CLINICAL STUDY DOCUMENT

Study Protocol

LUNG-DUAL-MO

Prospective Dual-Cohort Observational Study of Lung Cancer Treatment Response and Tumor Microenvironment Based on Multi-omics Analysis

Unique protocol ID	LCAI0812
Document version	English review draft 1.0
Document date	16 November 2026
ClinicalTrials.gov ID	Not yet assigned; insert the NCT number after registration, if available.
Source registration	ClinicalTrials.gov registration draft for the revised prospective dual-cohort observational study.
Ethics approval number	0978-01
Sponsor	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Principal investigator	Heshui Shi, Department of Radiology
Study period	September 2026 to September 2028

Study synopsis

Single-center prospective observational study with two predefined cohorts totaling 50 adults with lung cancer. Cohort A enrolls approximately 40 patients with advanced lung cancer planned to receive immunotherapy or immunotherapy-based systemic treatment and undergoing clinically indicated pretreatment tumor biopsy; residual tissue is analyzed principally by bulk RNA sequencing (bulk RNA-seq). Cohort B enrolls approximately 10 patients with stage IB lung cancer undergoing upfront curative-intent surgery without neoadjuvant anticancer therapy; residual fresh surgical tumor tissue is analyzed principally by single-cell RNA sequencing (scRNA-seq), with adjacent non-tumor tissue assessed when feasible. Cohort A evaluates associations between baseline clinical, imaging and transcriptomic features and immunotherapy response, progression-free survival and overall survival. Cohort B is an independent mechanistic cohort used to characterize tumor-cell states, immune microenvironment composition and cell-cell interactions related to prespecified molecular and/or imaging phenotypes.

Document status and provenance

This protocol is prepared from the supplied ClinicalTrials.gov registration draft. The source registration contains an internal inconsistency in cohort allocation: its cover and some design fields state approximately 35 participants in Cohort A and 15 in Cohort B, whereas the revised detailed description and outcome sections state approximately 40 in Cohort A and 10 in Cohort B. This protocol uses the revised 40/10 allocation as the working operational specification; the public registry should be reconciled before submission. This working copy does not by itself establish the actual recruitment status, ethics approval date, or first-enrollment date.

1 Study identification

Brief Title	Prospective Dual-Cohort Multi-omics Study in Lung Cancer
Official Title	Prospective Dual-Cohort Observational Study of Lung Cancer Treatment Response and Tumor Microenvironment Based on Multi-omics Analysis
Acronym and Study Type	LUNG-DUAL-MO; Observational.
Observational Study Model	Cohort
Time Perspective	Prospective
Protocol ID	LCAI0812

2 Study status

Record Verification Date	To be entered at registry submission.
Recruitment Status	To be confirmed from actual enrollment records before submission.
Study Start Date	September 2026; verify whether actual or anticipated and use the first participant enrollment date.
Primary Completion Date	September 2028; anticipated, subject to the actual follow-up schedule.
Study Completion Date	September 2028; anticipated, subject to confirmation of final survival follow-up.
Target Enrollment	50 participants total; operational allocation approximately 40 in Cohort A and 10 in Cohort B.

3 Sponsor, responsible party and collaborators

Responsible Party	Sponsor/Investigator according to the institution's registry-account arrangement; verify before submission.
Sponsor	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Principal Investigator	Heshui Shi; Department of Radiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Collaborators	None specified in the source registration.

Funding	Not specified in the source registration; enter verified funding information if applicable.

4 Oversight and ethics

Ethics Approval Number	0978-01
Ethics Review Status	Ethics approval is reported by the investigator. The approval date and exact board wording should be verified before public submission.
Board Name	Medical Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology; exact public wording to be verified.
Informed Consent	Written informed consent is required before enrollment and before research use of residual tissue.
Data Monitoring Committee	Not specified; generally not planned for this low-intervention observational cohort unless required by the institution.
FDA-regulated Drug	No.
FDA-regulated Device	No investigational device is assigned by the study.
Expanded Access	Not applicable.

