Clinical Evaluation of Genetron IDH1 PCR Kit in Glioma Patients

Study Document

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Content

1 Background

Glioma is the most common primary intracranial tumor. According to the ranking of tumor mortality published by the World Health Organization in 1998, malignant glioma is the second leading cause of death among tumor patients under 34 years old and the third among patients between 35 and 54 years old. In the past 30 years, the incidence of primary malignant brain tumors has been increasing year by year, with an annual growth rate of about 1.2%, especially in the elderly population. At present, WHO mainly relies on morphology for tumor classification and typing (Table 1). However, due to the heterogeneity of glioma and the subjective influence of pathologists, this method is highly subjective and one-sided. There is ample evidence that gliomas with the same or similar tissue characteristics can have different molecular genetic backgrounds. Molecular pathological classification based on tumor genetics can provide accurate and objective genetic information for tumor diagnosis, to help doctors understand the characteristics of each patient's tumor from a new perspective of molecular biology, so as to make more targeted treatment and rehabilitation programs.

Table 1 WHO pathological classification and classification of glioma

Tissue types	Class	Common subtype
Glioma of the brain	Class I	Pilocytic astrocytoma, subependymal giant cell astrocytoma embryonal dysplasia of neuroepithelial tumors
	Class II	Diffuse astrocytoma, mixed oligoastrocytomas, oligodendroglioma, pleomorphic xanthoastrocytoma, hairy myxoid astrocytoma
	Class III	Anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic oligoastrocytomas
	Class IV	Primary and secondary glioblastoma

Isocitrate dehydrogenase (IDH), located in the long arm of chromosome 2 (2q33), is a key rate-limiting enzyme in the tricarboxylic acid cycle, providing energy for cell metabolism and precursors of biosynthesis. There are three isomerases in the IDH gene family (IDHl, IDH2 and IDH3). IDH1 or IDH2 mutations lead to the accumulation of 2-hydroxyglutaric acid, another new metabolite, in cells, which can not only lead to abnormal histone modification, but also increase the activity of VEGF pathway, thereby inducing tumorigenesis. The data showed that IDH1/2 gene mutation was an early genetic mutation in the development of glioma. It usually occurs in young adults and

adolescents with diffuse glioma. IDH1/2 mutation had a certain distribution pattern corresponding to WHO classification, which was mainly distributed in grade II~III oligodendroglioma (69.2%), astrocytoma (79.8%), oligoastrocytomas (75.5%) and grade IV secondary glioblastoma (73%), but rare in primary glioblastoma (<5%) and childhood glioblastoma.

IDH1 mutations accounted for more than 90% of IDH1 and IDH2 gene mutations, among which IDH1 R132H (CGT>CAT, arginine replaced by histidine) mutations were the most common, accounting for about 90% of IDH1 mutations.

The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines for Central Nervous System Cancer (V1, 2015) include the presence or absence of IDH1 or IDH2 gene mutations as one of the indicators to assess the risk type of patients with low-class glioma. The Chinese Guidelines for molecular Diagnosis and Treatment of glioma (2014) also included IDH1 or IDH2 gene mutation as the preferred molecular marker for molecular pathological typing (grade I evidence).

At present, the gold standard method for clinical detection of IDH gene mutation is nucleic acid sequencing. However, this method is time-consuming and laborious to determine the detection results, and the operation process is complex, so it is difficult to widely apply this technique in clinical practice.

2 Research Purpose

By using this product to detect the IDH1 R132H gene mutation in the cancer cells of patients with glioma, compare it with the results of nucleic acid sequence determination, and combine the results of clinicopathological typing to confirm and evaluate the safety and effectiveness of this product.

3 Trial Design

3.1 Overall Design of the Trial

This study followed the principle of synchronous blind method. Enrolled cases were coded and compared with the nucleic acid sequence determination of the control method (gold standard). Results were determined independently according to the critical value or interpretation requirements provided by each method, and relevant statistics were conducted.Combined with the results of clinicopathological classification, the incidence of IDH1 gene R132H mutation in different subtypes was counted to evaluate the clinical application performance of this product

The total number of samples for clinical research should be no less than 1,000 cases, and clinical trials should be carried out in no less than 3 (including 3) provincial health care institutions or specialized hospitals to ensure that the results are statistically significant. All samples should have corresponding basic clinical information, including: patient visiting No. / medical record number/specimen number, age, sex, pathological diagnosis result, etc.

3.2 Test design and study method selection

3.2.1 Sample collection

Tissue sections fixed in neutral formaldehyde and embedded in paraffin were detected.

3.2.2 Sample data

(1) Sample information

A) Patient visiting No. / medical record No. / specimen No., age, sex, and pathological diagnosis result

B) HE staining results of samples, indicating tumor area.

(2) Pathological examination confirmed glioma, other brain tumors or normal tissues.

