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Supplementary information

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Early detection of multiple cancer types using multidimensional cell-free DNA fragmentomics

In the format provided by the authors and unedited

Supplementary information

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Supplementary Note

Study design and participants of the case-control study

In the model construction phase, plasma samples were retrospectively collected from multiple participating research centers, which included Fudan University Shanghai Cancer Center, Peking Union Medical College Hospital and China-Japan Friendship Hospital, Changzheng Hospital, The First Affiliated Hospital of Guangzhou Medical University, Sun Yat-sen University Cancer Center, Jiangsu Cancer Hospital, Jiangsu Provincial People's Hospital, The Second Affiliated Hospital of Air Force Medical University, Nanjing Drum Tower Hospital, Sun Yat-sen Memorial Hospital, and repeated contributions from The First Affiliated Hospital of Guangzhou Medical University, Jiangsu Provincial People's Hospital, and The First Affiliated Hospital of China Medical University. In the independent validation phase, participants were prospectively enrolled from a variety of research centers, ensuring a broad regional representation and diversity of the patient population in China. The centers included were Zhongshan Hospital affiliated with Fudan University, The First Affiliated Hospital of Guangzhou Medical University, which contributed on multiple occasions, Sun Yat-sen University Cancer Center, Sun Yat-sen Memorial Hospital, Shanghai Pulmonary Hospital, Tianjin Medical University Cancer Institute and Hospital, Beijing Obstetrics and Gynecology Hospital, Hunan Cancer Hospital, The Second Xiangya Hospital of Central South University, Fujian Medical University Cancer Hospital, Jiangsu Provincial People's Hospital, The Third Affiliated Hospital of Harbin Medical University, The Second Affiliated Hospital of Zhejiang University School of Medicine, General Hospital of Eastern Theater Command Qinhuai Medical Zone, Sun Yat-sen University Cancer Center, and The First Medical Center of PLA General Hospital.

For the independent validation set, the analysts who performed sequencing, quality control, and classification analyses were blinded to the clinical details, such as cancer or non-cancer status. Likewise, the results of the sequencing, including the determination of cancer or non-cancer status and predicted tissue of origin, along with the quality control results, were kept undisclosed to the executive team responsible for gathering the clinical information, up until the point of clinical data lock. Each research center had received approval from its local institutional review board.

Inclusion criteria

Non-cancer participants were required to meet the following inclusion criteria: a minimum age of 20 years and the ability to provide written informed consent. For cancer participants, eligibility was determined based on a minimum age of 20 years and a confirmed diagnosis (pathologically or radiologically) of any stage of the following cancer types: breast, cervical, colorectal, endometrial, esophageal, gastric, liver, lung, ovarian, pancreatic, prostate, bile duct, or lymphoma.

Exclusion criteria

Individuals with a known current or previous diagnosis of cancer were excluded from the non-cancer participant group. The exclusion criteria for cancer and non-cancer participants are as follows: pregnant women; individuals who had undergone an organ transplant or a non-autologous bone marrow or stem cell transplant; individuals who had received a blood transfusion within 30 days prior to the MCED test; individuals who had experienced an acute infection or inflammation within 14 days prior to the blood sampling; individuals who had taken medication with anti-tumor effects within 30 days prior to the blood sampling.

Additionally for cancer patients, individuals who were with concurrent or heterozygous for other malignant tumors or multiple primary tumors, with pre-cancerous lesions confirmed by pathology of tissue biopsy or surgical specimens, or currently undergoing or had previously received

treatment for their current cancer including, but not limited to, surgical oncology treatment for any purpose, local or systemic radiotherapy, targeted therapy (including anti-angiogenic drugs), immunotherapy, cancer vaccines, and hormone therapy.

Feature Analysis for Cancer Detection and Tissue of Origin Classification

Copy Number Variations (CNVs) and Fragment Size Ratio (FSR)

Supplementary Figures S1A and S1B illustrate significant differences in CNVs and FSR between cancer and non-cancer samples. These variations are foundational in identifying cancer-specific genomic alterations, which are crucial for the Detection-of-Cancer (DOC) classifier.

Fragment Size Distribution (FSD)

Supplementary Figure S1C highlights that specific FSD features significantly differentiate cancer patients from non-cancer patients in our model construction cohort. For instance, colorectal cancer samples show notable differences in the 150–154 bp fragment range on chromosome 13q, a region frequently aberrant in colorectal cancers, corroborating findings by Neklason et al(*1*).. Similarly, esophageal cancer samples exhibit distinctive fragment distributions in the 115–119 bp range on chromosome 3q, aligning with known alterations reported by Rao et al(*2*). Lymphoma samples display significant differences in the 135–139 bp range on chromosome 9p, consistent with aberrations detailed by Bentz et al(*3*). These examples, presented in Supplementary Figure S2A, demonstrate how specific FSD features contribute to distinguishing both cancer presence and tissue of origin. The fragmentation patterns are hypothesized to result from the interplay between tumor-specific chromatin architecture and nuclease accessibility.

Nucleosome Profiling (NP)

Supplementary Figure S1D showcases several genes where NP features differentiate between

cancerous and non-cancerous samples. These features are associated with cancer proliferation or suppression(*4-7*). Changes in nucleosome occupancy at transcription factor binding sites (TFBS) reflect aberrant epigenetic regulation across different cancer types (Supplementary Figure S2A). For example, altered nucleosome profiles are observed around the TFBSs of KDM2B(*8*) in pancreatic cancers and CXXC1(*9*) in liver cancers. In lymphoma samples, enhanced nucleosome protection around the BATF TFBS is noted, consistent with BATF's role in lymphoid cell differentiation and its dysregulation in lymphoma pathogenesis(*10*). These NP features significantly enhance the accuracy of the TOO classifier by capturing cancer-specific and tissue-specific chromatin alterations, resulting from aberrant transcription factor activity and chromatin remodeling in cancer cells.

Fragment-based Methylation (FM)

Supplementary Figure S1E presents differences in FM values between cancerous and non-cancerous samples, showing significant trends correlating with higher cancer stages. Cancers such as liver, lung, and lymphoma, which exhibit distinct patterns of CpG methylation, also display notable differences in FM values (Supplementary Figure S2C). These findings suggest that FM features effectively contribute to both the DOC and TOO classifiers by capturing cancer-specific methylation alterations.

In summary, the integration of CNVs, FSR, FSD, NP, and FM features provides a comprehensive framework for distinguishing cancerous from non-cancerous samples and accurately identifying the tissue of origin. These multidimensional features enhance the robustness of the DOC and TOO classifiers, underscoring the importance of combining genomic and epigenomic data in cancer diagnostics.