The study is observational. Anticancer therapy, imaging, laboratory testing, clinically indicated pretreatment biopsy in Cohort A, and surgical resection in Cohort B are selected according to routine clinical care and are not assigned by the study. No participant undergoes biopsy or surgery solely for research.

5 Study description

5.1 Brief Summary

Lung cancer is biologically heterogeneous across advanced treatment settings and resectable disease. This prospective dual-cohort observational study integrates clinical, pathological, imaging and multi-omics information using complementary tissue sources. In the advanced-disease cohort, pretreatment biopsy tissue is profiled with bulk RNA-seq to identify molecular features associated with immunotherapy response and prognosis. In the stage IB surgical cohort, fresh resection tissue is profiled with scRNA-seq to resolve tumor-cell states, immune microenvironment composition and cell-cell interactions that may provide mechanistic context for key molecular or imaging phenotypes.

5.2 Detailed Description

Approximately 50 adults with lung cancer will be consecutively enrolled at Union Hospital into two predefined observational cohorts. Cohort A includes approximately 40 patients with advanced lung cancer who are planned to receive immunotherapy or an immunotherapy-based systemic regimen and undergo clinically indicated tumor biopsy before treatment. After routine pathology and clinically necessary testing are completed, residual biopsy tissue may be used for bulk RNA-seq and related molecular analyses with written consent. Participants are followed for treatment exposure, objective response, disease progression and survival.

Cohort B includes approximately 10 patients with stage IB lung cancer who proceed directly to curative-intent surgical resection without neoadjuvant anticancer therapy. After routine pathological requirements are met, residual fresh surgical tumor tissue, and adjacent non-tumor tissue when feasible, may undergo scRNA-seq and related immune-

microenvironment analyses. Cohort B is intended for mechanistic interpretation rather than assessment of immunotherapy response. The two cohorts are analytically linked through prespecified molecular, pathway and/or imaging phenotypes while retaining their distinct clinical populations and outcome structures.

6 Conditions and keywords

Conditions	Lung Cancer; Advanced Lung Cancer; Stage IB Lung Cancer
Keywords	lung cancer; advanced lung cancer; stage IB lung cancer; multi-omics; bulk RNA sequencing; single-cell RNA sequencing; tumor microenvironment; immunotherapy; treatment response; progression-free survival; overall survival; biomarkers

7 Study design

Observational Study Model	Cohort
Time Perspective	Prospective
Sampling Method	Non-probability sample; consecutive enrollment of eligible patients in routine clinical care.
Number of Cohorts	Two predefined prospective observational cohorts; no randomized treatment groups and no control arm.
Primary Purpose	Biomarker association and translational mechanism research within routine clinical care.
Site	Single center: Union Hospital, Tongji Medical College, Huazhong University of Science and Technology.
Target Enrollment	50 participants total; operational allocation approximately 40 in Cohort A and 10 in Cohort B.

8 Exposure, biospecimens and study procedures

8.1 Exposure and treatment assignment

No study treatment is assigned. In Cohort A, immunotherapy or immunotherapy-based systemic treatment is selected by the treating team according to routine clinical practice and is recorded as an observational exposure. In Cohort B, upfront surgery is selected as standard clinical care; participants receive no neoadjuvant anticancer treatment before tissue acquisition. The study does not alter treatment selection in either cohort.

8.2 Biospecimen collection and multi-omics analyses

- Cohort A tumor tissue is obtained from a clinically indicated pretreatment biopsy; Cohort B tumor tissue is obtained from a clinically indicated curative-intent surgical resection.
- Clinical diagnostic needs take priority. Tissue is first allocated to routine pathology, immunohistochemistry and clinically necessary molecular testing.
- With written consent, residual biopsy tissue from Cohort A and residual fresh surgical tissue from Cohort B may be used for research analyses.

