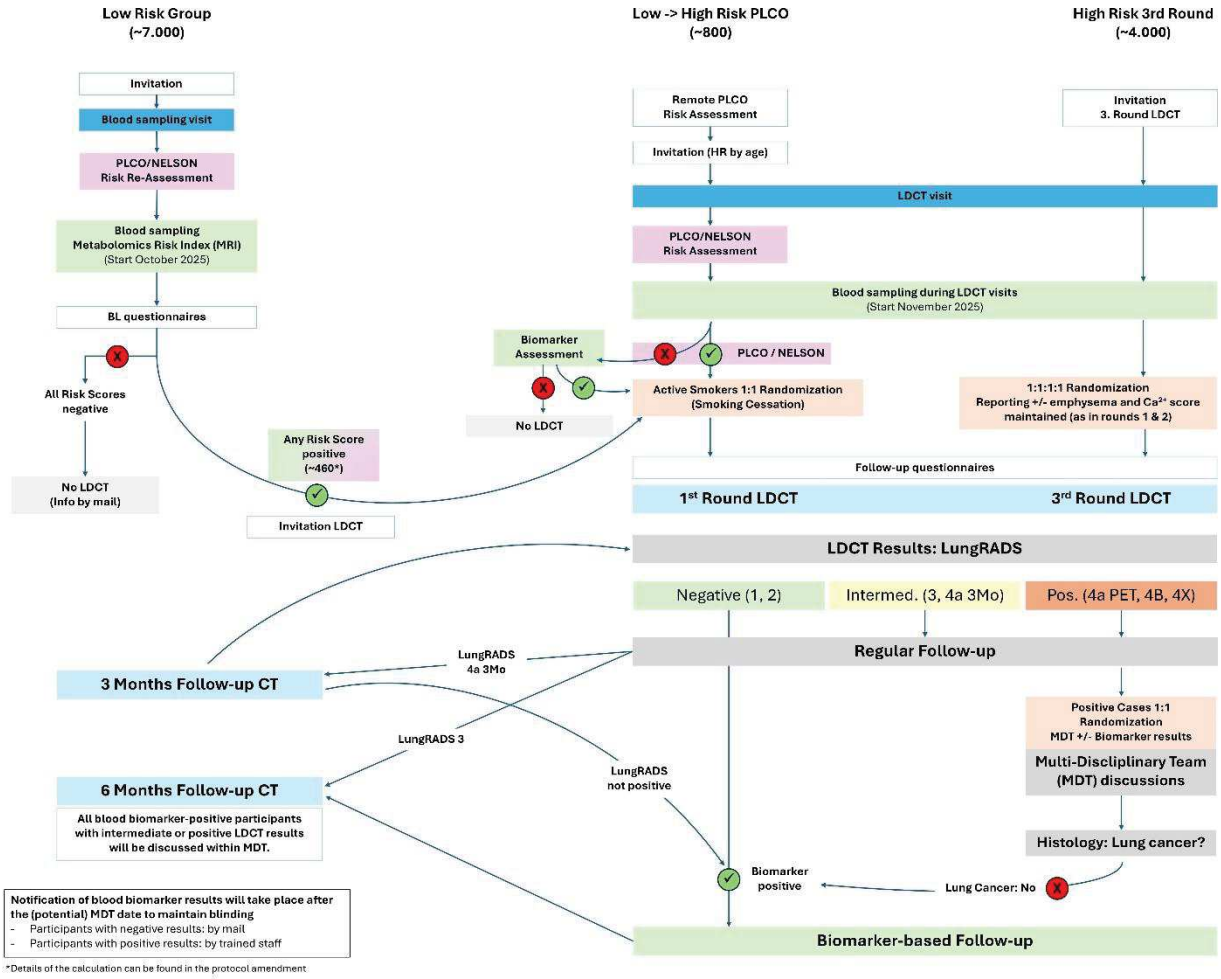

Figure 2 Procedural flow chart of the study amendment

Visit type 1: risk assessment and blood sampling of low risk participants

After invitation, low risk participants willing to provide a blood sample will be able to schedule an appointment at their preferred study site. On site, participants will be informed by qualified medical staff and their written consent will be obtained. Afterwards, participants' variables will be collected to re-assess their $PLCO_{M2012}$ and NELSON score. Participants revealing a $PLCO_{M2012}$ a risk score ≥1.58 % (6 years) or positive NELSON score will be handled as high risk participants and can schedule an appointment for LDCT screening (visit type 2). Blood samples for biomarker analysis of early LC detection will be obtained from all low risk participants, and blood samples will be shipped to BioMark Diagnostics (Richmond, Canada) for early LC detection via the metabolic blood test. After receiving the test results, low risk participants with a negative blood test will be informed about their test results via letter and/or email. Low risk participants with a positive blood test will be informed personally about their positive results by trained local staff after the date of their (potential) MDT. As they need to be informed that they have been reassessed to be in the high-risk group to be able to schedule an LDCT exam (visit type 2), they will be told initially that a reassessment of their general personal data at their initial visit has scored them into the high-

risk group in order to keep them blinded for potential randomisation for the MDT if they have a positive LDCT..