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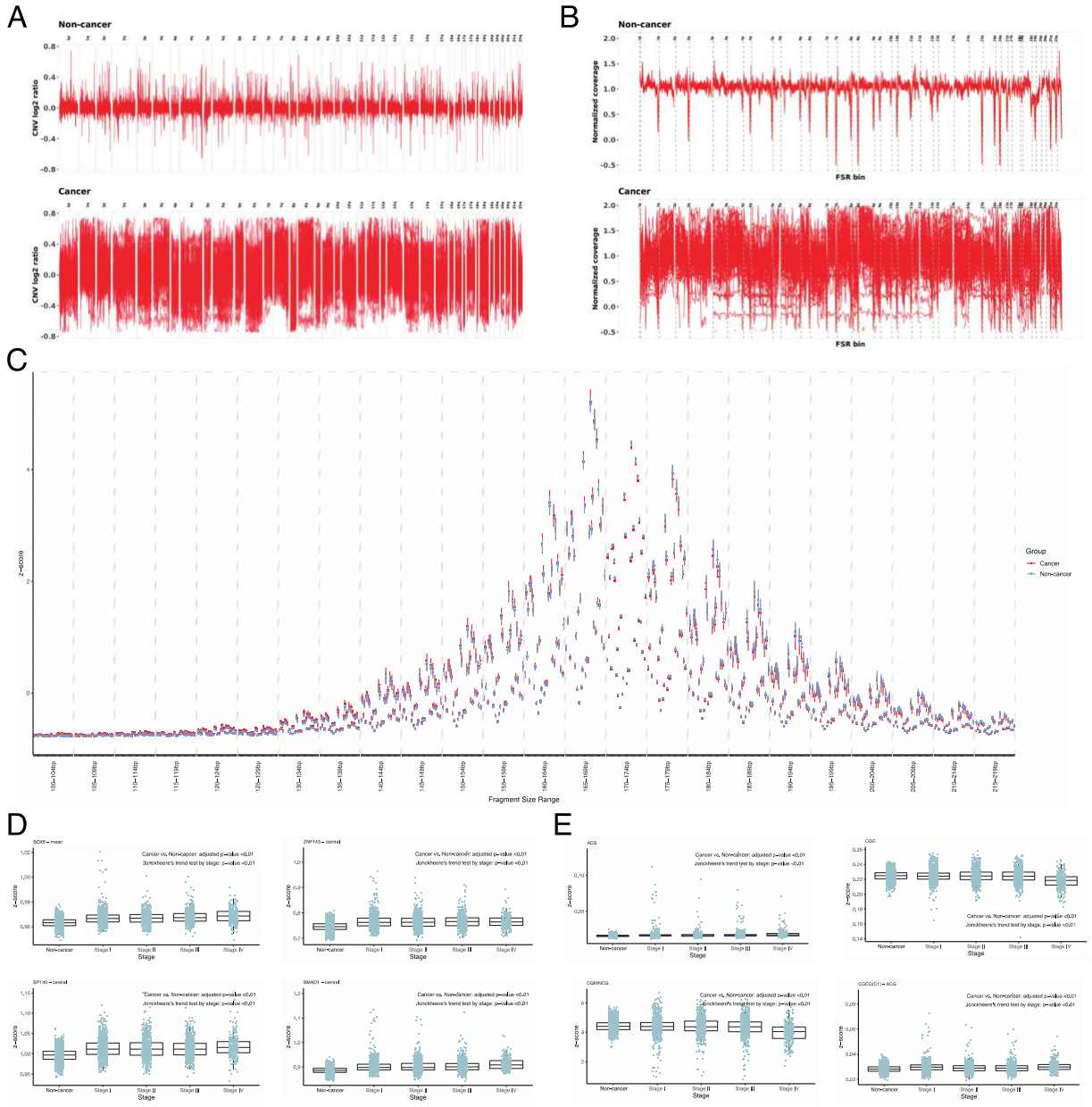
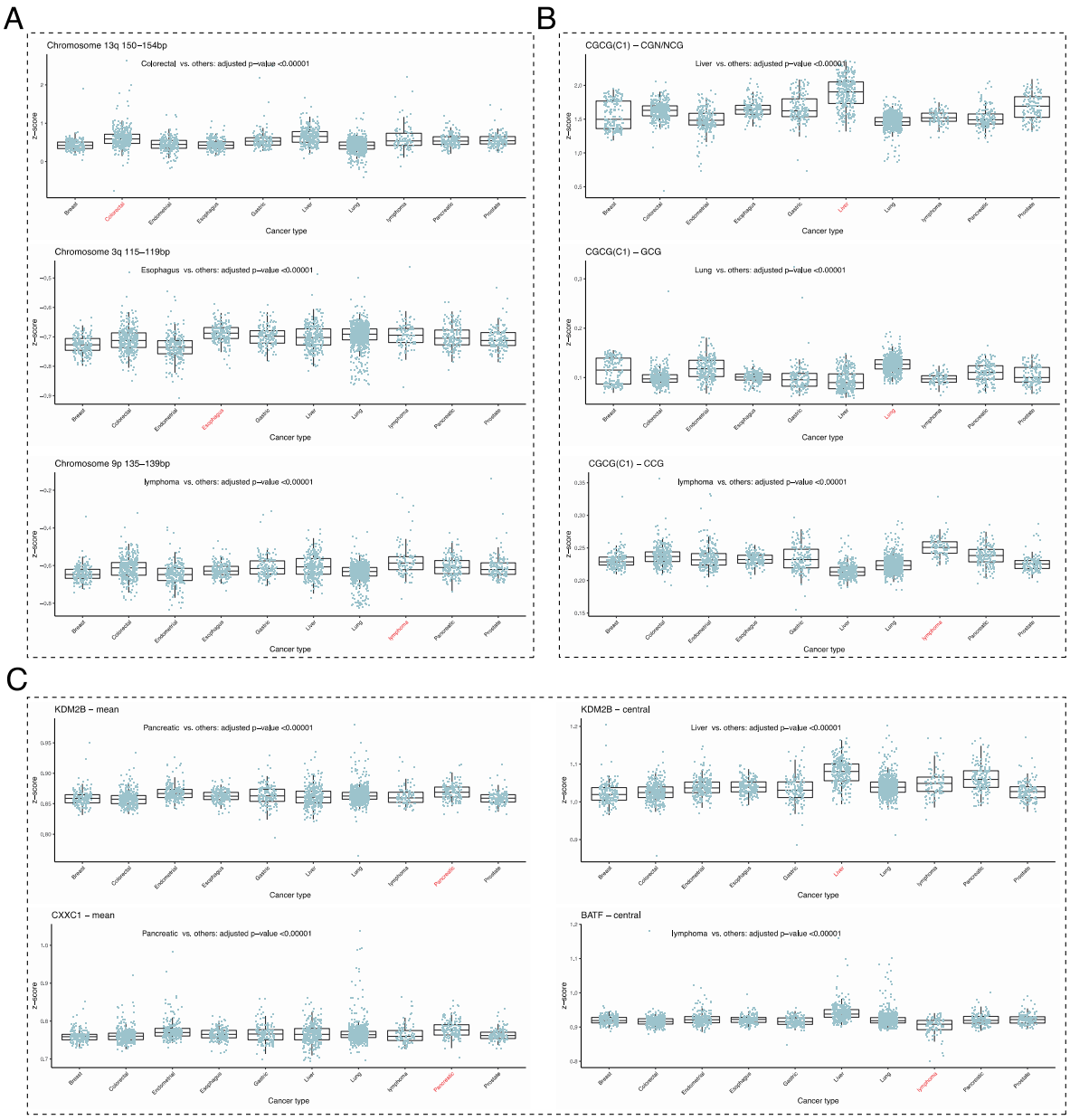
Supplementary Figures



Supplementary Figure 1. (A) Copy Number Variation (CNV) profiles for non-cancer and cancer samples in model construction cohort. (B) Fragment Size Ratio (FSR) distributions in non-cancer and cancer samples in model construction cohort, highlighting distinct fragmentation patterns associated with cancer. (C) Fragment Size Distribution (FSD) analysis, showing significant differences between cancer and non-cancer patients in model construction cohort. (D) Nucleosome

Profiling (NP) features across various genes, demonstrating differences in nucleosome occupancy at transcription factor binding sites between cancerous and non-cancerous samples in model construction cohort. (E) Fragment-based Methylation (FM) values, indicating significant methylation differences between cancer and non-cancer samples in model construction cohort, correlated with cancer stages.



Supplementary Figure S2 (A) Detailed FSD features on chromosomes 13q, 3q, and 9p, illustrating cancer-specific fragmentation patterns in colorectal, esophageal, and lymphoma samples in model construction cohort. (B) NP features for genes KDM2B, CXXC1, and BATF, showing altered nucleosome profiles in pancreatic, liver, and lymphoma cancers, respectively, in model construction cohort. (C) FM values in liver, lung, and lymphoma cancers in model

construction cohort

STUDY TITLE:	A Prospective, Multicenter Cohort Study of the MCED Test CanScan in an Average Risk Asymptomatic Population
INTERNAL SHORT TITLE	Jinling Cohort
NCT ID:	NCT06011694
ETHICS REF:	XZ-2021032, 20230106-k076
SPNSOR:	Nanjing Geneseeq Technology, Inc. 128 Huakang Rd, Nanjing, Jiangsu, China 210032
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PROTOCOL DATE:	January 4th, 2023
VERSION	5.0

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Protocol Approval Signature Page

Protocol Title	A Prospective, Multicenter Cohort Study of the MCED Test CanScan in an Average Risk Asymptomatic Population
Date	January 4th, 2023
Version	5.0

Reviewed and Approved by:

Reviewed and Approved by:

Reviewed and Approved by:

_____	_____	_____
Principal Investigator Printed Name	PI Signature	Date

Table of Content

Document Control Page

Study Name
A Prospective, Multicenter Cohort Study of the MCED Test CanScan in an Average Risk Asymptomatic Population
Study Short Title
Jingling Cohort

Protocol Version
5.0
Version Date
January 4th, 2023

Sponsor
Nanjing Geneseeq Technology, Inc.

Study Design
Prospective observational multicenter cohort, no intervention

Study Participants
15,000 eligible individuals aged 45-75 in Nanjing China

Planned Study Period
2022.06-2028.05

Protocol Revisions

Version:	Version Date:	Changes:
JL_cohort_protocol_v1.0	2021.04.29	First version
JL_cohort_protocol_v2.0	2021.06.25	Updated secondary analysis; Updated design of the Questionnaire and the Jinling cohort official app
JL_cohort_protocol_v3.0	2021.09.06	A criteria of cancer-related physical examination abnormality was established according to China Guideline for Cancer Screening and CSCO guidelines; add data authorization into the informed consent
JL_cohort_protocol_v4.0	2022.04.11	Add The Fourth Affiliated Hospital of Nanjing Medical University as a study site.
JL_cohort_protocol_v5.0	2023.01.04	Sample size increased and set to 15,000.

1 Background

Cancer is a leading cause of mortality in the world; in 2022, there were an estimated 20 million new cancer cases and 9.7 million deaths[1]. Cancer is a major public health problem in China with about 4.8 million new cancer cases and 2.6 million deaths in 2022[2]. Lung, breast, colorectum, and prostate cancers are the most common cancers[1]. The treatment and prognosis for different stages of cancer have significant differences. Curative treatment is the main goal for early-stage cancers as they have a high cure rate. However, prognosis is poor for advanced cancers, with a far lower 5-year survival rate compared to treating cancers in early-stage. The 5-year survival rate for lung cancer is 65% for stage I and 5% for stage IV. The 5-year relative survival rate for stage I breast cancer approaches 100%, but declines to 28% for stage IV breast cancer[3]. The cost of treatment for advanced cancer far exceeds the cost of treatment for early cancer. However, many cancers are asymptomatic or mildly symptomatic; disregard or inaccessibility of cancer screening misses the opportunity for cancer detection at an early stage. Since many cancers are diagnosed at an advanced stage, treatment options are limited and have a poor prognosis. Detection of cancer at an earlier stage can enable more effective treatment and more favorable prognosis, at lower cost.

Several screening methods have been used for specific cancers, reducing cancer-specific mortality. The United States Preventive Services Task Force (USPSTF) currently recommends breast, cervical, colorectal, and lung cancer-specific screening interventions for the at-risk population[4]. Colonoscopy is the gold standard for

colorectal cancer screening. Colorectal cancer can also benefit from stool tests. Breast cancer screening relies mostly on imaging techniques such as mammography and ultrasonography. HPV detection and cervical cytology can be applied for cervical cancer. Low-dose computed tomography (LDCT) is offered for lung cancer screening. Though these screening methods have increased survival rates, they still have some limitations, including radiation exposure, high cost, invasiveness, and scheduling challenges, therefore leading to poor adherence. In United States, adherence to colonoscopy is 13.2% for colorectal cancer screening [5]. The Positive Predictive Value (PPV) of these methods are not high enough with high false positives rate, leading to potential overdiagnosis. The PPV for mammograms is only 4.4%[6]. Though these screening methods are not perfect, their application has improved the survival rate. Many cancers still lack a consensus on adequate screening methods, which account for 59% cancer deaths. Thus, there remains a substantial need for simple, accurate, and convenient methods that are available for a larger part of the population and can detect a wider range of cancers.