- Bulk RNA-seq is the principal planned omics assay for Cohort A. scRNA-seq is the principal planned omics assay for Cohort B.
- Related molecular, immunohistochemical, immune-microenvironment or imaging analyses may be performed when sample quality, tissue quantity and technical conditions permit.
- Research biospecimens and molecular data are coded and managed according to institutional requirements.

Biospecimen Retention	Samples with DNA/RNA or derived molecular material may be retained only as permitted by signed consent and institutional approval.
Cohort A Specimen	Residual pretreatment biopsy tissue and derived bulk RNA/molecular data.
Cohort B Specimen	Residual fresh surgical tumor tissue, with adjacent non-tumor tissue when feasible, and derived single-cell molecular data.

9 Outcome measures

9.1 Clinical outcomes for Cohort A

Objective Response Rate (ORR)	Cohort A only. Proportion achieving complete or partial response according to the clinically applied tumor-response assessment criteria documented in routine care; anticipated assessment within 12 months.
Progression-Free Survival (PFS)	Cohort A only. Time from initiation of immunotherapy or immunotherapy-based treatment to documented disease progression or death from any cause; follow-up up to approximately 24 months.
Overall Survival (OS)	Cohort A only. Time from treatment initiation to death from any cause; participants alive at last contact are censored at the last known alive date; follow-up up to approximately 24 months.

9.2 Mechanistic and exploratory outcomes

Cohort A clinical/imaging associations	Associations between demographic, pathological, imaging, staging and treatment variables and ORR/PFS/OS.
Cohort A bulk transcriptomic features	Associations of bulk RNA-seq and related molecular signatures with ORR, PFS and OS.
Cohort B single-cell characterization	Characterization of tumor and immune cell states, immune microenvironment composition and cell-cell interactions.
Cross-cohort integration	Exploratory mapping of prespecified gene sets, pathways, molecular signatures and/or imaging phenotypes identified in Cohort A to cellular states observed in Cohort B.

10 Eligibility

Sex	All
Minimum Age	18 years
Maximum Age	No upper age limit specified
Healthy Volunteers	No

10.1 Cohort-specific inclusion criteria

- Age 18 years or older in either cohort.
- Cohort A: clinically staged advanced lung cancer with planned immunotherapy or an immunotherapy-based systemic anticancer regimen.
- Cohort A: pretreatment tumor biopsy is planned for clinical care and sufficient residual tissue is expected to remain after routine diagnostic requirements for bulk RNA-seq or related analyses.
- Cohort B: stage IB lung cancer planned for upfront curative-intent surgery without neoadjuvant anticancer therapy, with sufficient residual fresh surgical tissue expected after routine pathology for scRNA-seq.
- Reasonably complete clinical, pathological and imaging information. Cohort A must support treatment-response and survival follow-up; Cohort B must have complete perioperative and pathological data.
- Voluntary participation with signed written informed consent.

10.2 Cohort-specific exclusion criteria

- Cohort A: anticancer treatment directed at the target lesion before the pretreatment research biopsy sample is obtained.
- Cohort B: any neoadjuvant systemic therapy, radiotherapy or other anticancer treatment before surgical tissue acquisition.
- Another active malignant tumor likely to confound the planned molecular or clinical analyses.
- Cohort A: severe active infection, clinically important autoimmune disease, or long-term immunosuppressive treatment that materially affects interpretation of immunotherapy-related analyses.
- The clinically obtained biopsy or surgical specimen is unavailable for research after routine diagnostic needs are met, or residual tissue does not meet basic assay quality requirements.
- Clinical, pathological or treatment information is severely incomplete and prevents analysis of key study measures.
- Unable to comply with planned follow-up.

