SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-Time PCR Test

Device Trade Name: *therascreen*® PDGFRA RGQ PCR Kit

Device Procode: OWD

Applicant's Name and Address: QIAGEN GmbH
QIAGEN, Strasse 1
40724 Hilden, Germany

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P210002

Date of FDA Notice of Approval: June 29, 2023

II. INDICATIONS FOR USE

The QIAGEN *therascreen*® PDGFRA RGQ PCR Kit is a real-time qualitative *in vitro* diagnostic assay for the detection of the D842V somatic mutation in the *PDGFRA* gene using genomic DNA extracted from Gastrointestinal Stromal Tumor (GIST) patient's formalin-fixed paraffin-embedded (FFPE) tumor tissue.

The *therascreen* PDGFRA RGQ PCR Kit is intended for use as a companion diagnostic test, to aid clinicians in identification of patients with GIST who may be eligible for treatment with AYVAKIT™ (avapritinib) based on a *PDGFRA* mutation detected result. FFPE tumor specimens are processed using the QIAamp® DSP DNA FFPE Tissue Kit for manual sample preparation and the Rotor-Gene® Q (RGQ) MDx instrument for automated amplification and detection.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the *therascreen* PDGFRA RGQ PCR Kit labeling.

V. DEVICE DESCRIPTION

The following components comprise the overall device:

- QIAGEN QIAamp DSP DNA FFPE Tissue Kit
- QIAGEN *therascreen* PDGFRA RGQ PCR Kit
- QIAGEN Rotor-Gene Q (RGQ) MDx Instrument with automated data analysis and results interpretation using Rotor-Gene AssayManager (RGAM) software, Gamma MDx plug-in, and *therascreen*_PDGFRA_FFPE_MDx Assay Profile.

Specimen Preparation

FFPE blocks from patients with GIST are cut into 5 µm sections and mounted onto glass slides. A hematoxylin and eosin (H&E) stained slide is used to confirm that there is tumor present. Two non-stained tissue sections are scraped from the slide for Deoxyribonucleic Acid (DNA) extraction.

DNA is manually extracted and purified using the QIAGEN QIAamp DSP DNA FFPE Tissue Kit. The FFPE sample is deparaffinized with xylene, the xylene supernatant is then removed, and any residual xylene is extracted with ethanol. The sample is lysed under denaturing conditions with proteinase K for one hour at 56°C. The sample is heated for one hour at 90°C to reverse formalin cross-linking of genomic DNA (gDNA). The sample is passed through a silica-based membrane so that the gDNA binds to the membrane and any contaminants are removed. The membrane is washed multiple times with buffers (using a centrifuge to increase flow rates through the membrane). Purified gDNA is eluted from the membrane using elution buffer (ATE) from the QIAamp DSP DNA FFPE Tissue Kit.

PCR Amplification and Detection

The QIAGEN *therascreen* PDGFRA RGQ PCR Kit contains reagents for the detection of the D842V mutation in exon 18 of the Platelet-Derived Growth Factor Receptor A (*PDGFRA*) gene, and a short, conserved sequence in exon 15 of the *PDGFRA* gene which is used as a Control Reaction.

The *therascreen* PDGFRA RGQ PCR Kit uses real-time polymerase chain reaction (PCR) with the following technologies for sequence related amplification and detection: TaqMan Probes, Locked Nucleic Acid (LNA) primers, and LNA based TaqMan Probe.

PCR uses forward and reverse primers to hybridize to a specific DNA sequence to amplify it. In addition to the primers, the TaqMan probes are labelled with differently colored fluorophores. The probes, which are labeled with a 5' fluorophore and a 3' quencher, also hybridize to the target sequence between the primers. While the probe remains intact, the probe sequence holds the fluorophore and quencher in close proximity, preventing the generation of fluorescence. The probe binds to its complementary sequence in the amplicon after each cycle of PCR.

During the extension step, the probe is degraded by the 5'-3' exonuclease activity of the *Taq* polymerase. The degradation of the probe leads to the dissociation of the fluorophore

and quencher, allowing them to freely disperse into the reaction mix, and facilitating the generation of light by the fluorophore. This fluorescent signal is detected by the PCR platform.