(3) Number of samples: each sample contains 10 slices with a thickness of 10μm, and the tumor content is not less than 50%.

3.2.3 Sample storage

FFPE samples should be stored at room temperature.

3.2.4 Kit source:

(1) The kits were provided by Genetron Health (Beijing) Co., Ltd.

(2) Test instrument: BIO-RAD: CFX96 Real-Time PCR Detection System or LIFE: 7500 Fast Dx Real Time PCR Instrument.

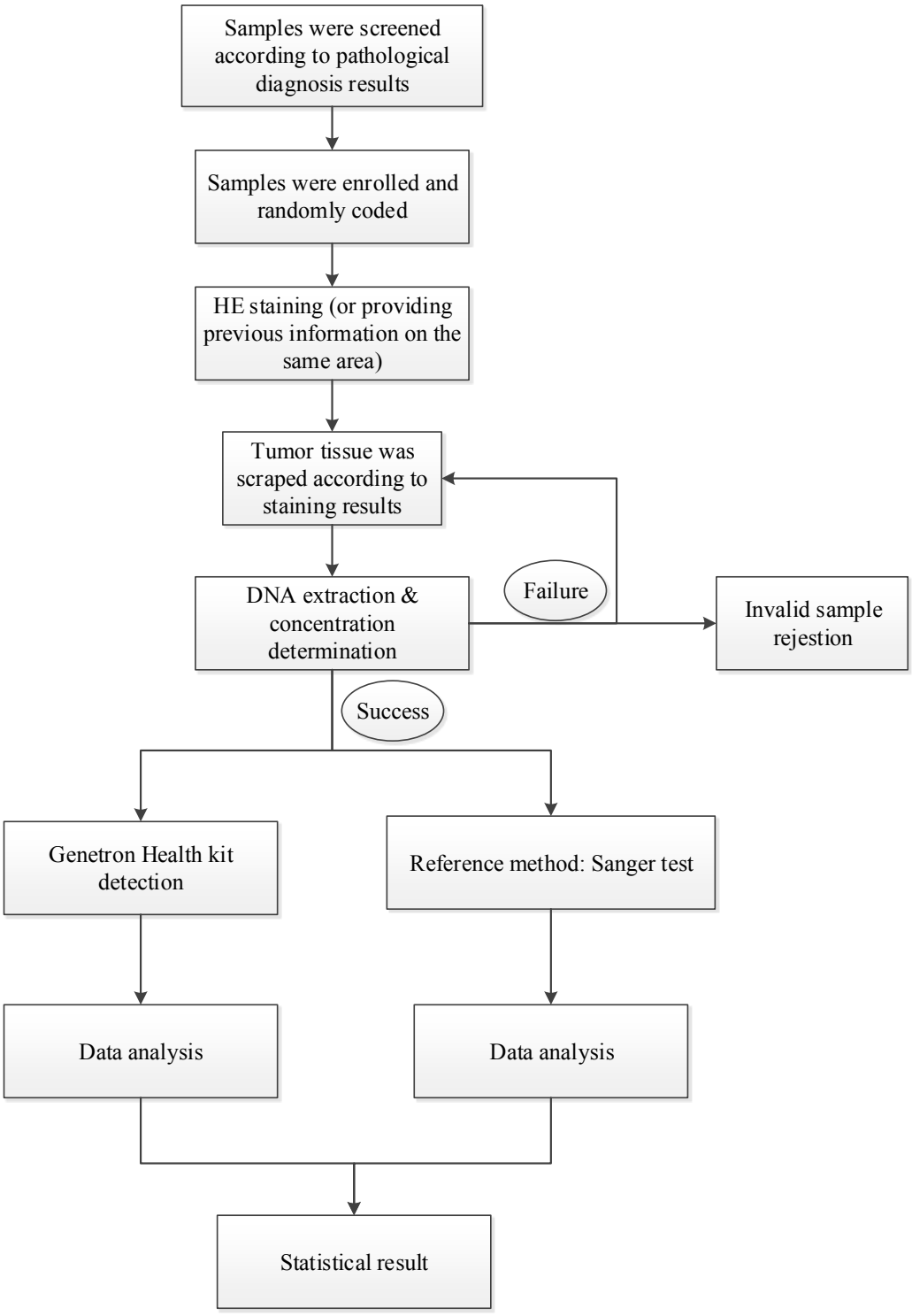
3.2.5 Reference method

Sanger sequencing was used as the control 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.

3.2.6 Testing process

The test samples were tested in strict accordance with the kit operation instructions.

3.3 Study technology route



3.4 Quality control method

(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 1 to 3 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 test kit detection and reference 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 test report must be complete, which must include date, test items, test results and their normal range. The person concerned should sign. Each test is set up control, and strictly in 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 fluorescence PCR technology and the applicable instruments, operating methods, and technical performance etc. of the kit.

