The 900th Hospital of the Joint Logistics Support Force Research Protocol Involving Human Subjects

(For Prospective Studies)

Study Title	Development and Validation of a SERS-based Diagnostic Approach for Leptomeningeal Metastasis in EGFR-mutant Advanced NSCLC: a Prospective Multicenter Study
	The 900th Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army, Fujian Cancer Hospital, and Fujian Provincial People's Hospital
Study Period	January 2026 – June 2027

1 Abstract

Leptomeningeal metastasis (LM) occurs in up to 9.4% of patients with EGFR-mutant NSCLC and carries a natural history of less than 3 months. Early and accurate diagnosis is critical for improving prognosis. However, the current gold standard—CSF cytology—has a sensitivity of only 60–70% and is constrained by sample volume, testing frequency, and processing time, leading to frequent diagnostic delays. There is an urgent clinical need for a rapid, highly sensitive, and low-volume diagnostic approach. SERS enables sensitive detection of subtle molecular alterations in proteins, nucleic acids, and lipids in biofluids and has demonstrated high diagnostic performance in liquid biopsies for various cancers. Yet, no study has applied SERS to LM diagnosis. We hypothesize that CSF from EGFR-mutant NSCLC patients with LM harbors specific Raman spectral "molecular fingerprints" that can be captured by SERS; these signature signals, when combined with machine learning algorithms, can overcome the sensitivity limitations of conventional cytology and enable high-precision LM diagnosis.Expected Outcomes: A rapid, highly sensitive SERS-based diagnostic methodology and predictive model for LM in EGFR-mutant NSCLC, along with a proprietary technical protocol ready for clinical translation, which will support targeted therapy decision-making and improve patient outcomes.

2 Background & Objectives

2.1 Background

Lung cancer is one of the most prevalent and deadliest malignancies globally, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of cases. Leptomeningeal metastasis (LM) is a severe complication of advanced NSCLC, characterized by the diffuse infiltration of malignant cells into the subarachnoid space of the brain and spinal cord. It has an incidence of about 3% in advanced NSCLC and is associated with a particularly poor prognosis, with a median survival of less than 3 months if untreated[1].

Epidermal growth factor receptor (EGFR)-mutant lung cancer is a common subtype of NSCLC. Due to prolonged survival from targeted therapies and the poor permeability of most drugs across the blood-brain barrier, the incidence of LM is significantly higher in

this population, reaching up to 9.4%[2]. Currently, the clinical diagnosis of LM remains challenging with suboptimal accuracy, highlighting an urgent need to develop improved diagnostic strategies[3].

The diagnosis of LM involves clinical symptoms, imaging, and laboratory findings. Cytological examination of cerebrospinal fluid (CSF) is the gold standard, which can be supplemented by typical neurological symptoms and characteristic MRI findings[4-5]. However, clinical presentations are diverse and often non-specific, including headaches, nausea, vomiting, and various neurological deficits.MRI, with gadolinium enhancement, is the standard neuroimaging method. The EANO-ESMO guidelines classify MRI findings into four types: linear (Type A), nodular (Type B), both (Type C), or neither (Type D). 3D T1-WI is optimal for identifying nodular lesions. While MRI has a sensitivity of 70-87% and specificity of 75-94% for LM, CSF cytology is still required. However, its sensitivity is only 60-70%, influenced by sample volume, testing frequency, and processing time.

At the molecular level, CSF ctDNA sequencing for EGFR mutations is a promising diagnostic and therapeutic guide[6]. However, challenges such as the invasiveness of CSF collection, low ctDNA concentrations, and the high costs of PCR/NGS limit its widespread clinical application. Therefore, a rapid and highly sensitive diagnostic method is urgently needed.

Raman spectroscopy (RS) is a non-invasive, highly specific molecular detection technique that can sensitively detect tumor-related biochemical changes. Surface-enhanced Raman spectroscopy (SERS), an advanced variant, offers a highly sensitive analytical platform. SERS has shown potential in various tumor diagnoses, such as nasopharyngeal carcinoma and breast cancer[7-10]. However, its application in diagnosing LM has not yet been reported.

In summary, the major challenges in the diagnosis of leptomeningeal metastasis (LM) in EGFR-mutant lung cancer are as follows: the current gold standard for LM diagnosis is cerebrospinal fluid (CSF) cytology, which requires a relatively large sample volume and has an accuracy of only 60–70%. Therefore, there is an urgent need to develop a highly sensitive and rapid diagnostic approach. To address this, the present study aims to establish a diagnostic methodology for LM in EGFR-mutant lung cancer based on

surface-enhanced Raman spectroscopy (SERS), develop a diagnostic model, and improve the diagnostic accuracy of LM, thereby laying the foundation for subsequent clinical treatment.

2.2 Purpose

This prospective, multicenter study is designed to establish a SERS-based diagnostic methodology and develop a diagnostic model for LM in EGFR-mutant lung cancer, with the aim of improving diagnostic accuracy and providing a more reliable foundation for subsequent therapeutic decisions.