The probe used in the control reaction is labeled with 6-carboxyfluorescein (FAM) and the probe used in the mutation specific reaction is labeled with Texas Red. Texas Red and FAM absorb and fluoresce at different wavelengths:

- Texas Red: A fluorophore that excited at 596 nm and emits at a wavelength of 615 nm. This fluoresces in the Orange RGQ channel.
- FAM: A fluorophore that excited at 495 nm and emits at a wavelength of 520 nm. This fluoresces in the Green RGQ channel.

The Control Reaction Mix contains a forward and reverse primer and labeled probe (detected in the Green Channel) to amplify a sequence of exon 15 of the *PDGFRA* gene. DNA input is based on the Control Reaction result which is used to determine if an appropriate level of amplifiable DNA is present in the sample and is a factor in the analytical calculations that determine mutation status. All samples must be tested with the Control Reaction to ensure that they give Ct values within a specified range to ensure that there is enough amplifiable DNA to proceed with analysis, but not so much as to overload the assay. The Control Reaction determines whether the quality and quantity of DNA is sufficient for the working range of the assay. The interpretation of the results obtained from the Control Reaction Ct is presented below in Table 1. Any samples that do not give Ct values within this range are invalidated by the RGAM software plug-in and associated assay profile.

Table 1: Control Reaction Working Range

Control Ct value	Interpretation	Action
> 30.99	Quantity of amplifiable DNA is not sufficient for mutation analysis	Additional samples should be extracted and tested
< 20.85	Quantity of amplifiable DNA is too high for mutation analysis	Dilute with the sample diluent water supplied in the kit
Within 20.85-30.99	Quantity of amplifiable DNA is suitable for mutation analysis	No action required, sample is suitable

The PCR cycling parameters used for assessing the DNA sample are:

- Hold at 95°C for 15 minutes to activate the *Taq* polymerase;
- PCR for 40 cycles of 95°C for 30 seconds, to denature, and 61°C for 1 minute, to anneal/extend.

If the control assay Ct falls within range, then the sample is analyzed for the presence of the mutation by analyzing the values obtained in the mutation channel and completing the ΔCt calculation. If the control assay Ct is not within range, the sample is considered invalid and any results obtained may not be used to make a mutation status evaluation. This assessment is performed automatically by the RGAM software and associated plug-in and assay profile.

The *therascreen* PDGFRA RGQ PCR Kit contains reagents that allow PCR amplification and qualitative detection of the mutation listed in Table 2.

Table 2: Mutation Detected by the *therascreen* PDGFRA RGQ PCR Kit

Mutation	Exon	Base change	COSMIC ID*
D842V	Exon 18	2525A>T	COSM736
Control Reaction	Exon 15	N/A	N/A

*COSMIC ID taken from the Catalogue of Somatic Mutations in Cancer: https://cancer.sanger.ac.uk/cosmic

Test Controls

The *therascreen* PDGFRA RGQ PCR Kit contains two controls: A Positive Control (PC) and a No Template Control (NTC), which have been designed to detect fault conditions.

No Template Control (NTC): An NTC test contains nuclease-free water and is required in each RGQ run. The NTC serves as a control to assess potential contamination during assay set up.

Positive Control (PC): A PC test is required in each RGQ run. The PC Tube comprises a plasmid which carries both regions of the *PDGFRA* gene detected by the assay (Mutant and Control). Detection of both targets within acceptable ranges confirms the proper functioning of the reaction mix in the kit.

Instrument and Software

The *therascreen* PDGFRA RGQ PCR Kit is designed to be used with the Rotor-Gene Q MDx (RGQ) instrument, which is a real-time PCR analyzer designed for rapid thermal cycling and real-time detection of PCR assays. The RGQ incorporates a centrifugal rotary design for thermal cycling where a rotor, containing each tube, spins in a chamber of moving air, keeping all samples at a uniform temperature. Samples are heated and cooled in a low-mass-air oven according to a software-determined cycle that initiates the different phases of the PCR cycle. In the RGQ, fluorophores are excited from the bottom of the sample chamber by a light-emitting diode. Energy is transmitted through the thin wall at the bottom of each PCR tube. Emitted fluorescence passes through the emission filters on the side of the chamber and is detected by a photomultiplier tube. Detection is performed as each tube aligns with the detection optics; tubes spin past the excitation / emission optics every 150 milliseconds. The fluorescence signals indicate the progress of the PCR reactions. The Rotor-Gene Q MDx has six channels (six excitation sources and

six detection filters). Two of these channels, green and orange, are used with the *therascreen* PDGFRA RGQ PCR Kit.