11 Recruitment, follow-up and data collection

Participant Source	Cohort A: oncology, respiratory, interventional and related departments. Cohort B: thoracic surgery and related departments.
Recruitment Method	Consecutive screening during routine clinical care after ethics approval.
Planned Period	September 2026 to September 2028.
Baseline Data	Age, sex, smoking history, pathological type, clinical stage, laboratory and imaging information. Cohort A records planned immunotherapy-based treatment; Cohort B records planned upfront surgery and absence of neoadjuvant therapy.

Follow-up Data	Cohort A: objective response, disease progression, survival status and relevant clinical events. Cohort B: perioperative, pathological and available follow-up information; immunotherapy-response outcomes are not assigned.

Withdrawal may occur at participant request, after later discovery of ineligibility, after a serious event making continued participation inappropriate, after loss to follow-up, or after study termination by the sponsor/institution/ethics committee.

12 Statistical analysis overview

Analyses will respect the two-cohort structure. Cohort A clinical, pathological, imaging, treatment and bulk RNA-seq measurements will be summarized descriptively. Treatment-response groups may be compared using t tests or Mann-Whitney U tests for continuous variables and chi-square or Fisher exact tests for categorical variables as appropriate. Logistic regression may explore associations with objective response. Kaplan-Meier methods will estimate PFS and OS, and Cox proportional hazards regression may be used for exploratory survival associations when event counts permit.

Bulk RNA-seq will undergo assay-appropriate quality control, differential expression, pathway enrichment and signature analyses with multiple-testing correction. Cohort B scRNA-seq analyses will characterize cell types and states, differential programs, immune composition and cell-cell communication. Cohort B participants will not be labeled as immunotherapy responders or nonresponders. Cross-cohort integration will focus on prespecified gene sets, pathways, molecular signatures and/or imaging phenotypes identified in Cohort A and mapped to cellular states observed in Cohort B. Findings will be interpreted as exploratory and hypothesis-generating.

13 Sample size rationale

The planned enrollment is 50 participants. The revised detailed description uses approximately 40 participants in Cohort A and 10 in Cohort B. The allocation is feasibility based, reflecting the availability of eligible advanced-disease biopsy tissue for bulk RNA-seq, the logistical and cost requirements of high-quality fresh surgical tissue for scRNA-seq, and the exploratory translational purpose of the study. No formal hypothesis-driven power calculation is specified.

14 Data quality, privacy and data sharing

- Clinical and molecular data are stored in a coded electronic database with password protection and access limited to authorized study personnel.
- Direct identifiers are stored separately from research data; published results will not contain directly identifiable participant information.
- Data entry is subject to logic checks, periodic review and traceable correction logs.
- Tumor samples are labeled, stored and tracked according to standardized procedures.
- Future use of remaining tissue or derived molecular data is limited to uses permitted by participant consent and ethics approval.
- Public sharing of participant-level or omics data is not promised unless institutional approval and consent permit it.

15 Contacts and location

Central Contact	To be confirmed before public registry submission.
Overall Study Official	Heshui Shi; Principal Investigator; Department of Radiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology.
Lead Facility	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Location	Wuhan, Hubei, China

Appendix A Operational schedule

Pre-activation	Verify ethics documentation, consent language for residual tissue and omics analyses, laboratory workflows, sample processing SOPs, database fields and registry status.
Cohort A baseline	Consent; record baseline clinical/pathological/imaging variables; clinically indicated biopsy; routine pathology and required clinical testing; retain eligible residual tissue for bulk RNA-seq.
Cohort A treatment and follow-up	Record clinically selected immunotherapy or immunotherapy-based regimen, response assessments, progression, subsequent therapy and survival through the planned follow-up.
Cohort B surgery	Consent; verify no neoadjuvant anticancer therapy; curative-intent surgery; routine pathology; retain eligible fresh residual tumor tissue and adjacent non-tumor tissue when feasible for scRNA-seq.
Final analysis	Complete omics quality control, cohort-specific analyses and prespecified cross-cohort integration after clinical follow-up and molecular datasets are sufficiently complete.