3 Study Design

This prospective, multicenter study aims to address the low sensitivity of cerebrospinal fluid (CSF) cytology (60–70%) in diagnosing leptomeningeal metastasis (LM) in patients with EGFR-mutant lung cancer. We will establish a diagnostic methodology and develop a predictive model using surface-enhanced Raman spectroscopy (SERS) technology to improve diagnostic accuracy and guide subsequent clinical management.

4 Study Population

Lung cancer patients harboring EGFR mutations who develop leptomeningeal metastasis

Eligibility Criteria

4.1 Inclusion Criteria

1)Age ≥ 18 years;2)Histopathologically confirmed diagnosis of non-small cell lung cancer (NSCLC);3)Molecular pathology indicating the presence of EGFR-sensitive mutations, including EGFR exon 19 deletion (19del), EGFR L858R point mutation, and other uncommon sensitive mutations;4)Presence of malignant cells confirmed by cerebrospinal fluid (CSF) cytology;5)Patients may be treatment-naïve or have received prior systemic anti-tumor therapy;6)Availability of complete medical records, including demographic characteristics, pathological reports, molecular pathology results, and imaging data;Voluntary participation with signed informed consent, and ability to comply with the entire study procedure.

4.2 Exclusion Criteria

1)Pathologically confirmed mixed histology containing small cell lung cancer (SCLC) components;2)Presence of other primary malignancies other than lung cancer;3)Molecular pathology indicating concurrent other driver mutations, including ALK, ROS1, or other oncogenic alterations;4)Co-existing neurological diseases unrelated to the tumor;5)Severe cardiac or pulmonary dysfunction;6)Pregnancy or lactation.

5 Sample Collection & Handling

5.1 Sample Collection: Samples are typically obtained via lumbar puncture, with the conventional puncture site being the L3–L5 intervertebral space. Approximately 5–10 mL

of cerebrospinal fluid (CSF) is collected, aliquoted into 1.5 mL EP tubes, and the collection time and patient information are recorded. The samples should be transferred to a –80°C freezer for storage within 1 hour.

5.2 Sample Transport:

CSF samples are stored at –80°C and transported using dry ice to maintain the temperature throughout the shipment.

6 Statistical Analysis

In this study, statistical analyses were performed according to the study protocol. Principal Component Analysis (PCA) was first applied to the data for dimensionality reduction, followed by Partial Least Squares (PLS) to further reduce data dimensionality. The PLS scores were then input into a Linear Discriminant Analysis (LDA) classifier, and Leave-One-Out Cross-Validation (LOOCV) was used to estimate the predictive performance. Spectral acquisition and analysis were further conducted using the WIRE 2.0 software package (Renishaw) to delineate cerebrospinal fluid (CSF) spectral variation signatures associated with leptomeningeal metastasis of lung cancer. Receiver Operating Characteristic (ROC) curves were employed to evaluate the predictive performance of the models.

In addition, the biochemical assignments of Raman characteristic peaks at various positions were performed to identify the characteristic peaks and their corresponding biochemical molecules, along with the relative peak spectral intensities. Student's t-test was used to compare the significance of SERS peak intensity differences between different groups, thereby identifying differentially expressed biochemical molecules among groups and constructing a "molecular fingerprint" for leptomeningeal metastasis of lung cancer. Finally, Logistic regression analysis was conducted to build a Logistic regression predictive model for leptomeningeal metastasis of lung cancer based on various peak-spectral characteristic factors, and ROC curves were used to assess the predictive performance of the model.

7 Quality Assurance

(1) Study protocol finalization: The clinical study protocol shall be jointly discussed and reviewed by all investigators participating in this clinical study. After reaching a consensus and making necessary revisions, the protocol shall be submitted to the Ethics Committee for approval.

(2) Investigator qualifications: All investigators participating in the clinical study must possess the requisite professional expertise, qualifications, and competencies for clinical research. After qualification review, the investigator team shall remain relatively stable throughout the study.

(3) Pre-study training: Prior to the commencement of the clinical study, training shall be provided to ensure that all research personnel have a full understanding and awareness of the study protocol and the specific definitions of all indicators. The entire clinical study process shall strictly comply with relevant operational standards. Training shall be conducted for the responsible personnel and participating staff at each participating site before the study begins, covering topics including disease diagnosis and treatment, inclusion and exclusion criteria, and specific implementation issues of the project.

(4) Measures to ensure subject compliance: All patients are early-stage lung cancer

patients with good compliance; subject compliance shall be monitored throughout the study.

(5) Criteria for determining abnormal laboratory findings: The normal reference ranges of the local testing facility shall be adopted as the criteria.

(6) All observations and findings in the clinical study shall be verified to ensure data reliability and to confirm that all conclusions drawn from the study are derived from the original data. Corresponding data management measures shall be implemented during both the clinical study phase and the data processing phase.

8 Ethical Considerations

This study was approved by the ethics committee of the 900th Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army(ethics approval No.2026-018).

9 References

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