Cycling parameters, data analysis, and results interpretation for the *therascreen* PDGFRA RGQ PCR Kit are performed by the RGAM Software, Rotor-Gene AssayManager Gamma MDx plug-in and *therascreen* PDGFRA RGQ PCR Kit Assay Profile (therascreen_PDGFRA_FFPE_MDx). Therefore, no manual analysis is required.

The RGAM Software is a core software which provides general functionality including: PCR run set up, cycler control and management of experiment data, results, assay profiles, and system configuration.

The Gamma MDx Plug-in extends the functionality of RGAM by providing cycle threshold (Ct) value calculation, data analysis, and normalization features.

Assay specific functionality, for example cycling conditions, thresholds and analysis cut-offs, and control ranges, is implemented by the *therascreen* PDGFRA Assay Profile.

The RGAM software plug-in and associated assay profile ensure that a user interface with restricted user options is displayed to the user and contains all the information required for automatic real-time PCR analysis including time and temperature profiles, data quality controls, and data analysis algorithms. The software suite also allows printing of test reports and creates result files in the software's file system.

In addition, the RGAM software plug-in and associated assay profile perform a quality check using Automatic Data Scan (AUDAS) that focuses on parameters of the respective fluorescence curves from which Ct values will be determined.

The AUDAS check is intended to identify problems that occur during the real-time PCR amplification that potentially generate non-typical curve shapes due to saturation, background noise, spikes, baseline dips, and sloping curves related to the real-time PCR instrument parameters or due to a problem linked to the assay itself. The curves in such situations are automatically invalidated to avoid generating misleading results.

Interpretation of Results

The first cycle at which the instrument can distinguish the amplification generated fluorescence as being above the background signal is called the Ct. The RGAM software interpolates fluorescence signals between any two recorded values. Ct values can therefore be any number (not limited to integers) within the range of 0 to 40.

Ct values generated by the Control and Mutation reactions indicate the quantity of assay specific input DNA. Low Ct values indicate higher input DNA levels and high Ct values indicate lower input DNA levels. Validity of controls and samples is determined based on the Ct values generated during a run.

Run Validity Criteria

For a *therascreen* PDGFRA RGQ PCR Kit run to be accepted as valid, the RGAM software, plug-in and associated assay profile require run data for the PC and NTC to meet specified criteria. The PC and NTC validity criteria are shown below. Each test run performed with the *therascreen* PDGFRA RGQ PCR Kit must meet all the validity criteria listed below (Table 3).

Table 3: Run, Sample Validity and Call Criteria

Sample	Target	RGQ Channel	Ct Acceptable Ranges*
Positive Control	Control	Green (FAM)	25.55 – 31.37
	D842V	Orange (Texas Red)	24.09 – 29.69
NTC	All	Green (FAM) and Orange (Texas Red)	Has no value
Test Sample	Control	Green (FAM)	20.85-30.99

* Ranges are inclusive (i.e., include the values shown)

If a run fails any of the validity criteria, the RGAM software displays the corresponding validity rule related to the failed control and does not provide test results for samples in the RGAM report. If all run validity criteria are correct, the RGAM software generates a report that confirms the respective controls validity and then displays sample results.

The individual sample results in each test run are accepted as valid if the RGAM software obtains Ct values for the PDGFRA assay. If a sample fails to generate a Ct value for the PDGFRA mutant (orange) channel, then the RGAM software checks the Ct value obtained in the green (control) channel to ensure the quantitative PCR (qPCR) reaction validity. If the RGAM fails to detect a signal within the validity criteria range in the green (control) channel, the sample is reported as invalid and no PDGFRA mutation status results for that sample are reported.

Determination of Sample Status

If the Control reaction Ct falls within range, then the sample is analyzed for the presence of the mutation.

If the Control reaction Ct falls outside of the higher end of this range (i.e., >30.99), the quantity of DNA is not sufficient for mutation analysis, so the sample should be retested. If the quantity of DNA is still insufficient upon re-test, re-extraction from FFPE sections is required. If this is not possible the sample will be reported as indeterminate.