Blood-based liquid biopsy is a promising method for cancer early detection in a non-invasive manner[7]. Cancer cells release cancer-specific biomarkers into the blood. These biomarkers can be used for cancer detection with the information on the tissue of origin. Liquid biopsy has potential for multi-cancer early detection (MCED) by analyzing biomarkers in the body fluids; this is different from traditional methods, which can only detect one cancer at a time. Cell-free DNA (cfDNA) is most commonly extracted for liquid biopsy. cfDNA is fragmented DNA released into peripheral blood

after cell apoptosis and necrosis, containing information on its origin. cfDNA released from cancer cells refers to circulating tumor DNA (ctDNA), which carries biosignatures of cancer, including mutations, copy number alternations, methylation, and fragmentation. Several MECD tests have been developed based on different signatures of ctDNA[8].

2 Geneseeq's CanScan MCED test

Geneseeq Technology Inc. has developed an innovative liquid biopsy early screening technology, MERCURY, utilizing a multi-dimensional model based on various features of cfDNA, including copy number variation (CNV), nucleosome profiling, and fragmentomic profiles, derived from low-coverage whole genomic sequencing (WGS). MERCURY was capable of single cancer or multi-cancer detection with high performance, validated by a series of DECIPHER studies[9-17]. Liver, colon, and lung cancer early detection has sensitivity of 96.8% and specificity of 98.8%, sensitivity of 98% and specificity of 94.8%, sensitivity of 94.7% and specificity of 98%, respectively. The MCED blood test, CANSCAN applies MERCURY technology, and is able to identify thirteen cancer types, including breast, cervical, colorectal, endometrial, esophageal, gastric, liver, lung, ovarian, pancreatic, prostate, bile duct, and lymphoma. These cancers cover about 66% of new cancer cases and 73% of cancer-related mortalities worldwide[1]. In China, these percentages translate to about 70% of new cancer cases and 80% of cancer-related mortalities[2]. CANSCAN contains the test assay and bioinformatics analysis pipeline, combining WGS to detect cfDNA and an AI machine learning model to analyze cancer signals. With one tube of blood,

CANSCAN can detect multiple cancers and predict the tissue of origin. The test is sensitive and convenient with a lower cost compared to traditional screening methods. CANSCAN is recommended for individuals aged 45 and over with an average risk of cancer, as complement to routine cancer screening tests. CANSCAN received CE certification from the European Union and Breakthrough Device designation from the U.S. Food and Drug Administration (FDA) successfully in 2023.

Previous case-control studies showed CANSCAN was able to detect multiple cancers with high sensitivity and specificity[12]. A large prospective study is indispensable in evaluating the performance and clinical utility of an MCED test. The prospective cohort (the JINLING study, NCT06011694) will present the results in the intended use population for the MECD test.

3 Study Design

The Jinling Cohort is a large-scale prospective, multicenter cohort study that will enroll 15,000 eligible individuals aged 45-75 in Nanjing China. The study aims to assess the performance and the clinical utility of the liquid biopsy MCED test CanScan in an average risk asymptomatic population.

Residents from local communities in the Jiangbei New Area of Nanjing will be invited to participate. After obtaining informed consent, each eligible participant will undergo peripheral blood collections for the CanScan MCED test, a health questionnaire, and annual routine physical exams once a year for three consecutive years. Participants will be followed up two additional years to collect health outcomes through telephone/text and electronic health records. The duration of the entire

assessment is up to 60 months. Figure 1 provides an overview of the entire study design.

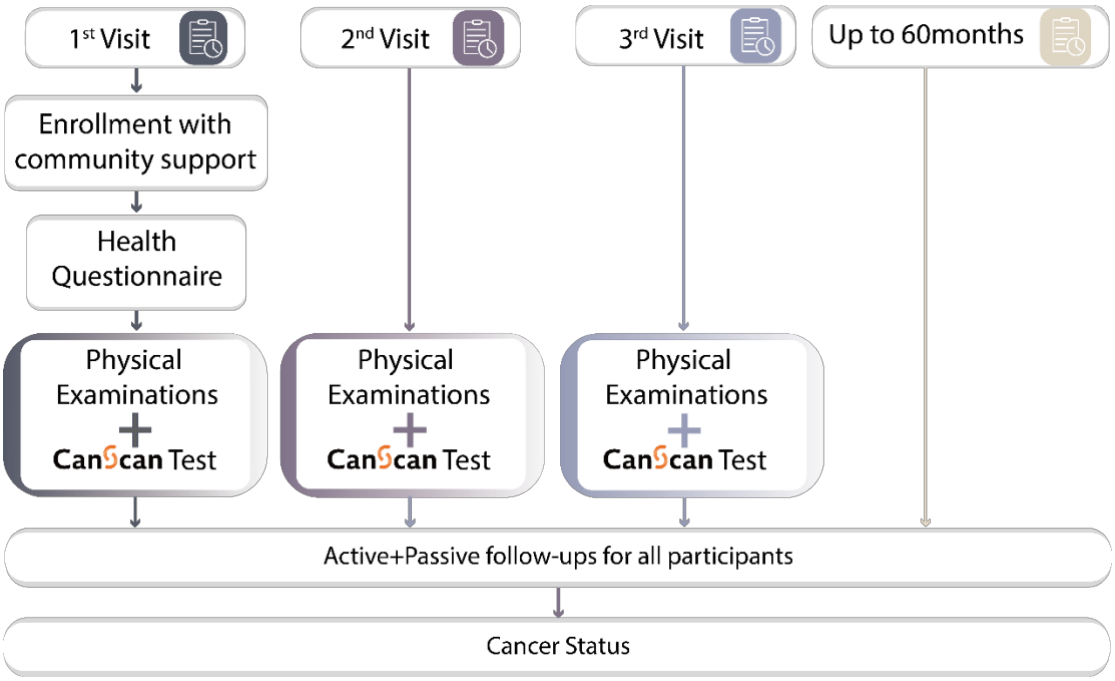

Figure 1. An overview of the study design

3.1 Objectives and Outcome Measurements

Objectives	Outcome	Timepoint
Primary Objective		
To evaluate the performance of a MCED test for the detection of cancer	Sensitivity Specificity Positive Predictive Value Negative Predictive Value	Assessed up to 60 months
Secondary Objectives		
To evaluate the performance of a MCED test for the identification of tissue of origin of cancer	Tissue of origin prediction accuracy	Assessed up to 60 months
To evaluate the performance of a MCED test for the detection of cancer by participant subgroups	Sensitivity Specificity Positive Predictive Value Negative Predictive Value	Assessed up to 60 months
To evaluate the yield with MCED by participant subgroups	Number of true positives / number of patients referred by physical examinations	Assessed up to 60 months

To evaluate the time to diagnosis by comprehensive physical examinations	Number of days between physical examination and cancer diagnosis	Assessed up to 60 months
To evaluate the yield of non-cancer diagnoses and no diagnoses following physical examinations	Number of patients without cancer diagnoses/number of patients referred by physical examinations	Assessed up to 60 months
To evaluate participants' attitude and perception towards MCED blood test	Adherence to annual blood exams and adherence to physical examination	Assessed up to 60 months

3.2 Health Outcome

Health outcomes will be acquired and determined from participants' follow-up questionaries via telephone/text and electronic health records. Health outcomes include cancer diagnosis, including cancer-related death, non-cancer diagnosis, no diagnosis, and death from non-cancer causes. Each participant will be categorized in one of the outcome groups while the categorization may vary during the study period.

3.2.1 Cancer Diagnosis

Cancer diagnosis is determined by the classification system of International Classification of Diseases for Oncology (ICD-O). It involves pathologic confirmation of an invasive solid or hematologic malignancy, with ICD-O codes of /3, /6, or /9, as well as premalignant conditions with an elevated risk of malignancy indicated by an ICD-O code of /2 (e.g., villous adenoma of colorectum, high-grade squamous intraepithelial lesion). If a participant is diagnosed with or dies from cancer or one of the premalignant conditions defined above, s/he will be categorized to the cancer diagnoses group. Conversely, other premalignant conditions (e.g., tubular adenoma of

colorectum, cervical intraepithelial neoplasia grade 1, gastric polyps) will not be considered as cancer.

A set of cancer diagnoses information will be collected, if available. It will include the type and location of cancer, and the cancer stage (which is coded according to the Union for International Cancer Control TNM or a cancer specific staging system, as appropriate), the date of diagnosis, the date of surgery, the name of the tumor sample, tumor size, pathologic diagnosis, pathologic subtype, pathologic histology, metastasis information, date of the pathology report, and the primary reason for the hospital visit, to help categorize the diagnosis pathway.

3.2.1.1 Cancer Diagnosis Pathway

The primary reason for the hospital visit categorizes the cancer diagnosis pathway into two categories, namely screening and clinical suspicion only, with each category being explained below:

- Screening: the primary reason of the hospital visit associated with the cancer diagnosis is abnormalities shown in the current physical examinations.
- Clinical suspicion only: the primary reason of the hospital visit associated with the cancer diagnosis is solely on clinical suspicion, without a specific screening protocol.

3.2.2 Non-cancer diagnosis

Non-cancer diagnosis includes the participants who are diagnosed with major non-cancer diseases that are not associated with cancer.

3.2.3 No diagnosis

Participants who have neither cancer diagnoses nor non-cancer diagnoses.

3.2.4 Non-cancer related death

If a participant pass away because of non-cancer causes during the study period, s/he will be categorized under non-cancer related death. Deaths due to cancer are categorized under cancer diagnoses.

4 Participants Identification

4.1 Study Participants

Residents from local communities in the Jiangbei New Area of Nanjing will be invited to participate.

4.2 Inclusion Criteria

1 Participants must be between the ages of 45 and 75.

2 Participants must be willing and able to undergo blood sample collection, complete health questionnaires, and attend comprehensive physical exams for three consecutive years.

3 Participants must be residents of Nanjing.

4 Participants must fully understand the study and be able to provide written informed consent.

4.3 Exclusion Criteria

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

1. Pregnant women are excluded from participation.
2. Individuals with a history of cancer or a current diagnosis of cancer are not eligible.

3. Individuals who have had an organ transplant or a non-autologous (allogeneic) bone marrow or stem cell transplant are excluded.
4. Individuals who have received a blood transfusion within 30 days prior to the blood draw are not eligible.
5. Individuals who have had an acute infection or inflammation within 14 days prior to the blood draw are excluded.
6. Individuals who have taken medication with anti-tumor effects within 30 days prior to the blood draw are not eligible.
7. Individuals who, in the judgment of the researchers, will not be able to comply with the protocol procedures are excluded.

