Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	#1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	#5
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set 1.**Primary Registry and Trial Identifying Number** ClinicalTrials.gov ID: NCT03651986 2. **Date of Registration in Primary Registry**	

August 29th, 2018

3.**Secondary Identifying Numbers**

N/A

4.**Source(s) of Monetary or Material Support**

Monetary and Material Support: AnchorDx Medical Co., Ltd.

5.**Primary Sponsor**

National Clinical Research Center for Respiratory Disease, First Affiliated Hospital, Guangzhou Medical University

6.**Secondary Sponsor(s)**

AnchorDx Medical Co., Ltd.

7.**Contact for Public Queries**

Central Contact Person: Bo WANG;

Telephone: +86-15920405979

Email: bo_wang@anchordx.com

Address: Unit 502, No.8, Luoxuan 3rd Road, International Bio-Island, Guangzhou, China, 510300

8.**Contact for Scientific Queries**

Central Contact: Wenhua LIANG, MD

Telephone: +86-13710249454

Email: liangwh1987@163.com

Address: No.151, Yanjiang Road, The First Affiliated Hospital of Guangzhou Medical University, Yuexiu District, Guangzhou, China, 510120

9.**Public Title**

Diagnostic and Monitoring Significance of Circulating Tumor DNA (ctDNA) Methylation Analysis by Next-

Generation Sequencing in Benign and Malignant Pulmonary Nodules

10.**Scientific Title**

The differential diagnosis and surveillance of pulmonary nodules with high-throughput DNA methylation sequencing of circulating tumor DNA: a prospective, observational, and multicenter clinical trial

11.**Countries of Recruitment**

China only

12.**Health Condition(s) or Problem(s) Studied**

Pulmonary nodules diagnosed by chest CT or low-dose computed tomography (LDCT) scans

13.**Intervention(s)**

This study is an observational study and will not interfere with the diagnosis and treatment of patients.

A blood-based assay for differentiating benign and malignant pulmonary nodules early using circulating tumor DNA (ctDNA) methylation analysis by next-generation sequencing (NGS) will be done at the end of this trial.

14.**Key Inclusion and Exclusion Criteria**

Inclusion Criteria：

1). Either sex, age ⩾ 18 years;

2). Patients with positive pulmonary nodules detected by standard-dose or low-dose CT screening:

2.1) The diameter of positive pulmonary non-calcified nodules is 5 to 30 mm;

2.2) Three imaging types of pulmonary nodules will be included, solid nodules, part-solid nodules (mixed ground-glass nodules) and pure ground-glass nodules.

3). Patients with newly positive pulmonary nodules detected by baseline CT were initially diagnosed or received within 60 days before enrollment;

4). Participants who are willing to fill in *The Subjects' Lung History Questionnaire*;

5). Patients accept follow-up for 2 to 3 years and cooperate with the relevant imaging, serological or surgery and other examination;

6). Ability to fully understand the informed consent, agree to participate in the study and sign the informed consent.

Exclusion criteria:

1). Pregnant or lactating females;

2). Patients underwent any diagnostic puncture therapy, such as percutaneous lung biopsy, transbronchial biopsy or surgery prior to enrollment;

3). Receive any blood transfusion therapy within 30 days prior to enrollment;

4). Patients with cancer confirmed pathologically within 2 years prior to enrollment except non-melanoma skin cancer;

5). Inability to understand or obtain informed written consent.

15.**Study Type**

1). Type of study: observational

2). Study design: a stratified case-cohort design with the purpose of assessing the diagnostic and surveillance values of circulating tumor DNA (ctDNA) methylation markers for pulmonary nodule differentiation

3). Phase: n/a

16.**Date of First Enrollment**

October 26th, 2018

17.**Sample Size**

1). Number of participants that the trial plans to enroll in total: 10560

2). Number of participants that the trial has enrolled: 7000

18.**Recruitment Status**

Recruiting: participants are currently being recruited and enrolled

19.**Primary Outcome(s)**

1). The name of the outcome: The efficacy of the blood-based ctDNA methylation assay comparing with pathologic diagnosis, the gold standard, and CT/LDCT diagnosis, including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

2). The metric or method of measurement used: targeted high-throughput DNA methylation sequencing of circulating tumor DNA (ctDNA)

3). The timepoint(s) of interest: n/a

20.**Key Secondary Outcomes**

1). Outcome Name: The diagnostic performance of the combination of routine tests and ctDNA methylation analysis by NGS in differentiating benign and malignant pulmonary nodules, including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

2). The metric or method of measurement used: targeted high-throughput DNA methylation sequencing of circulating tumor DNA (ctDNA)

3). The timepoint(s) of interest: n/a

21.**Ethics Review**

		1). Status: Approved (Approval Number: 2018-81) 2). Date of approval: August 29th, 2018 3). Name and contact details of Ethics committee(s): Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University Phone: +86-20-83382991 Email: gyfyyec@163.com Address: No.151, Yanjiang Road, The First Affiliated Hospital of Guangzhou Medical University, Yuexiu District, Guangzhou, China, 510120 22.**Completion date** The completion of data collection is anticipated by March 2023. 23.**Summary Results** n/a 24.**IPD sharing statement** 1). Plan to share IPD: No 2). Plan description: n/a	
Protocol version	#3	Date and version identifier Version 1.0, March 24th, 2018	
Funding	#4	Sources and types of financial, material, and other support	#25
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	#25
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	#4

Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	#25
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	#6-#9
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	#6
Objectives	#7	Specific objectives or hypotheses	#13
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	#10-#12
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	#11
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centers and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	#14-#15
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	#15
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	#16

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	#8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	#24
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	#13-#14
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (*e.g.*, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n/a
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n/a

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	#20-#21
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	#11-#12
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	#20

Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	#11-#12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	#18-#19
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n/a
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	#21
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	#23-#24
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	#20

Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	#20
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	#20
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	#22
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	#22
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	#25

Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	#22-#23
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions]	#22-23
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	#22-23
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	#22-23
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorized surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

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*As the checklist was provided upon initial submission, the page number/line number reported may be changed due to copyediting and may not be referable in the published version.