If the Control reaction Ct falls outside of the lower end of this range (i.e., <20.85), the sample is too concentrated and will overload the mutation assay. In order to obtain a valid sample result, the sample must be diluted. Samples should be diluted on the basis that diluting by half will increase the Ct by 1. Samples should be diluted using the water provided in the kit (Water for Dilution [Dil.]).

The difference in Ct values (ΔCt) between the Control reaction and the mutation-specific reaction (mutation assay) is a qualitative measure of PDGFRA mutation status and is calculated as:

ΔCt = [Mutation reaction Ct value] – [Control reaction Ct value]

Samples are classed as mutation positive if they give a ΔCt less than or equal to the cut-off ΔCt value identified for the assay. Above this value, the sample may either contain less than the percentage of mutation able to be detected by the *therascreen* PDGFRA RGQ PCR Kit (beyond the limit of detection of the assay), or the sample is mutation negative, both of which would be reported as "No Mutation Detected".

The Mutation Assay ΔCt Cut-off is shown below in Table 4.

Table 4: ΔCt Cut-off of PDGFRA Kit

Mutation	ΔCt Cut-off
D842V	9

On the RGAM report each sample is assigned with a status as follows:

Invalid:

- If one of the AUDAS checks failed
- **or** if one of the run control criteria failed
- **or** if the Control reaction Ct was outside of the acceptance range

Mutation Detected:

- If all AUDAS checks passed
- and if all run control criteria were met
- and if the Control reaction Ct was within the acceptable range
- and if the PDGFRA mutant signal is equal to or below the predefined ΔCt cut-off

No Mutation Detected:

- If all AUDAS checks passed
- and if all run control criteria were met
- and if the Control reaction Ct was within the acceptable range
- and if the mutant signal is above the predefined ΔCt cut-off

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA cleared or approved assays for the testing of FFPE GIST tumor tissue for the PDGFRA D842V mutation status for the selection of patients who are eligible for treatment with AYVAKIT (avapritinib).

VII. MARKETING HISTORY

The *therascreen* PDGFRA RGQ PCR Kit has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform appropriately, or failure to correctly interpret test results may lead to incorrect PDGFRA D842V mutation results, which could impact patient treatment decisions. A false positive test result may lead to treatment with AYVAKIT (avapritinib) rather than current standard of care treatments. A false negative test result may prevent a patient from receiving AYVAKIT (avapritinib), a potentially life extending targeted therapy. Either of these outcomes could adversely impact the survival or quality of life of a patient with advanced GIST.

For the specific adverse events that occurred in the clinical study evaluating the efficacy of AYVAKIT (avapritinib), please see AYVAKIT (avapritinib) FDA approved package insert which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The specific performance characteristics of the *therascreen* PDGFRA RGQ PCR Kit (henceforth referred to as PDGFRA Kit) were determined by studies using FFPE clinical specimens from patients with GIST randomized in the BLU-285-1101 trial and/or commercially procured FFPE tissue specimens obtained from patients with a gastrointestinal stromal tumor (GIST). No cell line materials have been used.

1. Correlation to Comparator Method/Accuracy

The accuracy of the PDGFRA Kit was demonstrated relative to a validated next generation sequencing (NGS) assay using FFPE clinical specimens from patients with GIST randomized in the BLU-285-1101 trial for which there were sufficient quantity and quality of specimen available for testing with the NGS comparator assay and from procured clinical GIST FFPE samples. The PDGFRA Kit and NGS testing was performed on DNA samples extracted from 217 clinical FFPE GIST samples (166 clinical trial samples and 51 procured clinical samples). Of the 217 FFPE samples, twelve clinical trial and four procured clinical samples could not be evaluated by the NGS assay, and seven clinical trial samples could not be evaluated using the PDGFRA Kit. The remaining 194 samples (147 clinical and 47 procured samples) produced valid results for analysis of analytical concordance. Samples with both NGS and PDGFRA Kit valid results were analyzed to assess overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA) based on the agreements between the two methods for mutation status. The percentages, together with the corresponding two-sided exact 95% CI, are summarized below.

The results demonstrate point estimates of PPA, NPA, and OPA of 98.44%, 100% and 99.48%, respectively (Table 5).