5 Protocol Procedures

5.1 Sponsor

Sponsor name: Nanjing Geneseeq Technology, Inc.

Address: 128 Huakang Rd, Nanjing, Jiangsu, China 210032

Email: cohortstudy@geneseeq.com

5.2 Study Sites

The study has two sites:

(1) The Nanjing Jiangbei Hospital: 552 Geguan Rd, Nanjing, China 210044

(2) The Fourth Affiliated Hospital of Nanjing Medical University: 298 Nanpu Rd, Nanjing, China 210031

5.3 Recruitment

Recruitment will take place in Jiangbei New Area of Nanjing, China. Staff members from the local Public Health Service and nurses from the physical

examination center of the 2 study sites will assist in the recruitment process. Residents from local communities will be invited to participate.

5.4 Eligibility Assessment

Participants will need to fill out a Participant Eligibility Questionnaire to determine their eligibility for enrollment. It is essential that each participant meets all the inclusion and exclusion criteria outlined in the protocol without exception.

5.5 Registration

Each eligible participant will be asked to register in the Jinling cohort official app and be given a unique identification number, which will be used for future appointments, study visits, follow-up texts/telephone calls, as well as sample labelling and tracking.

5.6 Study Visit

Registered eligible participants can schedule study visits to either of the two study sites through the Jinling cohort official app. The availability of appointments is based on the capacity of the physical examination centers. Each participant is required to complete three study visits, one per year. They can schedule the first visit at any time after registration, but appointments for the second and third visits must be made at least 11 months after the previous visit. The procedures of the first study visit and the visits at the following years will be different and are listed on the following table.

Table 1 Procedure of the Study Visits

Procedure	**1st Study Visit**	**2nd Study Visit**	**3rd Study Visit**
Informed consent	X		
Health Questionnaire	X		
Physical examination	X	X	X

Sample Collection for CanScan MCED test	X	X	X
Genetic testing	X		

5.6.1 1st Study Visit

5.6.1.1 Informed Consent

The participants must personally sign and date the latest approved version of the Informed Consent Form before any study specific procedures is performed. The detailed content of the consent will be explained to the participants both verbally and in writing. It will be made clear that participants have the right to withdraw from the study at any time and for any reason, without any negative impact on their future care, legal rights, or any requirement to justify their decision to withdraw. Participants will receive a copy of the signed Informed Consent, while the original document will be kept securely at the study site.

The Informed Consent Form will authorize the investigators limited access to participant's electronic health records, which will be used to determine the health outcome. It will also contain a discreet clause about the possible use of any remaining samples for further exploratory research that goes beyond the scope of this study. This additional research may involve further biological analyses of the samples and the development of new techniques for analyzing biological data, which could help advance the MCED test. Participants will be informed that while their involvement in this additional research is not explicitly optional, they are free to decline participation

and can withdraw their consent at any time and for any reason throughout the duration of the study.

5.6.1.2 Health Questionnaire

After signing the Informed Consent Form, participants will be assisted to complete the Health Habits and Disease History Questionnaire (see Annex 1 for details), referred to as Health Questionnaire hereafter, through the Jinling cohort official app. Their response process will be recorded unless declined. The assistance and recording aim to improve the accuracy of participants' responses and will also be utilized for the purpose of quality control.

The Health Questionnaire will collect participants' data, including self-reported demographics (e.g. age and sex), lifestyle factors (e.g. smoking status, alcohol use, dietary habits, living environment, etc.) and health history (e.g. family history of cancer-related disease history, history of other related diseases), which will be used in cancer risk group classification.

5.6.1.3 Physical examination

Participants will undergo comprehensive physical examinations. The physical examinations include mandatory cancer screening tests for nine types of cancer (breast, colorectal, cervical, prostate, lung, liver, kidney, thyroid and pancreas cancer). The tests consist of standard cancer screenings recommended by the China Guideline for Cancer Screening (CGCS) or U.S. Preventive Services Task Force (USPSTF), as well as non-standard screenings for cancers that currently lack CGCS or USPSTF recommendations.

For a comprehensive list of items included in the physical examination, please refer to Annex 2.

Upon completion of their physical examinations, participants may opt for cancer diagnostic evaluations based on the clinical judgment of their physicians. This clinical judgment is contingent upon each participant's health profile and the outcomes of their physical examinations upon standard of care. The complete physical examination results will be collected from the two study sites for analysis.

5.6.1.4 Sample Collection for CanScan MCED Test

During the physical examination, participants will also provide approximately 10ml of blood into EDTA cfDNA tube. Samples will be couriered to a central laboratory at the sponsor’s site. The CanScan MCED test will be performed for each participant but will not be disclosed to the participants or their healthcare providers. This ensures that the CanScan MCED test results will not influence physicians’ standard of care procedure. Additionally, the MCED test (CanScan, Geneseeq) will also be performed without prior knowledge of the participants' clinical outcomes to prevent any potential bias.

5.6.1.5 Genetic Testing

Genetic testing will be conducted using the Geneseeq Prime 425-gene panel on the white blood cells, with an average coverage depth of 300X. Although the results of the MCED test will not be shared with participants, individuals will still be informed if their genetic testing results indicate a heightened hereditary risk of cancer.

5.6.2 2nd and 3rd Study Visit

At the second and the third study visits, the participants will only be asked to complete the physical examination and sample collection for the CanScan MCED test. The itinerary of the physical examination is the same as the physical examination during the first visit.

A participant may also be assisted to complete a follow-up questionnaire if s/he has not completed the annual questionnaire by his/herself.

5.7 Follow-ups

Follow-up procedures will take place from enrollment up to 60 months. It will be conducted through two main methods: active and passive follow-up. Data is to be collected within 3 months of enrollment to identify health outcomes.

Active follow-up will be performed every three months for those without diagnosis at 3 months and will involve direct contact with participants through follow-up questionnaires by text messages or telephone calls. The way of follow up of a participant is determined through the risk of disease assessed by the physician after a comprehensive consideration of the result of physical exam, lifestyle factors, and health history. The participant will be reached by telephone calls if s/he is at a high risk. Otherwise, the participant will be reminded via text messages to complete a follow-up questionnaire (see Annex 3) through the Jingling cohort official app. This proactive approach aims to gather up-to-date information on the participants' health status, any new diagnoses of cancer, treatments received, and overall well-being.

The passive follow-up will consist of a retrospective review of electronic health

records, which will only take place with the participants' explicit informed consent. This consent will allow researchers to access and analyze the medical data, (see Section Cancer Diagnosis for details) kept within the Jiangsu Commission of Health's healthcare system. Details about cancer diagnoses and cancer-related diseases, along with the treatment received, will be accessed and analyzed. Information regarding cancer history before enrollment will also be collected, facilitating a more comprehensive determination of the participants' eligibility of the study. Participants who had cancer history before enrollment will be kept in the study, although fail to meet the enrollment requirements, but will be excluded from analysis (see analysis population for details). The retrospective review will be conducted every 6 months up to 60 months.

The results of the active follow-up will corroborate those of the passive follow-up. If necessary, participants will be contacted by phone to further verify the accuracy and validity of their cancer diagnosis information.

5.8 Withdraw or Termination

Participants will be informed that they can withdraw from the study at any time, for any reason, without facing any negative consequences regarding their future care, legal rights, or an obligation to justify their decision. Study site staff will make every reasonable effort to encourage participants to remain in the study; however, participants have the right to withdraw if they desire.

A participant may be removed from the study by the study site if, although they initially consented and enrolled, they are found to not meet all the required inclusion or exclusion criteria.

For withdrawn participants, their blood samples and associated data will be retained for further analysis unless declined by the participants.

The sponsor has the authority to terminate the study at any time for safety, data quality, new external information or administrative reasons, after notifying the Investigators. Investigators will also be informed by the Sponsor if the study is put on hold, completed, or closed.

5.9 Definition of End of Study

The end of the study is defined as the moment when all final data from the last participant has been collected.

6 Statistics and Analysis

This study plans to enroll 15,000 asymptomatic participants aged 45-75. Since the Jinling Cohort is a prospective observational study without intervention, and the interim analyses will not affect the subsequence of the study; the statistical analysis plan (SAP) is provided within the study protocol.

6.1 Statistical Analysis Plan (SAP)

6.1.1 Analysis Populations

- Enrolled population: all consented participants
- Analysis population: Analysis population: all consented eligible participants with at least the 1st study visit, including valid physical examination result and MCED test result, and with at least 3 months follow-up.
- Performance population: all analyzable participants either with cancer diagnoses or without cancer diagnosis. Cancer diagnosis information should be

confirmed with sufficient clinical data at the time of analysis, up to 60 months.

6.1.2 Data Collected

Categories of data collection will include the following:

- Participant identifiers (e.g. enrollment id)
- Demographics (e.g. sex, age)
- Baseline clinical information (e.g. smoking, alcohol use, family history of cancer, personal history of disease)
- Physical examination results
- CanScan MCED test results
- Genetic testing results
- Cancer related diagnosis information

6.1.3 Description of the Statistical Methods

The characteristics of participants will be summarized using descriptive statistics, with appropriate figures and/or tables. These summaries will be stratified by demographic factors, cancer risk groups, presence or absence of cancer-related abnormalities, health outcomes, cancer diagnosis pathways, cancer types and stages and MCED test results. A few separate sets of performance estimates will be generated: three early interim estimates, which will be derived at various time points during the progression of this cohort study, and a comprehensive estimate at the study's conclusion. The purpose of the interim estimates is to evaluate the progression of the cohort study and monitor the performance of the MCED test. Since the cohort study is observational

and does not involve any interventions to the participants, the interim estimates will not inform the subsequence of the study. Therefore, these interim estimates will not affect the overall statistical power of the study.

