Clinical Evaluation of Genetron D842V PCR Kit in GIST Patients

Study Document

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Content

1 Background

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor in gastrointestinal tract, biologically manifested from benign to malignant, usually expressed as CD117 and DOG1 positive in immunohistochemical testing, showing differentiated Cajal cells. Most cases have c-kit or platelet derived growth factor receptor alpha (PDGFRA) gene activation mutation, and a few cases involve other molecular changes, including SDHX, BRAF, NF1, K/N-RAS and PIK3CA gene mutations. These mutations force the protein kinase into an increasingly active state. Since other available treatments mainly bind to inactive protein conformations, certain primary and secondary mutations usually cause therapeutic resistance and disease progression. In unresectable or metastatic GIST, the clinical benefits of current treatments may vary depending on the type of mutation. Mutation detection plays an important role in tailoring treatments for potential disease drivers and is recommended in expert guidelines.

The full name of the protein encoded by PDGFRA gene is platelet derived growth factor receptor alpha α. As a cell surface receptor tyrosine kinase, PDGFRA can be activated after binding with its corresponding ligand PDGF, and then activate the phosphorylation pathways of phosphatidylinositol, cAMP and various proteins to regulate cell division and proliferation. Abnormal gene activation may cause tumorigenesis and promote tumor angiogenesis. PDGFRA mutation is closely related to GIST. Currently, up to 85% of patients with GIST have PDGFRA or KIT gene mutations. These mutations are associated with the production of abnormal KIT and PDGFRA proteins, which drive cancer. The two proteins can usually be shut down by imatinib (Glivec) and similar drugs, so as to block the activity of proteins. However, the PDGFRA Exon 18 mutant is very special. It changes the shape of PDGFRA protein, thus preventing the protein from binding to drugs. Meanwhile, clinical studies found that Avapritinib was able to bind to all mutated PDGFRA proteins tested and inhibit their activity in cancer cells. Since the most common mutant of PDGFRA is D842V mutant on Exon 18, the qualitative detection of D842V mutant on PDGFRA

in patients with GIST can be used to assist the concomitant diagnosis of Avapritinib in clinical drug treatment.

Genetron D842V PCR Kit is used for qualitative detection of D842V mutation in Exon 18 of PDGFRA gene in formalin-fixed paraffin-embedded (FFPE) tissue samples of patients with gastrointestinal stromal tumors (GIST), and for concomitant diagnosis of Avapritinib tablets in drug therapy. The result of this kit is for the related sites only, used as a reference for clinicians to choose proper tumor-targeted drugs for patients with GIST, and cannot be used as the only basis for individualized treatment of patients. Clinicians should thoroughly evaluate the detection results based on factors such as patients' condition, drug indications, treatment response and other laboratory indicators.

2 Study Purpose

The primary purpose of this study is to validate and evaluate the safety and effectiveness of Genetron D842V PCR Kit by comparing the results of this kit to those of the simultaneous detection with Sanger sequencing method, calculating the coincidence rate and consistency between the test reagent and the comparison method.

3 Study Design

3.1 Overall Study Design

This trial will be conducted in accordance with the principles of the synchronous blind method. The selected cases will be coded, and the selected samples will be detected with the test reagent and comparison method. When the comparison results are inconsistent with the detection results of Sanger sequencing method, the detection results of sanger will be acceptable. Results will be interpreted independently according to the critical value or interpretation requirements provided by each method, and relevant statistics will be carried out to evaluate the performance of the investigation kits in clinical application.

3.2 Sample Screening

3.2.1 Basis to Determine Sample and Sample Size

$$n = \frac{\left[Z_{1-\alpha/2}\sqrt{P_0(1-P_0)} + Z_{1-\beta}\sqrt{P_T(1-P_T)}\right]^2}{(P_T - P_0)^2}$$

Where, n represents the sample size; $Z_{1-\alpha/2}$ and $Z_{1-\beta}$ are fractions of standard normal distribution, P_0 is the target value of the evaluation indicator, and P_T is the expected value of the evaluation indicator for in vitro diagnostic reagents.

According to the results of preclinical studies, the target value of positive coincidence rate between test reagent and contrast reagent is up to 85% with the expected value up to 99%, the minimum sample size of positive control group is estimated to be 32 cases; if the target value of negative coincidence rate is up to 85% with the expected value up to 90%, the minimum sample size of negative control group is estimated to be 363 cases.