Table 5: Agreement in Mutation Status between *therascreen* PDGFRA RGQ PCR Kit and NGS (using NGS as the Reference Method)

Measure Of Agreement	Frequencies	Percent Agreement (%)	Lower Two-sided 95% Confidence Limit	Upper Two-sided 95% Confidence Limit
Overall Percent Agreement	193/194	99.48	97.16	99.99
Positive Percent Agreement	63/64	98.44	91.60	99.96
Negative Percent Agreement	130/130	100.00	97.20	100.00

The OPA, PPA, and NPA were also evaluated including the seven invalid samples (samples without PDGFRA Kit results), with corresponding two-sided exact 95% CI. The results demonstrate point estimates of PPA, NPA, and OPA of 96.92%, 95.59% and 96.02%, respectively (Table 6).

Table 6: Agreement in Mutation Status between *therascreen* PDGFRA RGQ PCR Kit and NGS (using NGS as the Reference Method) - Including PDGFRA Kit Invalid Samples

Measure Of Agreement	Frequencies	Percent Agreement	Clopper-Pearson (Exact) Binomial Lower Two-sided 95% Confidence Limit	Clopper-Pearson (Exact) Binomial Upper Two-sided 95% Confidence Limit
Overall Percent Agreement	193/201	96.02	92.31	98.27
Positive Percent Agreement	63/65	96.92	89.32	99.63
Negative Percent Agreement	130/136	95.59	90.64	98.36

2. **Analytical Sensitivity**

   a. **Analytical Sensitivity – Limit of Blank (LoB)**

   The LoB of the PDGFRA Kit was established by testing DNA extracted from 53 individual wild-type (WT) FFPE samples with two replicates per sample for each of three PDGFRA Kit lots (generating 318 data points total). Of the 318 replicates, 273 fell within the control working range and were included in the analysis. The LoB was defined as the highest measurement result that corresponds to the upper 95th percentile in the WT samples. The lowest value of the three LoB estimates (one from each *therascreen* PDGFRA Kit lot) was determined to be the LoB value.

The LoB value for the mutation assay (in terms of ΔCt) detected by the *therascreen* PDGFRA RGQ PCR Kit was determined as 9.854 ΔCt and was therefore above the cut-off value of ΔCt ≤ 9 determined for the mutation assay.

b. Analytical Sensitivity – Limit of Detection (LoD)

The LoD for the PDGFRA Kit, defined as the minimum percentage of mutant DNA in a background of WT DNA that can be detected with a 95% probability as determined by a probit analysis, was determined using five mutation positive clinical samples normalized to six different percent mutant DNA or mutant allele frequency (%MAF) levels (using clinical WT DNA as diluent) in two DNA input levels – low (29 ± 1.5 Ct) and medium (26 ± 1.5 Ct). The %MAF of each individual sample was determined previously by digital droplet PCR. The evaluation was performed with two different PDGFRA Kit lots for samples at a Ct value of 29.00 and two kit lots for samples at a Ct value of 26.00. In total, 756 data points were generated. The LoD was determined to be 9% MAF, the maximum observed value (in terms of %MAF) across all kit lots.

The LoD was confirmed using DNA samples extracted from four clinical mutant FFPE GIST specimens, which were normalized using clinical wild-type samples to two target Ct values (26.00 and 29.00 [± 0.5 Ct]) followed by MAF dilution of the mutant samples to 9% MAF (two clinical WT FFPE GIST specimens were used as diluent). The %MAF of each individual clinical sample was determined previously using digital droplet PCR. Each mutant sample was combined with the WT sample to generate five replicates at 9% MAF in each DNA input background across three different PDGFRA Kit lots. 358/360 DNA sample replicates were evaluated and upper one-sided exact 95% confidence limit calculated. The LoD was confirmed at 9% MAF as the upper one-sided exact 95% confidence limit for each DNA input level was determined to be greater than 95%.

c. Analytical Sensitivity - Control Working Range and ΔCt Cut-off

i. Control Working Range

The objective of this study was to set an appropriate Control Reaction Ct working range for use in assessing DNA sample validity. The Control Reaction Ct working range, defined as the amount of total amplifiable DNA in a sample, was determined using a total of 53 wild-type clinical FFPE GIST samples and 10 clinical mutant (D842V mutation positive) FFPE GIST samples with two replicates per sample for each of three PDGFRA Kit lots. In total, the samples generated 378 data points. To determine the Control Ct working range, the distribution of the Ct values of the control assay (green channel) was assessed from all of the samples evaluated in the study.

