Clinical Evaluation of Genetron Lung Cancer Panel in Non-small Cell Lung Cancer Patients

Study Document

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Content

1 Background

Lung cancer is currently one of the most common malignant tumors in the world, and Non-small Cell Lung Cancers (NSCLC) accounts for 80%-85% of all lung cancer patients, among them about 75% of them are in the middle and advanced stage when they are discovered, and the 5-year survival rate is very low. At present, the treatment of lung cancer patients is based on chemotherapy combined with targeted drug therapy in clinical. The effective rate of first-line chemotherapy is only about 30%, and it has high toxicity and adverse reactions, so its application is limited.

Targeted drug therapy-related genes are key genes in the process of lung cancer cell differentiation, proliferation, apoptosis, migration, etc. These gene mutations will continue to activate related signaling pathways, leading to the occurrence and development of tumors, mutations in these genes can be detected in tumor cells of almost all histological types of lung cancer patients. Targeted therapy can provide a basis for the diagnosis and postoperative drug adjuvant treatment of NSCLC patients, and the formulation of drug selection and treatment plans for advanced patients based on the genetic mutations of NSCLC patients.

Wherein, in the treatment of non-small cell lung cancer, the main molecular targeted therapy is epidermal growth factor receptor (EGFR). The efficacy of molecularly targeted drugs is significantly different between different lung cancer patients of the same tissue type and different lung cancer pathological tissue types. The EGFR mutation rate in Asian patients with advanced lung adenocarcinoma is as high as 50%, and there are many EGFR tyrosine kinase inhibitor (TKI) is listed in China and used for clinical treatment. In addition to EGFR, anaplastic lymphoma kinase (ALK) fusion gene and ROS1 fusion gene are also divided into subgroups of NSCLC molecules with specific clinical and pathological characteristics, thereby providing new molecular targets for the treatment of lung cancer. In addition, biomarkers related to lung cancer include HER2, KRAS, BRAF V600E gene mutations, and MET exon 14 skipping mutations. At present, the molecular classification of lung cancer plays an important role in guiding clinical treatment plans, drug selection, and establishing an individualized treatment model for molecular classification. Patients with specific genotypes can only benefit from the corresponding targeted drugs. Therefore, effective and accurate detection of tumor gene status and accurate molecular

subtype differentiation of NSCLC patient populations have become an important basis for patient treatment, prognosis evaluation, and efficacy monitoring.

At present, the routine detection methods for clinical gene detection are fluorescent quantitative PCR, nucleic acid sequence determination, FISH and so on. However, to carry out the above-mentioned gene detection at the same time requires the combination of multiple technology platforms and multiple methods. The large sample size requirements, the complex operation process, and the long detection cycle pose very high challenges to personnel, technology, equipment, etc., which is a serious departure from the clinical goal of quickly and accurately obtaining patient clinical medication information.

The Genetron Lung Cancer Panel is developed by Genetron Health (Beijing) Co., Ltd.. It is used to detect EGFR, KRAS, BRAF, HER2, PIK3CA, ALK, ROS1, MET gene mutations or fusions in non-small cell lung cancer patients. The Genetron Lung Cancer Panel is prepared in a clean production workshop that meets the requirements of the "In Vitro Diagnostic Reagent Production Implementation Rules", and has passed the self-test.

2 Research Purpose

The main purpose of this study is: by evaluating the Genetron Lung Cancer Panel to compare the results of simultaneous detection with similar products on the market and the Sanger sequencing method, and to calculate the coincidence rate and consistency of the assessment reagents and the comparison method. The safety and effectiveness of the product are confirmed and evaluated. Meanwhile, the accuracy of the Genetron Lung Cancer Panel for the following drug companion diagnostic tests: EGFR gene 19 exon deletion and L858R point mutation in gefitinib tablets, erlotinib hydrochloride and icotinib hydrochloride tablets, T790M point mutation in methanesulfonate acid osimertinib tablets, ALK gene fusion and ROS1 gene fusion in crizotinib capsules.