6.1.3.1 Primary Analysis

The primary focus of the analysis is to assess the performance of the CanScan MCED test in detecting cancer. The MCED test results will be compared to their true cancer status which will be determined pathologically or radiologically, specifically in the performance population. The performance of the cancer signal detection of the CanScan MCED test will be evaluated by its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The sensitivity and specificity will be examined using a 2x2 contingency table analysis, by comparing the results detected by the MECD test with the true cancer status determined pathologically or radiologically. An example is given below:

		Cancer status		
		Cancer	Non-cancer	Total
MECD test	Detected	TP	FP	TP+FP
	Not Detected	FN	TN	FN+TN
	Total	TP+FN	FP+TN	TP+FN+FP+TN

Detected: the result of MECD test is positive, with the signals from cancer
Not Detected: the result of MECD test is negative, without the signals from cancer

Sensitivity is the proportion of participants with a positive test result among all cancer participants.

Sensitivity = TP/(TP+FN)×100%

Specificity is the proportion of participants with a negative test result among all

non-cancer participants.

Specificity = TN/(FP+TN)×100%

Positive Predictive Value (PPV) is the proportion of cancer participants among all participants with a positive test result.

PPV = TP/(TP+FP)×100%

Negative Predictive Value (NPV) is the proportion of non-cancer participants among all participants with a negative test result.

NPV = TN/(TN+FN)×100%

The evaluation of the MCED test's performance will be conducted on the performance population, utilizing point estimates along with 95% confidence intervals (CIs). Two-sided 95% CIs will be calculated for proportion estimation using the Wilson score method, unless otherwise noted. Results will be presented in tables and figures that include the corresponding CIs.

6.1.3.2 Secondary Analysis

Like the primary analysis, the secondary analysis will be performed on the performance population. This analysis has six components:

1. Identification of tissue of origin (TOO):

Assess the MCED test's performance in identifying the tissue of origin for cancer.

TOO prediction accuracy is the proportion of participants with a correct prediction among true positive participants with a known origin.

TOO accuracy = Correct TOO prediction/True positive participants with known

origin

TOO accuracy will be evaluated for both the top 1 prediction (with the highest probability) and the top 2 predictions (with the second highest probabilities).

2. Stratification by subgroups:

Assess the MCED test's performance in detecting cancer across various demographic characteristics, cancer risk group, presence or absence of cancer-related abnormalities, cancer diagnosis pathways, cancer types and stages. Point estimates for PPV, NPV, sensitivity, and specificity will be determined, along with 95% CIs. Groups of cancer risk and presence/absence of cancer-related abnormalities are defined as follows:

- Cancer risk group definition

Participants will be divided into two groups: elevated risk group and average risk group. This classification is based on the risk levels associated with lung cancer, liver cancer, breast cancer, esophageal cancer, stomach cancer, and intestinal cancer. A participant is placed in the elevated risk group if they exhibit an elevated risk level for any of the cancers listed above. The criteria for cancer-specific risk levels are based on the criteria established by National Cancer Center of China and are defined in Annex 4.

- Cancer-related physical examination abnormality:

The mandatory physical examination includes cancer screenings for nine cancers (breast, colorectal, cervical, prostate, lung, liver, kidney, thyroid and pancreas cancer). A criteria of cancer-related physical examination abnormality was established according to the China Guideline for Cancer Screening and Chinese Society of Clinical

Oncology (CSCO) guidelines as shown in Annex 5.

3. Comparison of true positives:

Evaluate the differences in the ratio of true positives among various subgroups through a significance test.

4. Time to diagnosis:

Analyze the time from physical examination to cancer diagnosis, stratified by different cancer diagnosis pathways.

5. Comparison of cancer rate:

To evaluate the yield of non-cancer diagnoses and no diagnoses following physical examinations.

6. Evaluate attitude and perception towards MCED blood test:

Analyze the adherence to the annual blood draw and physical examination.

6.1.3.3 Exploratory Analysis

1. Lead time:

Plasma samples will be collected continuously from each participant over three years. The level of tumor cfDNA in the blood tends to increase as the tumor progresses. Continuous testing of cfDNA over a three-year period will help us gauge how much earlier the MCED test can identify cancer compared to traditional screening methods, thus further validating the performance characteristics of this technology.

6.1.4 Sample Size Determination

The number of participants needed is estimated based on the incidence of cancer in China, and the sensitivity and the specificity of the MCED test.

To determine the sample size, we employed a single-group target value methodology, expressed as follows:

$$n = \frac{\left[Z_{1-\alpha}\sqrt{\pi_0(1-\pi_0)} + Z_{1-\beta}\sqrt{\pi_1(1-\pi_1)}\right]^2}{(\pi_1 - \pi_0)^2}$$

where π_1 represents the expected primary evaluation indicator for the technology being assessed, which in this case refers to sensitivity and specificity, and π_0 is the target value of the indicator.

Preliminary results indicate that the expected sensitivity of the CanScan MCED technology is 85%. With a target sensitivity difference of 15%, the target value is set at 70%. Setting α at 0.025 (one-sided) and 1-β at 0.8 means that 64 cancer incidences are required. Given a total incidence rate of 0.55% over a five-year follow-up period and a follow-up dropout rate of 20%, the necessary total sample size amounts to 14,517 participants.

The expected specificity of the MERCURY technology is 95%. With a target specificity difference of 5%, the target value is established at 90%. Using the same α and β values mentioned earlier, we estimate that 239 healthy individuals are needed, leading to an overall required sample size of 334. Therefore, to fulfill the statistical requirements for validating the sensitivity and specificity of the CanScan MCED technology, approximately 15,000 qualified participants are needed for this project.

7 Data Management

7.1 Source Data

Source data are all information, original records of findings, observations, or other activities in a clinical trial. These original documents and data records include, but are not limited to, participants' demographics, baseline lifestyle and disease history information (from health questionnaire), physical examination results (from two study sites), cancer-related diagnosis information (from follow-up), and genetic testing results and CanScan MCED test results (from the CAP/CLIA laboratory at the Nanjing Geneseeq Technology, Inc., China).

7.2 Documentation

The source data from the health questionnaire will be recorded in the Jinling cohort official app during the interview at the study visit, along with the interview recording. Any changes to the questionnaire results, including the interview records, will be recorded and can be traced through the official app.

Each study site will also document sample information, including the participant's ID and sex, the amount of whole blood collected, the date and time of the collection, and the storage temperature.

The CAP/CLIA laboratory at the Nanjing Geneseeq Technology, Inc. in China will establish comprehensive records for the entire experimental procedures involved in the CanScan MCED testing, from plasma isolation and cfDNA extraction, to sequencing and analysis. All measurements will be recorded in metric units and will be traceable to national metrological standards. All experimental records will be signed by the responsible researcher. The laboratory is responsible for performing any necessary

verification and calibration of its instruments to ensure proper functionality and result reliability. During the experiment, observations and intermediate data will be updated in real-time. After the experiment is complete, the experiment records will be finalized as they are, providing an accurate and valid representation of the experiment.

Corrections will be made using only single strikethrough, alongside the operator's initials and the date of the correction to clearly indicate the rectified information. Apparent outliers will be verified and reviewed, and comments will be added when necessary. All observations during the study will be verified to ensure the reliability of the results. Any conclusion made during the study will be traceable to the raw data and will be supported by statistical analysis.

Cancer related diagnosis information will be collected either through follow-up questionnaires or electronic health records retrieved from the Jiangsu Health Commission's healthcare system. The source date will be recorded in the Jinling cohort official app.

7.3 Record Retention

The participants' clinical information and the original written informed consent will be kept by the study sites. Each study site is responsible in retaining the original records in an appropriate and confidential state for a minimum of 10 years after the conclusion of this study.

The receiving, storage, distribution, use, and retraction of the instrument, reagents, and consumables will be controlled and documented using applicable records by the CAP/CLIA laboratory at the Nanjing Geneseeq Technology, Inc. in China. The laboratory is responsible in retaining the experimental records in an appropriate and

confidential state for a minimum of 10 years after the conclusion of the study.

A copy of the aforementioned records and the sequencing results will be collected by the sponsor and retained in an appropriate and confidential state for a minimum of 10 years after the conclusion of this study.

8 Quality Assurance

8.1 Staff Training

Before the initiation of the Jinling cohort, the sponsor will coordinate with the principal investigator at each study site to implement a training program. The training aims to help the researchers become familiar with the procedures of blood collections and health questionnaire interviews, as well as to standardize the recording of the results of physical examinations and follow-ups. The competence of the researchers, facilities, and instruments will be verified.

8.2 Sample Collection and Testing

Blood samples of approximately 10 ml will be collected from each participant using EDTA tubes (Becton Dickinson, USA) at each study site during each study visit. These samples will then be transported to the central laboratory (Nanjing Geneseeq Technology Inc., China) at room temperature (preferably between 4 °C and 28 °C) for CanScan MCED testing. Blood samples will be centrifuged at 16,000x for 10 minutes within four hours post-collection. Plasma samples will be anonymized with participant ID for the CanScan MCED test.

8.3 Blindness

The clinical information will be blinded to the operators who perform sequencing and analysis. Results will be unblinded to perform statistical analysis in accordance with the procedures described in this protocol.

8.4 Audits

During the study, an inspector appointed by the sponsor will perform regular on-site interim inspection at each study site. This ensures compliance with the study's protocol and the standard of the sample information records.

9 Ethics and Regulatory Requirements

9.1 Approvals

The protocol and informed consent document must obtain written approval from the Ethics Committee of the Nanjing Jiangbei Hospital and the Fourth Affiliated Hospital of Nanjing Medical University before the study can proceed.

The investigators will be responsible for explaining the benefits and risks of participating in the study to each enrolled participant. It will be made clear that participants have the right to withdraw from the study at any time and for any reason, without any negative impact on their future care, legal rights, or any requirement to justify their decision to withdraw. Participants will receive a copy of the signed and dated Informed Consent, while the original document will be kept at the study site.

9.2 Other Ethical Considerations

The CanScan MCED test will be performed for each participant, but the results will not be disclosed to the participants or their healthcare providers. This ensures that the CanScan MCED test results will not influence physicians' standard of care procedures.

9.3 Ethics Declaration

This study is guided by the "Measures for the Ethical Review of Biomedical Research Involving Humans" (2016) of the National Health and Family Planning Commission, the World Medical Association's (WMA) Declaration of Helsinki (2013),

and the Council for International Organizations of Medical Sciences' (CIOMS) International Ethical Guidelines for Biomedical Research Involving Human Subjects (2016). It will be strictly conducted according to the approved protocol and related operational standards by the ethics committee to ensure the scientific nature of the research and to protect the health and rights of the participants.