The final Control Reaction Ct working range was set at a Ct value of 20.85-30.99.

ii. ΔCt Cut-off

ΔCt Cut-off for this assay was determined by testing DNA extracted from 53 clinical WT and 10 clinical mutation positive FFPE GIST specimens with two replicates per sample for each of three PDGFRA Kit lots (generating 378 data points total). Of the 378 data points generated, 333 were determined to be within the control working range and included in the cut-off analysis.

The cut-off was determined in terms of ΔCt values and was chosen with respect to the following parameters: false positive rate, false negative rate, and assay sensitivity. The ΔCt cut-off value was determined to be ΔCt ≤ 9.

3. Linearity – Effect of DNA input on ΔCt

To demonstrate that the performance of the PDGFRA Kit is consistent across the control working range (20.85-30.99 Ct), a nine-level serial dilution of a D842V mutation positive sample with varying DNA input levels (lower levels being outside of the control working range) and 13.5% MAF (1.5x LoD) was evaluated (shown in Table 7). There were no specimens available with a natural concentration corresponding to the lower limit of the control working range (20.85 Ct); therefore, the target Ct value for the highest concentration was set by the specimen with the highest available natural concentration (23.35 Ct).

Table 7: Dilution levels targeted in the study

Dilution level:	13.5% MAF Samples (1.5x LoD)	
	Target Green Ct	Green Ct Generated
1	23.35	23.88
2	24.43	25.02
3	25.51	26.24
4	26.59	27.52
5	27.68	28.73
6	28.76	30.18
7	29.84	31.79
8	30.92	33.39
9	32	34.93

The evaluation was performed using one PDGFRA Kit lot with six replicates tested per DNA input level. The data was analyzed using regression analysis to determine the linear range. For the assay to be determined as linear across the DNA input range, there should be no change across the range in ΔCt, i.e., there is no statistically significant linear, quadratic, or cubic effect. The assay did not show a statistically significant ($p>0.05$) linear, quadratic, or cubic trend; therefore, this assay showed no change in ΔCt across the tested DNA input range (data not shown).

4. Analytical Specificity

a. Analytical Specificity - Primer and Probe Specificity

The purpose of this study was to demonstrate the specificity of the primers and probes used within the PDGFRA Kit. Multiple analyses were conducted to ensure that each oligonucleotide (Oligo) used in the PDGFRA Kit binds only to the target sequence and not to sequences from other species or to non-target human genome sequences. Each primer and probe design was checked against the nucleotide (nr/nt) database and the Human Genomic and Transcript (Human G+T) database using BLAST. Although two of the top hits for the forward primer were from EPH receptor sequences, the BLAST results for the reverse primer and probe did not return any hits for this target and therefore any amplification of this target, and hence generating a false positive result from amplification of the EPH receptor sequence is extremely unlikely. Therefore, study concluded that the primers and probes do not produce non-specific amplifications against other species or off-target human genome sequences, nor do they non-specifically bind to each other.

b. Analytical Specificity – Interference

The effects of potential interfering substances introduced from the FFPE Extraction Kit (exogenous substances) or from the FFPE sample (endogenous substances) on assay performance were measured by comparison of ΔCt between interferent-spiked and control-spiked lysates of D842V mutation positive DNA samples, by comparison of the Control reaction Ct for WT DNA samples, and by comparison of mutation status calls. The endogenous substances evaluated were hemoglobin and triglycerides, and exogenous substances evaluated were 1) paraffin wax, 2) xylene, 3) ethanol, 4) extraction buffers (ATL, AL, AW1, AW2), and 5) Proteinase K.

Mutant and WT clinical samples that were spiked with exogenous interferents were first normalized to the control reaction Ct 26.00 (±0.5 Ct) and Ct 29.00 (±0.5 Ct). The mutant samples were then diluted with WT (also normalized to the corresponding control reaction Ct) to give the %MAF representing 1.5x LoD (13.5% MAF). To avoid removing any variability which may have been introduced by the interferent, samples spiked with

hemoglobin and triglycerides during the extraction process were used immediately following extraction.