3 Trial Design

3.1 Overall Design of the Trial

This trial follows the principle of synchronous blinding. The enrolled cases are coded, and the enrolled samples are detected with the Genetron Lung Cancer Panel and comparison methods. The

detection results are compared, and the samples that are inconsistent with the detection results of similar products on the market are reviewed by the Sanger sequencing method, when the site detection results compared with Sanger sequencing method are inconsistent, Sanger's detection results are recognized. The results were determined independently according to the cutoff values or interpretation requirements provided by each method, and relevant statistics were used to evaluate clinical application performance of
Genetron Lung Cancer Panel.

3.2 Clinical Trial Sample Screening

3.2.1 Sample Type and Size

The samples in this study are confirmed tissue biopsy samples, which are retrospective samples.

The total number of samples in this clinical trials is 1052. It is planned to be carried out in at least 4 clinical trial institutions, and 3 of them will enroll no less than 255 samples. In case of objective reasons, when the sample size is insufficient, the centers can make appropriate adjustments or increase clinical trial institutions under the premise of ensuring the total amount. It is planned that the ratio of positive samples shall not be less than 30%, and the rare mutation types shall be counted according to the clinical occurrence, which should be statistically significant. Gene locus below the above standards can no longer be included in clinical data statistics.

The analysis of the selected number of case samples is statistically significant, and the conclusions obtained are reasonable and effective, which can make a scientific evaluation of the Genetron Lung Cancer Panel and save social costs to the greatest extent. If all types of specimens are not received as planned due to other reasons, the final type and quantity of specimens shall be subject to the actual collected specimens after negotiation and agreement between the two parties.

3.2.2 Inclusion Criteria，Exclusion Criteria Rejection Criteria of Study Subjects

3.2.2.1 Inclusion Criteria

(1) Clinically diagnosed as non-small cell lung cancer samples (mainly lung adenocarcinoma and some lung squamous cell carcinoma, adenosquamous carcinoma, large cell carcinoma, etc.), and a small number of other cancer types are enrolled as interference samples.

(2) Collect some samples of relevant medical information before and after the use of targeted drugs. This part of the samples should have relevant previous molecular diagnosis results.

(3) Able to provide samples in time according to the requirements of the plan:

samples for DNA/RNA extraction: 10 pieces of 10 μm thickness for each sample or 10 pieces of paraffin rolls. Samples for FISH experiment: 5 slices of 3-4μm thickness.

(4) The pathological examination conforms to the types of tissue samples listed in the above table. The HE staining results show that the tumor content is not less than 50%, and the paraffin slice damage should be avoided.

(5) The sample should have corresponding basic clinical information, including: patient visiting number/medical record number/specimen number, age, gender, pathological diagnosis result, molecular diagnosis result (if any).

3.2.2.2 Exclusion Criteria

Those who do not meet any of the above conditions are excluded.

Reason: The included non-small cell lung adenocarcinoma samples and normal samples should have statistical significance, and the conclusions obtained should be scientific and valid. The selection of subjects for this study, while excluding research-related influencing factors, has no adverse effect on the health of the subjects.

3.2.2.3 Rejection Criteria

(1) Samples considered by the researcher to be unsuitable for continuing clinical trials, such as samples that have not been prepared in accordance with the required steps.

(2) The amount of DNA and RNA extracted from the sample is insufficient, and the clinic can no longer provide more tissues for the extraction of DNA and RNA samples.

(3) Samples whose results failed in repeated detections by the contrast method.

(4) Sample patients with incomplete clinical basic information (visiting number/medical record number/specimen number, age, gender, pathological diagnosis results).

(5) Samples that cannot be completed in a single test due to instrument or human factors (samples are contaminated during operation).

3.2.3 Sample Collection, Storage, and Transportation Methods

Sample collection

Samples are collected according to the pathology operation standard, and the paraffin-embedded pathological section samples should be determined to contain more than 50% of tumor cells.