This product is classified as Class III IVD reagent medical device, and the overall sample size of clinical trials should meet the requirements of current relevant laws and regulations. According to the Measures for the *Administration of Registration of In-Vitro Diagnostic Reagents* (CFDA [2014] No. 5) and the *Technical Guidelines for Clinical Trials of In-Vitro Diagnostic Reagents* (CFDA [2014] No. 16), the overall sample size of this clinical trial should not be less than 1,000. It is planned to carry out the trial in at least three clinical sites, with the enrollment of no less than 300 samples at each site. In case of objective reasons, when the sample size is insufficient, appropriate adjustments can be made among the sites to ensure the overall sample size. If the relevant laws and regulations change during the trial, the latest laws and regulations shall prevail, and the number of positive and negative control samples should be statistically significant.

3.2.2 Inclusion, exclusion and elimination criteria

3.2.2.1 Inclusion criteria

(1) The paraffin-embedded tissue samples of patients clinically diagnosed as gastrointestinal stromal tumors (GIST)are included, and a small number of other cancers or benign lesions in gastrointestinal sites are included as interference samples.

(2) Samples should have appropriate basic clinical information, including: The patient's unique traceable number, age, sex, pathological diagnosis results, etc.

(3) The samples are FFPE gastrointestinal stromal tumor tissue samples, and each sample can provide 5 paraffin sections or 5 paraffin rolls with a thickness of at least 5 μm.

(4) The content of tumor cells meets the requirements of test reagent and comparison method.

3.2.2.2 Exclusion criteria

Those who do not meet any of the above inclusion criteria will be excluded.

3.2.2.3 Elimination criteria

(1) The investigators consider it inappropriate to continue clinical trials, such as samples prepared without following the required procedures.

(2) Samples that cannot complete the whole detection process.

(3) Patients with incomplete sample information.

3.2.3 Sample collection, storage and transportation

Sample collection

The samples will be collected according to the standard pathological operating procedures, the paraffin-embedded pathological section samples should be tested for the content ratio of tumor lesion cells which should meet the requirements of test reagents.

Sample storage and transportation

The FFPE tissue samples within the retention period are transported at normal

temperature, and the extracted nucleic acid is recommended to be detected immediately. Otherwise, DNA should be stored at -20°C±5°C for no more than 6 months, and repeated freezing and thawing should be avoided.

3.2.4 Sample numbering

In principle, only one sample should be collected from one subject, and each subject should have a unique clinical trial number (secondary code). After sample enrollment, before the experimental procedures, the blinding personnel marks the secondary code outside the sample tube, and carefully records the comparison table between the primary code (the unique traceable number of the patient) and the secondary code as the blind codes. Blinding documents shall be kept independently by the blinding personnel until breaking of blindness. Blindness should not be broken under any circumstance during the trial.

During experimental procedures, only the secondary code of samples is reflected.

3.3 Basis to Determine Comparison Method

According to "Technical Guidelines for Clinical Trials of in Vitro Diagnostic Reagents", for newly developed IVD reagents, appropriate subjects will be selected, and the investigational IVD reagents will be simultaneously compared with the "gold standard" for diagnosis in a blinded way in accordance with *Technical Guidelines for Clinical Trials of In-Vitro Diagnostic Reagents*.

For products that have similar products approved for marketing, it is best to use investigational IVD reagents and commercially available products of the same kind as clinical samples for comparative study, so as to demonstrate the equivalency between investigational IVD reagents and commercially available products of the same kind. Based on this principle, for the sites with commercially available products, the commercially available kits will be used for the comparison method; for the sites without commercially available products, Sanger sequencing will be used for the comparison method. There is similar product on the market for the human PDGFRA gene D842V mutation detection kit (PCR-fluorescence probe method), and Sanger sequencing is used as a comparison method.

3.4 Study Duration and Reasons for Determination

This clinical study will last about 8 - 12 months from drafting protocol, project establishment, ethical review, agreement signing, specimen collection, detection until the end of experiment, report completion and stamping. If samples are collected smoothly and the sample size meets the trial requirements, the trial duration can be shortened; if it doesn't go smoothly, the duration can be extended appropriately.

4 Statistical Method

4.1 Positive, Negative and Total Coincidence Rates Between Test Reagent and Comparison Method

For each genetic locus, fourfold tables will be used to calculate the positive and negative coincidence rates, positive and negative predictive values, total coincidence rate and their 95% confidence intervals. Using statistical software, Kappa test is used to determine statistical significance of the two detection methods.

Table 1 Result Summary of Clinical Trial Comparing Test Reagent and Comparison Method

Test Reagent	Comparison Method		Total
	Positive	Negative	
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	A+B+C+D

Samples with mutant genotype detected are considered as positive, and samples without mutant genotype are considered as negative.