Samples tested with hemoglobin and triglycerides were spiked during the extraction process at the following concentrations: hemoglobin (2 mg/mL and 4 mg/mL) and triglycerides (3.7 µmol and 7.4 µmol). Samples tested with potential exogenous interferents were spiked at a concentration representing the highest (worst-case) feasible level of the interfering substance carryover into a sample. In total, eight replicates of each sample/interferent combination were tested with one PDGFRA Kit lot. All mutation status calls in the D842V mutation positive samples and WT samples were as expected. It should be noted that while the mutation status call was not impacted by the presence of these interferents, the samples used in this study were not considered borderline, i.e., near the clinical decision cutoff of ΔCt 9. In the presence of some potential interferents, however, statistically significant differences were observed with Ct values, which are described below.

The study demonstrated that none of the exogenous potential interfering substances tested had any impact on the performance of the PDGFRA Kit as the 95% confidence interval between samples spiked with interferent and the respective control samples were within ±2x intermediate precision of the assay, with respect to Ct, for WT and mutant samples, respectively. When evaluating hemoglobin and triglycerides as potential interferents, the study showed that hemoglobin had no impact on the PDGFRA Kit when testing with D842V mutation positive samples.

However, there was a statistically significant impact on the performance of the PDGFRA Kit observed in 1) WT samples spiked with low and high concentrations of hemoglobin, 2) D842V mutant samples spiked with low concentration of triglycerides, and 3) mutant and WT samples spiked with high concentration of triglycerides. The 95% confidence intervals between samples spiked with interferent and the respective control samples were outside ±2x intermediate precision of the assay. The presence of hemoglobin in WT samples at a concentration of at least 2 mg/ml and the presence of triglycerides at a concentration of 7.4 µmol have the potential to increase the Green channel Ct value closer to the upper limit of the control working range, increasing the risk of an invalid result. While the mutation status call was not impacted in the study performed, the presence of triglycerides in mutant samples at a concentration of at least 3.7 µmol have the potential to either increase or decrease the ΔCt value, potentially resulting in a different mutation call if the sample's mutation status is borderline to ΔCt 9. The results can be found in Table 8 below.

Table 8: Summary of the potential impact of the endogenous substances

Potential Interferant (Endogenous)	Concentration	Sample Type	Mean Control reaction Ct/ΔCt without interferent^ (SD)	Mean Control reaction Ct/ΔCt with interferent^ (SD)	95% Confidence Intervals of difference in Ct/ΔCt with interferent and without interferent	Potential Impact Control reaction Ct/ΔCt
Hemoglobin	2 mg/mL	Wild-type	25.29 (0.339)	26.25 (0.464)	0.584, 1.346	Higher Control reaction Ct value*
Hemoglobin	4 mg/mL	Wild-type	25.29 (0.339)	26.12 (0.557)	0.453, 1.214	Higher Control reaction Ct value*
Triglycerides	3.7 µmol	Mutant	-0.29 (0.043)	-0.33 (0.060)	-0.142, 0.064	Lower ΔCt value
Triglycerides	7.4 µmol	Mutant	-0.29 (0.043)	-0.24 (0.155)	-0.046, 0.161	Higher ΔCt value#
		Wild-type	26.01 (0.406)	27.19 (0.698)	0.664, 1.688	Higher Control reaction Ct value*

^ Analysis of interference was based on the 95% CI of the difference in control reaction Ct for wild-type samples and difference in ΔCt for mutant samples
* Increased risk of invalid result
# Risk of false negative in borderline samples

5. Cross-Contamination/Carry Over

The objective of this study was to demonstrate the absence of PCR cross contamination of the WT samples by mutant samples within the DNA extraction and run set-up procedure. The study focused on the DNA extraction of FFPE samples utilizing one kit lot of the FFPE extraction kit to identify any cross contamination associated with routine use of the PDGFRA Kit. Contamination could potentially occur at any stage of the testing procedure. This study was designed to investigate the probability of cross contamination during the whole testing procedure (DNA extraction and subsequent PDGFRA Kit).

This study was performed with D842V and WT FFPE sections from procured clinical GIST specimens. Two independent sets of samples referred to as "Set A" and "Set B" were extracted following a pre-defined extraction matrix. Two operators performed the extractions. Nine extractions were carried out from the D842V mutation positive specimen, and seven extractions were carried out from each of the three WT specimens. The extracts were tested across five PCR runs, alternating between a checkerboard layout of mutant sample replicates adjacent to WT sample replicates and a WT samples only layout. The five PCR runs were set up consecutively by the same operator using the same equipment and RGQ instrument, with no other runs set up using this instrument between these runs.