Sample storage and transportation

The formalin-fixed paraffin-embedded tissue within the validity period of the sample is transported at room temperature. The extracted nucleic acid is recommended to be detected immediately, otherwise the DNA should be stored below -18°C, the storage time should not exceed 7 months, and the number of freeze-thaw cycles ≤ 5 times; RNA should be stored below -70°C, the storage time should not exceed 3 months, and the number of freeze-thaw cycles ≤ 5 times.

3.2.4 Sample Code

In principle, one subject only provides one sample, and each subject should have a unique clinical trial number (secondary code). After the samples are entered into the group and before the experiment operation, the blind-editing staff will mark the secondary code outside the sample tube, and carefully record the primary code (patient visit number/medical record number/specimen number) and the correspondence table of the secondary code as the blind bottom save. The blind-editing file is kept independently by the blind-editing staff until the blind is solved. Under no circumstances should the blindness be solved during the tail.

During the experiment operation, only the secondary code of the sample is reflected.

3.3 Product Information for the Clinical Trial

3.3.1 Test Reagent

Reagent name: Genetron Lung Cancer Panel

Manufacturer: Genetron Health (Beijing) Co., Ltd.

Applicable instruments: gene sequencer DA8600, gene sequencer Genetron S5

3.3.2 Comparison Method

The specific sites and corresponding kits are shown in the following table

Table 1. Selection of comparison methods for each gene

Gene	Comparison method
EGFR	cobas EGFR Mutation Test V2
ALK	Vysis ALK Break Apart FISH Probe Kit
ROS1	Vysis 6q22 ROS1 Break Apart FISH Probe
ROS1*	ROS1 gene fusion detection kit (fluorescence PCR method)
KRAS	KRAS gene mutation detection kit (fluorescence PCR method)

PIK3CA	PIK3CA gene mutation detection kit (fluorescence PCR method)
BRAF	BRAF gene mutation detection kit (fluorescence PCR method)
MET	/
HER2	/

* On the basis of choosing the Vysis 6q22 ROS1 Break Apart FISH Probe kit as a comparison method for the ROS1 gene, add no less than 200 samples for ROS1 gene fusion detection kit (fluorescent PCR method) to perform the comparative study of companion diagnostic reagents.

Applicable instrument for comparison method

EGFR, KRAS, BRAF, ROS1 (for fluorescent PCR method): including but not limited to cobas® 4800 System, Stratagene Mx3000P, ABI7500, Bio-rad CFX96;

PIK3CA (for fluorescent PCR method): Stratagene Mx3000P

ALK, ROS1 (FISH method applicable): Break Apart FISH Probe platform;

MET, HER2 (Sanger method applicable): Life 3130/3130xl, 3730xl, 3500Dx, 3500xl Dx.

3.3.3 Review method

Sanger sequencing is used as the review method. Sequencing fragments cover the target nucleic acid segments, sites and corresponding types amplified by the assessment reagent. After the sequencing reaction is over, submit a representative sample sequencing map and result analysis data.

During the trial, different verification methods can be appropriately added according to the specific conditions.

(1) Product name for clinical trials: Sequencing Kit (Life: BigDye Sequencing Reaction Kit)

(2) Test equipment: Life 3130/3130xl, 3730xl, 3500Dx, 3500xl Dx

4 Statistical Analysis

4.1 The coincidence rate of the panel and the comparison method

For each gene locus, a four-grid table was used to calculate the positive coincidence rate, negative coincidence rate, positive predictive value, negative predictive value, total coincidence rate and 95% confidence interval of the assessment reagent and the comparison method. Using statistical software, Kappa test is used to determine whether the two detection methods are statistically significant.