The calculation formula is:

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

Negative compliance 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%

The calculation formula of 95% confidence interval: $p\pm1.96\times[\ p(1-p)/n]^{1/2}$ (where P

represents positive and negative coincidence rates, positive and negative predictive values and total coincidence rate, n presents the sample size. If p>0.9, Wilson score method will be used for correction).

4.2 Kappa test

Kappa test will be used to analyze the equivalence of test reagent and comparison method. Using statistical software, Kappa test will be used to compare the results of investigational reagents with gold standards, and k value will be calculated. When k>0.75, the investigational reagent and comparison method show 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 Method

Quality control should be applied at every stage of the clinical trial to ensure that all data are reliable and correct. The quality control method is as follows:

(1) Qualifications of researchers involved in the study Researchers involved in the clinical trial must have professional expertise, qualifications and competences in the clinical trial, and pass the qualification examination, and staffing should be relatively fixed.

(2) Training and preliminary tests: The sponsor is responsible for the training of researchers before the start of clinical trial, so as to help clinical researchers fully understand the clinical trial protocol, the detection of investigational products, the filling of original records and forms, etc.

(3) Laboratory quality control: Clinical laboratories should be strictly in accordance with the *Measures for the Administration of Clinical Gene Amplification Testing Laboratories in Medical Institutions* (MOH Office MA [2010] No. 194 or the current effective version) and other relevant administrative regulations of molecular biology laboratories and clinical gene amplification laboratories.

(4) Only when the quality control of investigational products meets relevant requirements can the test data be valid.

(5) Clinical trial monitoring: The monitor shall make a complete monitoring plan

before the start of the clinical trial, and monitor the clinical trial according to the monitoring plan to ensure that the clinical trial is carried out according to the clinical protocol, and ensure the intactness and accuracy of the original test data and records.

6 Provisions for Protocol Amendment

Any modification of the protocol during the trial shall be explained, and the time, reason, process and filing of the change shall be elaborated in detail, and its influence on the evaluation of the overall study results shall be demonstrated. If the investigators modify the protocol and case report form during the trial, it must be approved by the ethics committee before continuing the clinical trial.

7 Ethical issues and explanations involved in the clinical trial

The purpose of this clinical trial is to evaluate the performance of human PDGFRA gene D842V mutation detection kit (PCR-fluorescence probe method) in clinical application. The samples used in this clinical trial are the remaining pathological specimens detected during the treatment. These samples are only used for in vitro diagnosis without direct contact with patients and any harm to patients. These sample are only used to compare performance of the test reagent and the comparison method. The test results are only used for research and analysis related to this test, and will not guide the diagnosis and treatment of patients, therefore it will not have any adverse effects on human body. Informed consent can be exempted after being approved by the Ethics Committee.

This clinical trial is conducted in strict accordance with the *Declaration of Helsinki*, and the validation of this clinical trial involves relevant data of patients. For example: The patient's unique traceable number, age, sex, pathological diagnosis results, etc. The patient's relevant data will be kept confidential by the hospital and Genetron Health (Beijing) Co., Ltd, and the implementing party and verifier of the clinical trial are committed not to disclose the contents related to the subject specimens.

8 Data Processing and Record Storage

Investigators should record all the items on the clinical trial case report form truthfully, in detail and carefully according to the filling requirements, so as to ensure that the contents in the form are complete, true and reliable. All observations and findings obtained from the clinical trial should be verified to ensure the reliability of data and ensure that all conclusions in clinical trials come from original data. The clinical trial personnel should truly fill in relevant clinical information and the test results of clinical trials, and have them reviewed and signed by a specially-assigned person.

All original data records generated by this clinical trial shall be filed and preserved by the clinical sites, and the retention period shall be determined by the sites, but shall not be less than 5 years upon the completion of the clinical trial.

9 Responsibilities of Involved Parties

1. Sponsor's responsibility:

（1） Work with medical institutions to design and formulate clinical trial protocols, and sign clinical trial protocols and contracts agreed by both parties;

（2） Provide medical institutions with tested products and required contrast reagents free of charge, and provide necessary test equipment;

（3） Provide training for study staff.

2. Test facility's responsibility:

（1） Should be familiar with the relevant information provided by the implementing party and familiar with the use of the tested products;

（2） Work with the implementing party to design and formulate clinical trial protocols, and sign the clinical trial protocols between both parties;

（3） Propose clinical trial reports and be responsible for the correctness and reliability of the reports;

（4） The information provided by the implementing party shall be kept confidential, and the test results and process shall not be disclosed to any third party.

10 Study Technology Roadmap