10 Privacy Protection

10.1 Laws & Regulations

Strictly abide by the "Personal Information Protection Law of the People's Republic of China" and "Regulations of the People's Republic of China on Administration of Human Genetic Resources". Ensure the lawful and ethical acquisition of human genetic resource-related data and samples.

10.2 Data Collection

The collection of peripheral blood, health questionnaire, lifestyle factor, and disease history will mainly be completed in the physical examination center of the two study sites. These centers are designed to maintain confidentiality, with limited access to sensitive information and strict compliance with privacy regulations. Carrying out these data collection activities in such a controlled environment will help protect participants' privacy.

All personal privacy information of enrolled participants, project-related documents, reports, or information is strictly confidential. Identity masking is performed during the sample collection process of plasma; all experimental and analytical procedures in the central laboratory utilize sample testing IDs for processing, without involving any privacy information, such as participants' name, age, ID number,

and telephone number.

10.3 Data Utilization

The blood samples, genetic testing data, lifestyle factor, and disease history of enrolled participants will be used for scientific research after identity masking, including uploading data to public scientific databases or providing it to related universities and institutions for analysis, publishing it in academic papers or using it for drug research and development. Any public reports related to this research will not disclose the personal identity and other privacy information of the participants.

11 Funding

The cost of the physical examinations will be funded by the sponsor, who will also be responsible for the logistics and storage of samples, DNA extraction, library construction, library quality control and sequencing for the CanScan MCED test, and genetic testing for the participants.

The local Public Health Service in Jiangbei New Area of Nanjing, China will provide in-kind support for participant recruitment. Jiangsu Commission of Health and National Healthcare Big Data (East) Center will assist with follow-up by providing the healthcare database.

12 Contractual Arrangements

The Sponsor will set up formal agreements or contracts with the participating parties formally. The division of responsibilities for each party outlined in the study protocol will be documented.

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Annex 1：Health Questionnaire V5.0_2023.01.04

Health Habits and Disease History Questionnaire

1. What is your highest level of education?

1) Not formally enrolled in school 2) Did not graduate from elementary school 3) Graduated from elementary school 4) Graduated from Junior high school 5) Graduated from high school or equivalent 6) Graduated from college 7) Graduated with a bachelor's degree 8) Graduated with a master's degree or higher

2. What is your marital status?

1) Unmarried 2) Married 3) Divorced 4) Widowed 5) Cohabiting 6) Separated

3. What is your occupation?

1) Personnel of government agencies and enterprises 2) Production personnel of agriculture, forestry, animal husbandry, fishery and water conservancy 3) Operators or related personnel of production and transportation equipment 4) Personnel of commerce and service industry 5) Farmers 6) Professional technician 7) Military personnel 8) Personnel of housekeeping service 9) Unemployed 10) Others, please specify _____

4. Have you ever worked in any of the following occupations or been exposed to any of the following substances (listed below) for 12 months or more during your employment?

Occupation or substance exposure	Have you worked at your current job for 12 months or more? (1. Yes 2. No (**skip to Q5**))	Are protective masks or other protective measures used at work? (1. Yes 2. No)
Asbestos (e.g. asbestos tiles, asbestos-cement walls, insulation boards, asbestos-cement pipes, etc.)		
Roasting		
Slaughtering or meat packing		
Petroleum or chemical industry		
Mining		
Cotton and linen processing		
Waste recycling		
Iron and steel casting		

Painting and coating		
Sandblasting		
Pesticide production		
Chef		
Rubber/tires		
Welding		

5. What is your gender?

1) Male (skip to Q12) 2) Female (skip to Q6)

6. How old were you when you had your first menstrual period? ___ years old

7. Have you stopped menstruating?

1) Yes; and what is your menopause age? 2) No

8. Have you ever been pregnant?

1) 1. Yes 2) No (skip to Q11)

9. What are the pregnancy outcomes and the associated number of times?

1) Number of spontaneous abortions 2) Number of induced abortions 3) Number of stillbirths 4) Number of live births (ask Q10 if answered, skip to Q11 otherwise)

10. How old are you when your first child was born? ___ years old

11. Have you ever taken birth control pills?

1) Yes; how many years have you taken birth control pills? ___ years 2) No

12. Do you currently smoke?

1) Smoke every day 2) Smoke, but not every day (skip to Q14) 3) Used to smoke, but do not anymore (skip to Q15) 4) Never smoked (skip to Q21)

13. At what age did you start smoking every day? ___ years old

14. How many cigarettes do you currently smoke per week on average? ___

15. Did you used to smoke every day?

1) Used to smoke every day, ___ cigarettes per day 2) Used to smoke, not every day, ___ cigarettes per week (skip to Q17)

16. At what age did you start smoking every day in the past? ___ years old

17. Excluding the years you quit smoking, how many years have you smoked in total? ___ years

18. Have you ever quit smoking in the past?

1) Yes, within the past 12 months 2) Yes, more than 12 months ago 3) No (skip to Q21)

19. How long have you been stopped smoking?

1) ___years 2) ___months 3) ___weeks 4) ___days

20. What was the most important reason that motivated you to quit smoking?

1) Due to suffering from disease 2) Concern about future health (not yet sick) 3) Because of excessive financial burden 4) Because of family opposition 5) Doctor's advice 6) Other

21. On average, how many days per week are you exposed to secondhand smoke for at least 15 minutes, in your home, workplace, or in indoor public places?

1) Every day 2) 4-6 days per week 3) 1-3 days per week 4) No exposure (skip to Q25) 5) Don't know/can't remember (skip to Q25)

22. How many years have you lived or worked with a smoker in a smoky indoor environment? ___ years

23. Excluding years s/he has quit smoking, how many years has the smoker smoked in total?

1)___ years 2) Don't know

24. How many cigarettes does the smoker smoke on average per day?

1)___ cigarettes 2) Don't know

25. Have you drunk alcohol in the last 12 months?

1) Drank, within the past 30 days 2) Did not drink (skip to Q29) 3) Drank more than 30 days ago

26. How often did you drink alcohol in the past 12 months?

1) daily 2) 1-2 days/week 3) 5-6 days/week 4) 1-3 days/month 5) 3-4 days/week 6) less than 1 day/month

27. How many years have you been drinking alcohol regularly? ___ years

28. In the past 12 months, how often did you usually drink the following alcoholic beverages?

Alcohol	**Frequency**
Strong Chinese liquor (≥42%)	1. Do not drink
Light Chinese liquor (<42%)	2. Less than 1 time/month
Beer (4%)	3. 5-6 times/week
Wine (12%)	4. 1-3 times/month
Yellow wine (18%)	5. 1 time /day
Rice wine (18%)	6. 1-2 times/week
	7. 2 times/day
	8. 3-4 times/week
	9. 3 or more times/day

29. Do you drink tea?

1) Yes, currently (at least 1 time/week) 2) Yes, used to drink, but not anymore (skip to Q35) 3) Occasionally (less than 1 time/week) 4) Never drink tea (skip to Q35)

30. In the past 12 months, how often did you drink tea per week on average?

1) 1-2 days/week 2) 3-5 days/week 3) 6-7 days/week

31. How many years have you been drinking tea regularly? ___ years

32. Which variety do you drink most often?
1) Green Tea 2) Black Tea 3) Oolong Tea 4) Other
33. What strength of tea do you usually prefer?
1) Light tea 2) Moderate tea 3) Strong tea
34. What temperature of tea do you usually prefer?
1) Boiled tea 2) Hot tea 3) Warm tea
35. What is your preference for the temperature of your three meals throughout a day?
1) Scalding 2) Hot 3) warm 4) Cold
36. How often do you eat processed meats (e.g. canned meats, sausages, and cured tripe)?
1) 3 or more times/day 2) 2 times/day 3) 1/day 4) 5-6 times/week 5) 3-4 times/week 6) 1-2 times/week 7) 1-3 times/month 8) Less than 1/month 9) Never
37. How often do you eat Fresh vegetables?
1) 3 or more times/day 2) 2 times/day 3) 1/day 4) 5-6 times/week 5) 3-4 times/week 6) 1-2 times/week 7) 1-3 times/month 8) Less than 1/ month 9) Never
38.How often do you eat Fresh fruit?
1) 3 or more times/day 2) 2 times/day 3) 1/day 4) 5-6 times/week 5) 3-4 times/week 6) 1-2 times/week 7) 1-3 times/month 8) Less than 1/ month 9) Never
39. How often do you eat fried/smoked/grilled foods?
1) 3 or more times/day 2) 2 times/day 3) 1/day 4) 5-6 times/week 5) 3-4 times/week 6) 1-2 times/week 7) 1-3 times/month 8) Less than 1/month 9) Never
40.How often do you eat whole grains?
1) 3 or more times/day 2) 2 times/day 3) 1/day 4) 5-6 times/week 5) 3-4 times/week 6) 1-2 times/week 7) 1-3 times/month 8) Less than 1/month 9) Never
41. Are you an agricultural or a non-agricultural worker?
1) Non-agricultural worker (skip to Q42) 2) Agricultural worker (skip to Q45)
42. In the past year, did you mainly work in a seated or standing position, or did you mainly engage in physical labor?
1) Mainly seated (e.g. administrators, secretaries, full-time drivers, etc.) 2) Mainly standing or walking (e.g. salespersons, security guards, service workers, etc.) 3) Mainly general physical labor (e.g. plumbers, electricians, carpenters, masons, etc.) 4) Mainly heavy physical labor (e.g. loading and unloading, mining, steel making, etc.) 5) Retired or engaged in housework duties, or unemployed for more than one year, or physical disabled and unable to work (Skip to Q55)
43. How many days a week do you work? ___ days
44. How many hours do you work in an average day? ___ hours (skip to Q53)
45. Is there a clear distinction between busy and idle seasons for the agricultural work you do each year?
1) No (skip to Q49) 2) Yes
46. How many months does the busy agricultural season last each year? ___ months

47. During the busy agricultural season, what is the main way you work?

1) Manual 2) semi-Mechanized 3) Mechanized

48. On average, how many hours a day do you do agricultural work during the busy season? ___ hours

49. How many hours per week, on average, do you do agricultural work in the idle season? ___ hours

50. Apart from agricultural work, do you engage in any other formal work at the same time?

1) No (skip to Q53) 2) Yes

51. In the other work, do you mainly work in a seated or standing position or do you mainly engage in physical labor?

1) Mainly seated (e.g., knitting, sewing) 2) Mainly standing (e.g., janitor, store clerk) 3) Mainly general physical labor (e.g., carpenters, electricians, construction workers, etc.) 4) Mainly heavy physical labor (e.g., porters, miners, loading and unloading, etc.)