Table 2. Results statistics

Genetron Lung Cancer Panel	Comparison method		Total
	Positive	Negative	
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	A+B+C+D

A sample with a mutation genotype is judged as positive, and a sample with no mutation genotype is judged as negative

The calculation formula is:

Positive coincidence rate=A/(A+C)×100%

Negative coincidence rate = D/(B+D) ×100%

Positive predictive value (PPV)=A/(A+B)×100%

Negative predictive value (NPV)=D/(C+D)×100%

Total coincidence rate=(A+D)/(A+B+C+D)×100%

95% confidence interval calculation formula: $p\pm1.96\times[\ p(1-p)/n]^{1/2}$ (where p is the positive coincidence rate, negative coincidence rate, positive predictive value, negative predictive value, total coincidence rate, n is the sample size, if p>0.9, use Wilson score method for correction).

4.2 Kappa test

The Kappa test method was used to analyze the equivalence of clinical research reagents and comparison methods. Using statistical software, Kappa test was performed on the detection results of clinical research reagents and gold standard, and the k value was calculated. When k>0.75, clinical research reagents and comparison methods have good consistency.

$$Kappa=\frac{P_a-P_c}{1-P_c},\ Where\ P_{a=}\frac{A+D}{N},\ P_C=\frac{\frac{(A+B)(A+C)}{N}+\frac{(B+D)(C+D)}{N}}{N}$$

5 Quality Control

(1) The clinical verification institutions are all three-level medical institutions above the provincial level with the ability to conduct the clinical research technology and equipment to ensure the quality of clinical diagnosis.

(2) Each clinical trial institution has 1 project director, and 3 to 6 fixed project members. Strictly follow the requirements of the clinical trial protocol. The reporting institution appoints 1

inspector to monitor and cooperate with the team leader institution and the trail institution at any time. The technical personnel of the team leader institution keep close contact with each trail center at any time.

(3) The Genetron Lung Cancer Panel detection and comparison method detection are carried out in accordance with the blind method.

(4) Hospital laboratories participating in clinical trials should establish standard operating procedures and quality control procedures for experimental observation indicators. The person concerned should sign. Each detection is set up for comparison, and is carried out in strict accordance with the requirements of the manual.

(5) Training: Before the start of the experiment, conduct protocol training for all personnel participating in the experiment, and conduct training and assessment for clinical research staff, so that clinical trial personnel able to quickly become familiar with and master the semiconductor sequencing technology and the applicable instruments, operating methods, and technical performance etc. of the panel.

(6) This protocol has been determined through repeated communication and research between the experimental institution and the clinical institution, and must be strictly implemented. Any changes in the plan must be approved by the team leader (master) institution.

6 Ethical issues and explanations

The purpose of this trail is to evaluate the performance of Genetron lung cancer panel in non-small cell lung cancer patients using semiconductor sequencing method.The retrospective samples of this trial are pathological specimens that have been detected during the previous treatment process. They are limited to in vitro diagnosis, do not directly contact the patient, and will not cause any harm to the patient. It is only the reagent performance comparison with the contrast reagent. The test results are only used for research and analysis related to this test, and will not guide the diagnosis and treatment of patients based on this. Therefore, it will not have any adverse effects on the human body. Informed consent can be exempted after review and approval by the ethics committee.

This trial strictly complies with the "Declaration of Helsinki", and involves relevant patient data. Such as: patient visit number/medical record number/specimen number, age, gender, pathological diagnosis results, etc. The patient's relevant information will be kept confidential by the hospital

and Genetron Health (Beijing) Co., Ltd., the clinical trial implementer and clinical verification unit promise not to disclose the content related to the subject's specimen.

7 Data processing and record keeping

Researchers should fill in the trial case report form according to the requirements, truthfully, in detail, and carefully record the contents of the form to ensure that the content in the form is complete, true and reliable. All observations and findings in the trial should be verified to ensure the reliability of the data and ensure that the conclusions in the trial are derived from the original data. The trial personnel should fill in the relevant clinical information and the test results of the trial, and have a dedicated person to review and sign.

All the original data records generated in this trial are archived and preserved by the clinical trial institution, and the retention period is determined by the trial institution, but not less than 5 years after the completion of the trial.