All participants will:

- Be questioned whether they receive treatment for any cardiovascular conditions.
- Be assessed of current COPD treatment medication.
- Be asked to answer a 'consequences of screening' survey

Visit type 2: LDCT of high risk participants.

All high risk participants (either by initial scoring or by $PLCO_{M2012}$ and NELSON re-assessment or by positive blood test) will be invited to schedule an appointment for LDCT screening. In case of initially scored high risk participants, LDCT of this amendment reflects a third screening round, whereas in case of participants changing from low to high risk during conduct of this amendment LDCT reflects a first screening round.
On site, all high risk participants will be informed by qualified medical staff and their written consent will be obtained. Variables will be collected to confirm the high risk status of the high-risk subgroup with estimated $PLCO_{M2012}$ age adapted (4y) risk score based on the two risk scoring models (NELSON/PLCO) and the final LDCT eligibility (check for the initially defined inclusion and exclusion criteria, for details, see sections 3.3 and 3.4 within the initial study protocol which can be found on section 9. Attachments) will be assessed by qualified medical staff prior to the LDCT scan.

As described in the initial study protocol, all participants will:

- Be questioned whether they receive treatment for any cardiovascular conditions or whether they initiated treatment for any cardiovascular conditions during the study
- Be assessed for their smoking status
- Be assessed of current COPD treatment medication and treatment initiation during the study
- Be asked to answer a 'consequences of screening' survey

prior to undergoing LDCT. Lung LDCT assessment will be conducted as described in the initial study protocol and randomization to the four reporting groups will be maintained for participants initially scored as high risk. Importantly, all high risk participants (initial or by risk re-evaluation) will be randomized after receiving the initial LDCT scan if their LDCT result is positive (Lung RADS 4A PET, 4B, 4X). Randomization will not affect the procedure and the schedule of the cancer screening process but only determine the allocation of the participants to a biomarker reporting group, in which MDT discussions are supplemented by biomarker results, or a control group. Randomization ratio of 1:1 will provide equally sized groups of patients in both groups using the actual $PLCO_{M2012}$ score (by reassessment) as stratification factor. Patients being rated as low risk by $PLCO_{M2012}$ and NELSON reassessment during visit type 2 and who will be tested positively for LC blood biomarkers

afterwards will be excluded from this randomization since they can deduce their positive blood biomarker findings from the procedural steps and become unblinded. This randomization is independent of and will not affect the initial reporting group randomization.

Differing from the initial study protocol, participants changing from low to high risk will not be randomized into a reporting group, and coronary calcium score and emphysema score will always be reported in this group. Instead, participants of this group who reported to be current smokers will be randomized to an intensified smoking cessation program and a control group with standard smoking cessation counselling. Randomization ratio of 1:1 will provide equally sized groups of patients in both groups including age (5 year groups) and sex stratification.

Visit type 3: follow-up LDCT schedule

In accordance with the initial study protocol, follow-up LDCT scans will be scheduled according to the LDCT findings (LungRADS categories). In case of LungRADS 3, an additional LDCT will be performed after ~6 months. In case of LungRADS 4A 3months, an additional LDCT will be performed after ~3 months.

All participants with a positive LC blood biomarker test will receive a 2nd LDCT about 6 months after the baseline CT of the HANSE 3rd round or baseline round respectively to confirm the negative diagnosis according to the mod. Lung RADS 1.1 score used in the HANSE study, except for participants with a positive lung cancer diagnosis on histology. In case the first LDCT scan of a blood biomarker-positive participant reveals LungRADS 3, the regular ~6 months follow-up LDCT will be the same as the blood biomarker-based follow-up. All participants with positive blood biomarker results who are scored intermediate (LungRADS 3 or 4A 3months) or positive (LungRADS 4A PET, 4B, 4X) at their 6 months follow-up LDCT will be discussed in the MDT. Such patients will, however, not be randomized since their positive blood biomarker status is known at that time point.

Visit type 4: additional smoking cessation visits

In addition to behavioural counselling and pharmacotherapy (intensified program) and information about voluntary group courses (standard program), participants of both smoking cessation programs will be invited for additional visits at 1, 3, 6, and 12 months after the initial LDCT scan. Within these visits, urine will be analysed for cotinine as a measure of nicotine consumption and questionnaires (Fagerström test, craving survey, absence/sick days of the previous year, as well as QoL survey) will be completed by the participants.

6.1.3 Quality Control

No changes to initial protocol (for details, see section 9. Attachments).

6.2 Protection of Human Subjects

General details of the initial protocol remain unchanged (for details, see section 9. Attachments).

However, since the procedures described within this protocol amendment are not part of the initial protocol and hence not described within the initial patient information sheets and the informed consent forms, these documents will be adapted respectively, participants will be newly informed, and the written informed consent (re-consent) will be obtained before conduct of any of the procedures described within this protocol amendment.

6.3 Collection and Reporting of Adverse Events/Adverse Drug Reactions/Special Situations

No changes to initial protocol (for details, see section 9. Attachments).

7. LIST OF REFERENCES

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8. APPENDICES

No changes to initial protocol (for details, see section 9. Attachments).

9. ATTACHMENTS

KB-Observational+ESCR+Protocol+HANSE·