52. Apart from agricultural work, how many hours per week do you typically do for other works? ___ hours

53. In the past year, what was your typical method of commuting to work (including agricultural work)?

1) Walk 2) Ride a motorcycle 3) Ride a bicycle 4) By private/public transportation (car, subway, ferry) 5) Usually work at home/or by home

54.How many minutes do you usually spend each day commuting (round trip) to work (including agricultural work)? ____ minutes

55. In the past year, how often did you typically participate in physical activities in your spare time?

1) Never or almost never (skip to Q58) 2) 1-3 times per month (skip to Q58) 3) 1-2 times per week 4) 3-5 times per week 5) Exercise every day or almost every day

56. If you participate in weekly exercise, which type of exercise do you do most often?

1)Tai chi/Qigong/walking 2) Long-distance running/aerobics 3) Ball games (basketball, table tennis, badminton, etc.) 4) Fast walking/health exercises/Yangge 5) Swimming 6) Other (e.g., hiking)

57. In the past year, how many hours per week did you spend on leisure exercises? ___ hours

58. How often do you sweat or have a fast heart rate because of heavy physical activities, other than work (including agricultural work) and exercises?

1) Never or almost never (skip to Q60) 2) Less than 1/week (skip to Q60) 3) 1-2 times/week 4) 3-5 times/week 5) Daily or almost daily

59. How many hours per week on average do you spend on intensive physical activities? ___ hours

60. How many hours per day on average do you spend on various types of housekeeping activities (including babysitting)? ___ hours

61. In the past year, how many hours did you sit each day? ___ hours

62. In the past year, did your family cook at home?

1) Yes 2) No (skip to Q66)

63. How many years have you been cooking? ___ years
64. In the past year, which type of fuel did your household currently use most for cooking?
1) Gas from the public pipeline system 2) Tanks of liquefied petroleum gas (LPG) 3) Electricity 4) Kerosene/paraffin 5) Anthracite coal 6) Bituminous coal or lignite 7) Charcoal 8) Wood/fuelwood/crop residue 9) Animal manure
65. How often do your family use a cooker hood when at home?
1) Never 2) Sometimes 3) Often 4) Not sure
66. Does your family currently current have heating in the winter?
1) Yes 2) No (skip to Q68)
67. In the past year, which type of fuel did your family use for heating in the winter?
1) Centralized heating 2) Split natural gas heating 3) Electricity (electric heaters/heated air/air conditioners/electric blankets) 4) Anthracite coal 5) Bituminous coal or lignite 6) Charcoal 7) Wood/fuelwood/crop residue 8) Animal manure
68. Rate your perception of air pollution from 0 to 10, the lower the better. ___
69. How many years have you lived at your current residence? ___ years
70. How many years did you live at your previous residence? ___ years
71. Have you had a major organ transplant or hematopoietic stem cell transplantation?
1) Yes 2) No
72. Have you had a coronary artery bypass graft (CABG)?
1) Yes 2) No
73. Have you had heart valve surgery?
1) Yes 2) No
74. Do you have severe chronic kidney disease?
1) Yes 2) No
75. Do you have acute severe hepatitis or subacute severe hepatitis?
1) Yes 2) No
76. Do you have chronic stomach problems (e.g. gastric ulcers)?
1) Yes 2) No
77. Do you have chronic intestinal diseases (e.g. intestinal polyps, etc.)?
1) Yes 2) No
78. Do you have a serious benign intracranial tumor?
1) Yes 2) No
79. Do you have a mild malignant tumor?
1) Yes 2) No
80. Do you have severe encephalitis or meningitis sequelae?
1) Yes 2) No

81. Do you have severe ulcerative colitis?
1) Yes 2) No
82. Do you have a severe brain injury?
1) Yes 2) No
83. Do you have high blood pressure?
1) Yes 2) No
84. Do you have dyslipidemia/hyperlipidemia?
1) Yes 2) No
85. Do you have coronary heart disease?
1) Yes 2) No
86. Have you had a cerebral stroke?
1) Yes 2) No
87. Do you have hepatitis B?
1) Yes 2) No
88. Do you have diabetes?
1) Yes 2) No (skip to Q90)
89. Type of diabetes you have
1) 1. Type I 2) 2. Type II 3) 3. Gestational diabetes 4) 4. Other types, please specify _____
90. Do you have any other major illnesses?
1) Yes 2) No
91. Has any of your blood relatives ever had cancer?
1) Yes 2) No
92. Family history of malignant tumors

Types of malignant tumors	What were they diagnosed with? (multiple cancers can be selected)	What relatives have this malignant tumor? (select from the kinship code; multiple selections allowed)
Lung		
Colorectal		
Thyroid		
Gastric		
Liver		

Pancreatic		
Lymphoma		
Cervix		
Prostate		
Endometrial		
Esophageal		
Ovaries		
Brain tumor		
Head and Neck		
Kidney		
Nasopharyngeal Carcinoma		
Other, please specify		

93. When do you usually go to bed (in 24-hour format)?

94. How many minutes do you usually take to fall asleep after going to bed, starting from when you begin getting ready for sleep, excluding the time you spend reading, playing on your phone, and other recreational activities?

95. What time do you usually get up? ___

96. How many hours of actual sleep do you usually get (not counting the time spent in bed awake)? ___

97. Do you have difficulty falling asleep (cannot fall asleep within 30 minutes), frequently waking up at night, or early waking?

1) Never/rarely 2) Sometimes 3) Usually

98. Do your close relatives, partner, or friends complain about your snoring?

1) Never/rarely 2) Sometimes 3) Usually

99. Do you experience drowsiness when you don't want to sleep (e.g., at work, reading, driving)?

1) Never/rarely 2) Sometimes 3) Usually 4) Always

100. Do you take naps during the day?

1) No 2) <1/week, duration per session: _____ minutes 3) 1-2/week, duration per session: _____ minutes 4) ≥3/week, duration per session: _____ minutes

101. I hereby pledge that I have answered all the questions in this questionnaire truthfully and that the questionnaire will truly reflect my basic information, lifestyle, disease history, and family history.

Annex 2：Physical Examination Checklist V3.3_2021.09.06

Inspection items	Detail
Basic Inspection Procedure	
General Inspection	General Inspection: Height, Weight, BMI, Blood pressure, Pulse rate Surgery Examination: Skin, Superficial lymph nodes, Thyroid, Spine, Joints of extremities, Breasts
Gynecological Examination	Pelvic Examination
Leukorrhea	Leukocytes, Erythrocytes, Mycobacteria, Epithelial cells, Trichomonas
Cervical Cytology	Pap smear (general cytology test)
Laboratory Test Procedure	
Blood Routine (22 items）	Leucocyte
	GR% granulocyte
	LY% lymphocyte
	MONO% monocyte
	EOS% eosinophil
	Baso% basophils
	NEU Neutrophil Count
	LC Lymphocyte Count
	MONO Monocyte Count
	EC Eosinophil Count
	BC Basophil Count
	RBC red blood cell
	HGB hemoglobin
	HCT hematocrit

	PCV mean red blood cell physical examination
	MCH mean corpuscular hemoglobin
	MCHC mean corpuscular hemoglobin concentration
	RDW red blood cell volume distribution width
	blood platelet count
	MPV mean platelet volume
	PDW platelet distribution width
	MPV mean platelet volume
Blood Glucose Testing	Fasting blood glucose (GLU)
Lipid testing	Lipid profile (TC, TG, HDL-C, LDL-C), Atherosclerotic index (AI)
liver and gallbladder function	Serum alanine aminotransferase (ALT)
	Serum aspartate aminotransferase (AST)
	AST/ALT
	Total serum protein
	Serum alkaline phosphatase (ALP)
	Serum albumin (ALB)
	Serum Globulin (GLO)
	ALB/GLO
Kidney function	Blood urea nitrogen (BUN), Blood creatinine (CR), Serum uric acid test (UA)
Tumor marker (7 items)	Alpha fetoprotein (AFP)
	Cancer embryonic antigen (CEA)

	Glycated antigen 19-9 [(CA19-9)
	Squamous cell carcinoma antigen (SCCA/SCC)
	Glycoantigen 50 (CA50)
	Glycated antigen 15-3 [(CA15-3)
	Glycated antigen 125 [(CA125)] (female)
	Total prostate specific antigen (TPSA) (male)
	Free prostate specific antigen (FPSA) (male)
Urine Routine (11 items)	Urine vitamin C
	Urine specific gravity (SG)
	Urine urobilinogen (URO)
	Urine bilirubin
	Urine ketone bodies (KET)
	Urine occult blood (BLD)
	Urine protein (PRO)
	Urine glucose (GLU)
	Urinary calcium
	Urinary creatinine
	Urinary leukocytes
	Nitrite
	urine pH
	microalbuminuria
	urine albumin-to-creatinine ratio (uACR)
Fecal Occult Blood Test	Fecal occult blood test (FOBT)

Electrocardiography	Static electrocardiography (ECG)
Hepatitis B virus	Hepatitis B virus (HBV) qualitative test
Instrument Inspection Program	
CT (without film)	Lung (low dose)
Color ultrasonography (ultrasound)	Liver, Gallbladder, Spleen, Pancreas, Both kidneys
	Thyroid gland
	Breast (female)
	Uterus, Adnexa (female)
	Prostate (male)

Annex 3：Follow-up Questionnaire V4.0_2022.04.11

ID：	Name：	Age：
Sex：	Phone number：	ID number:
Yth follow-up in the Xth year		
Date	Follow-up method:	

1. (Single choice) The current basic health status of the participant:

□Healthy—skip to Q4

□Diagnosed with non-cancer—skip to Q4

□Diagnosed with Cancer—skip to Q4

□Deceased

2. (Date selection) Please select the date the participant passed away:

Q1 is selected for “Deceased” needs to record and then skip to Q3

3. (single choice question) Please fill in the reason why the participant is deceased:

□ Due to illness (tumor) – skip to Q11

□ Due to illness (non-tumor disease) – skip to Q9

□ Due to accident

Logical record: Recorded when “deceased” is selected in Q1

4. (Fill in the blank) Please fill in the type of diagnosed disease (except cancer):

Logic 1: Recorded when Q1 selects “diagnosed disease (except cancer)”

Logic 2: Recorded when Q3 selects “due to illness (non-tumor)”

Logic 3: Select any skip to Q10

5. (Date selection) Please select the time of diagnosis of the disease:

Record and end follow-up

6. (multiple choice) Select the type of cancer diagnosed, the stage of cancer, the time of diagnosis, and fill in the hospital where the cancer was diagnosed:

Logic 1: Record and end follow-up when Q1 selects “Diagnosed with cancer”

Logic 2: Record and end follow-up when Q3 selects “Due to illness (tumor)”

(multiple choice) Please select the type of cancer diagnosed	(single choice) Please select the stage of cancer diagnosed □ Stage I □ Stage II □ Stage III □ Stage IV	Time of diagnosis:	Hospital of diagnosis:

□Lung			
□Breast			
□Colorectal			
□Stomach			
□Liver			
□Esophageal			
□Cervical			
□Thyroid			
□Prostate			
□Endometrial			
□Ovarian			
□Brain tumor			
□Pancreatic			
□Blood tumor or lymphoma			
□Bladder			
□Kidney			
□Gallbladder			
□Nasopharyngeal			
□Melanoma			
□Head and neck			
□Sarcoma			
□Other			

7. (single choice) Since the last contact or follow-up, survey, during this period, has the participant's family members (including the participant's spouse, parents, children, siblings, etc., immediate and extended blood relatives within three generations) been diagnosed with cancer?

□ No

□ Yes - record the type of cancer and the time of diagnosis

8. (single choice) Since the last contact or follow-up, has there been a significant change in the participant's living environment since the last contact or follow-up survey? For example, frequent exposure to harmful substances (petrochemicals, cotton and linen processing, steel casting, pesticide production, painting and lacquering, etc.)

□ No change

□ There was exposure to these harmful substances in the previous living environment, but there is no exposure now

□ There was no exposure to these harmful substances in the previous living environment, and there is still exposure now

9. (single choice) Since the last contact or follow-up, has there been a significant change in the participant's working environment since the last contact or follow-up survey? For example: frequent exposure to harmful substances (petrochemical, cotton and linen processing, steel casting, pesticide production, painting and lacquering, etc.)

□ No change
□ There was exposure to these harmful substances in the previous work environment, but there is no exposure now
□ There was no exposure to these harmful substances in the previous work environment, but there is exposure now

10. (multiple choice) Apart from this follow-up questionnaire, has the participant received follow-up information from other channels?

□ Text message from Jinling cohort official app
□ Telephone call from Physical examination hospital
□ Telephone call from local Public Health Service
□ Face-to-face interview from local Public Health Service
□ None
□ Others

11. (Single choice) Since the last contact or follow-up, has the contact information of the participant changed since the last contact or follow-up survey?

□ No
□ Yes, updated contact information: _____

End follow-up

Remarks:

Signature of Surveyor:
Date：

Annex 4: Cancer Risk Group Definition

Lung cancer

Aged 50 or older, and

- Current smoker who smokes more than 30 packs per year or previous smoker who smoked more than 30 packs per year who quit smoking less than 15 years ago, or
- Non-smoking females who are exposed to second-hand smoke for more than 20 years, or
- Frequent exposure to harmful substances (petrochemicals, cotton and linen processing, steel casting, pesticide production, painting and lacquering, etc.)

Liver cancer

- Male or female age ≥ 50 who is a carrier of the hepatitis B virus, or
- Have a family history of liver cancer, or
- Diagnosis of liver cirrhosis, or
- Consume Chinese liquor daily for more than 30 years

Breast cancer

Female and

- Have a family history of breast cancer or ovarian cancer, or
- Presence of pathogenic mutations in BRCA1/BRCA2

Esophageal cancer

- Current smoker who smokes more than 30 packs per year or previous smoker who smoked more than 30 packs per year who quit smoking less than 15 years ago, or
- Chronic alcoholism for more than 30 years, or
- Preference for hot or pickled foods, or

- A family history of esophageal cancer

Stomach cancer

- Current smoker who smokes more than 30 packs per year or previous smoker who smoked more than 30 packs per year who quit smoking less than 15 years ago, or
- Non-smoking females who are exposed to second-hand smoke for more than 20 years, or
- Consume Chinese liquor daily for more than 30 years, or
- First-degree relatives with a history of gastric cancer

Intestinal cancer

Aged 50 or older, and

- First-degree relatives with a history of colorectal cancer (including non-hereditary familial colorectal cancer history and hereditary colorectal cancer history), or
- Suffering from chronic intestinal diseases or severe ulcerative colitis, or
- A positive fecal occult blood test, or
- SNP pathogenic mutations associated with colorectal cancer, including APC, MLH1, MSH2, MSH6, PMS2, EPCAM, MUTYH, BMPR1A, SMAD4, STK11, TP53, PTEN, POLD1, POLE, AXIN2, RNF43.

Annex 5: Physical Examination Related Abnormalities Definition

Cancer Specific Abnormality Site	Risk Criteria	Reference
Breast	a) Breast Nodule: Breast ultrasound reports low echo nodule/low echo area b) Breast BIRADS: Breast ultrasound reports BIRADS category 4/5/6	[1] Editorial Committee of Chinese Journal of Health Management, Expert Consensus on Basic Items of Health Examination (2022), Chinese Journal of Health Management, 2023, 17(9): 649-660, (2022) [2] Health Management Branch of Chinese Medical Association and Editorial Committee of Chinese Journal of Preventive Medicine, Expert Consensus on Management of Important Abnormal Results of Health Examination (Trial version), Chinese Journal of Health Management, 2019, 13(2): 97-101 (2019) [3] National Cancer Center, Guidelines for Cancer Screening in Chinese Population, (2021) [4] Hejie, Chen Wanqing, Shen Hongbing, Li Ni, Qu Chunfeng, Shi Jufang, Sun Feng, JiangJing, Cao GuangWen, Zhuang GuiHua, Peng Ji, Work Guidelines for Liver Cancer Screening in Chinese Population, (2022) [5] Hejie, Chen Wanqing, Li Ni, Cao Wei, Ye Dingwei, Ma Jianhui, Xing nianzeng, Peng Ji, Tian JinHui, Guidelines for Prostate Cancer Screening and Early Diagnosis and Treatment in China (2022, Beijing), Chinese Oncology, 31 (2022) 1-30 [6] Chinese Preventive Medicine Association, Chinese Lung Cancer Screening Standard (T/CPMA 013-2020), Chinese Journal of Oncology, 43 (2021) 1-7
Cervix	Pap smear reports atypical squamous epithelial cells/atypical squamous cells	
Intestines	Fecal occult blood test (FOBT) positive	
Liver	a) Liver Nodule: Liver ultrasound reports nodules/low echo/occupancy b) AFP: AFP≥200ng/ml	
Lung	a) Chest Nodule (i) Ground-glass nodule, diameter ≥	

	8mm, or (ii) Solid nodule (including mixed/ solid/ semi-solid/ partial solid/ solid component), diameter ≥ 6mm, or (iii) Nodule diameter ≥ 10mm	[7] Hejie, Li ni, Chen Wanqing, Wu Ning, Shen Hongbing, Jiang Yu, Li Jiang, Wang Fei, Tian Jinhui, Chinese Lung Cancer Screening and Early Diagnosis Guidelines (2021, Beijing)
Prostate	PSA: (i) TPSA > 4ng/ml, or (ii) TPSA≥2ng/ml &FPSA/TPSA <0.16	[8] Hejie, Chen Wanqing, Shen Hongbing, Li Ni, Qu Chunfeng, Shi Jufang, Sun Feng, Jiang Jing, Cao GuangWen, Zhuang GuiHua, Peng Ji, Chinese Guideline for Population-Based Liver Cancer Screening (2022, Beijing), Chinese Journal of Digestive Surgery, 21 (2022) 971-996
Pancreas	a) Pancreas Nodule: Pancreas ultrasound reports nodules/low echo/occupancy/solid	[9] National Health Commission, Guidelines for Diagnosis and Treatment of Renal Cell Carcinoma (2022), http://www.nhc.gov.cn/yzygj/s7659/202204/a0e67177df1f439898683e1333957c74.shtml
Kidney	Kidney Nodule: Kidney ultrasound reports nodules/low echo/occupancy	[10] The Superficial Organs and Peripheral Vascular Professional Committee of China Ultrasound Medical Engineering Society, Expert Consensus on Several Common Clinical Questions in Thyroid Ultrasound and Related Lymph Nodes (2018 Edition), China Journal of Ultrasound in Medicine, 35 (2019) 193-204
Thyroid	a) Thyroid Nodule: Thyroid ultrasound reports nodules/solid/low echo b) Thyroid TIRADS: Thyroid ultrasound reports TIRADS category 4/5/6	[11] National Health Commission, Guidelines for Thyroid Cancer Diagnosis and Treatment (2022) [12] China- Thyroid and Breast Ultrasound Artificial Intelligence Alliance, 2020 China Thyroid Nodule Ultrasonography Suspicious Malignant Evaluation Guidelines– C-TIRADS, Chinese Journal of Ultrasonography, 30 (2021) 185-200 [13] National Health Commission, Guidelines for Breast Cancer Diagnosis and Treatment (2022) [14] National Health Commission, Guidelines for Pancreatic Cancer Diagnosis and

Treatment (2022)

[15] Chinese Anti-Cancer Association Gynecologic Oncology Committee, Guidelines for diagnosis and treatment of cervical cancer(2021 Edition), Chinese Journal of Oncology, 31 (2021) 474-489