(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.

4 Evaluation method

4.1 Evaluation method for reagents to be tested

1) The Ct value of FAM channel of positive control substance was ≤18, and the amplification curve had obvious exponential growth period.

(2) The blank control FAM channel had no amplification curve, or the amplification curve was slightly oblique without significant exponential growth, and the Ct value of FAM channel was ≥24.

(3) Result determination: After determining Ct value and △Ct value according to the above steps, determine the result according to the following table.

Table 2 Result determination

Mutation type	Negative	Negative		Positive	
IDH1 R132H	There was no obvious amplification of FAM channels Or the Ct value is not displayed	Ct value < 25	△ Ct > 8	Ct value < 25	△Ct ≤ 8

4.2 Evaluation method for nucleic acid sequence determination

(1) The results of the positive reference substance tested on the machine for nucleic acid sequence determination were positive, and the results of the blank reference substance should be in line with expectations without mutation detection.

(2) Determination of reagent results: the sequencing software Mutation Surveyor was used to interpret the results. When 6695 G>GA, corresponding mutation is indicated; otherwise, no mutation is indicated.

4.3 Evaluate the conformity between the kit and the reference method

(1) If the results to be tested and the reference results are both positive or negative, it is regarded as in conformity.

(2) In the event that the result to be tested is positive and the reference result is negative, or the result to be tested is negative and the reference result is positive, it is recorded as non-conformity.

(3) If the result to be tested fails and the reference method has confirmed results, it shall be recorded as non-conformity. The reasons and results of the non-conformity shall be truthfully recorded, and the comparison results shall be repeatedly confirmed when necessary.

(4) Complete subsequent statistical analysis based on the above statistical results.

5 Statistical methods

Statistical analysis of clinical study data: Kappa test was used for statistical analysis of the comparison between the kit test results and gene sequencing test results, and then the difference between the results obtained by the two methods was compared. If Kappa > 0.6, it was proved that there was no statistical difference between the two methods in detecting IDH1 gene mutations in glioma samples.

6 Duration of clinical trials and its reasons

The clinical study lasted about 6-8 months from the drafting of the protocol, project approval, ethical review, agreement signing, specimen collection, the beginning of the test to the end of the trial, and the report and seal are completed. If the sample collection is smooth and the number of samples meets the test requirements, the clinical trial time can be shortened; if it is not smooth, the time can be appropriately extended.

7 Inclusion criteria, exclusion criteria and rejection Criteria

7.1 Inclusion criteria

(1) Be able to provide samples timely according to the requirements of the protocol.

(2) The pathological examination conforms to the type of tissue sample listed in Table 1, and the tumor content is not less than 50%.

(3) The sample should have corresponding basic clinical information, including: the patient's visiting No. / medical record No./specimen No., age, sex, and pathological diagnosis result.

7.2 Exclusion criteria

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

Rationale: Included glioma, other brain tumors, and normal samples should be statistically significant and the conclusions obtained are scientifically valid. The selection of subjects for this study, while excluding research-related influencing factors, has no adverse effect on the health of the subjects.

7.3 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) Samples with unqualified genomic DNA quality, such as:

① DNA concentration is less than 10ng/μL;

② Genomic DNA was severely degraded or had insufficient purity, and OD260/OD280 exceeded the range of 1.6~2.0.

(3) Samples whose repeated test results of reference methods failed.

(4) Samples with incomplete clinical basic information (patients' visiting No. / medical record No./specimen No., age, sex, pathological diagnosis result).

8 Provisions for amendments to clinical trial protocols

Any modification to the protocol during the test should be explained, and the time, reason, process and record of the modification should be elaborated in detail, and its impact on the evaluation of the whole study results should be demonstrated.

9 Ethical issues and explanations of clinical trials

This clinical trial verification test uses the patient's pathological specimens in the previous treatment process. It is limited to in vitro diagnosis, does not directly contact the patient, does not cause any harm to the patient, and only compares the performance of the reagent with the control reagent. The results are only used for study and analysis related to this test, and will not guide the diagnosis and treatment of patients based on this, so it will not have any adverse effects on the human body.

This clinical trial strictly complies with the "Declaration of Helsinki", and this clinical verification trial involves relevant patient data. Such as: Patient visiting No. / medical record No. / specimen No., age, sex, pathological diagnosis result, etc. The hospital and Genetron Health (Beijing) Co., Ltd. will jointly undertake the confidentiality of the patient information, and the clinical trial operator and clinical validation unit promise not to disclose the content related to the subject specimen.

Therefore, the clinical trial was verified to submit an informed consent exemption to the ETHICS Committee.

10 Data processing and record keeping

It is required to keep all experimental records during the clinical trial, the results should be objective, timely and serious, and the operator and operation time should be recorded. Medical institutions shall keep the clinical trial data for five years after the termination of the trial.