A total of 126 WT replicates were tested. The observed percentage of correct mutation status calls for WT samples was 100% ('no mutation detected,' i.e., WT sample), demonstrating no cross contamination of the WT samples by D842V

mutation positive samples sharing the same DNA extraction and run set up procedure.

6. **Repeatability and Reproducibility**

The objective of this study was to evaluate the precision of the PDGFRA Kit within-laboratory (repeatability and intermediate precision) and between-laboratories (reproducibility). The repeatability and reproducibility was investigated by testing DNA extracted from three mutant and three WT clinical GIST FFPE specimens. To assess repeatability, D842V mutation positive samples at two mutation levels (LoD and 1.5x LoD) and two DNA input levels (medium (control reaction Ct value of 26) and low (control reaction Ct value of 29)) and WT samples at medium and low DNA input levels were tested at one site (located in the United Kingdom). The samples were tested in triplicate with four runs per day across multiple days, RGQ instruments, and operators and using one PDGFRA Kit lot resulting in 108 data points per sample. To assess reproducibility, two runs per day were performed per operator (two operators per site) by two additional sites (both located in the USA) over six days to give an additional 108 data points for each additional site. One PDGFRA Kit lot (the same lot for all three sites) was used.

For each sample, the proportion of correct mutation calls along with the corresponding two-sided exact 95% confidence intervals are reported in Table 9 below, for repeatability.

Table 9: Repeatability - proportion of correct mutation status calls for Site 1

Grouping Variable(s)			Proportion		Two-Sided 95% Confidence Limit	
Sample	Green Channel Ct	Site	Fraction	Percentage	Lower	Upper
MT 1.5x LOD	Ct26	1	108 / 108	100.00%	96.64%	100.00%
	Ct29	1	108 / 108	100.00%	96.64%	100.00%
MT LOD	Ct26	1	108 / 108	100.00%	96.64%	100.00%
	Ct29	1	108 / 108	100.00%	96.64%	100.00%
WT	Ct26	1	108 / 108	100.00%	96.64%	100.00%
	Ct29	1	107 / 107	100.00%	96.61%	100.00%

For each sample, the proportion of correct mutation calls along with the corresponding two-sided exact 95% confidence intervals are reported in Table 10 below, for reproducibility. There was one incorrect (false negative) call for the mutant (MT) sample at 1.5x LoD and Ct29.

Table 10: Reproducibility - proportion of correct mutation status calls across all sites

Grouping Variable(s)		Proportion		Two-Sided 95% Confidence Limit	
Sample	Green Channel Ct	Fraction	Percentage	Lower	Upper

MT 1.5x LOD	**Ct26**	321 / 321	100.00%	98.86%	100.00%
	Ct29	323 / 324	99.69%	98.29%	99.99%
MT LOD	**Ct26**	324 / 324	100.00%	98.87%	100.00%
	Ct29	324 / 324	100.00%	98.87%	100.00%
WT	**Ct26**	324 / 324	100.00%	98.87%	100.00%
	Ct29	323 / 323	100.00%	98.86%	100.00%

A variance component analysis was used to estimate the standard deviation for between-run, between-day, between-operator, between-instrument, and between sample for the repeatability and reproducibility study. These estimates were reported along with the number of observations for the mean of ΔCt, Control (green) Ct, and Mutant (orange) Ct values. Results by variance components are presented below (Tables 11 and 12).

Table 11: Repeatability - Variance Components in terms of SD

Analysis Variable	Template	Sample Green Ct	Site	Number of Amplified	Number of Non-Amplified	Mean	Between Day	Between Run Key Order	Between Instrument	Between Operator	Between Sample	Residual	#Total
							SD	SD	SD	SD	SD	SD	SD
Delta Ct	**MT 1.5x LOD**	**Ct26**	**1**	**108**	**0**	**0.69**	0.0000	0.1623	1.1395	0.1810	0.0544	0.1880	0.8649
		Ct29	**1**	**108**	**0**	**0.76**	0.0000	0.0000	0.9674	0.0000	0.1538	0.4481	0.8302
	MT LOD	**Ct26**	**1**	**108**	**0**	**1.41**	0.0899	0.1203	1.3485	0.0000	0.0172	0.1933	0.9884
		Ct29	**1**	**108**	**0**	**1.68**	0.0000	0.1220	1.1478	0.0000	0.0679	0.6761	1.0658
Green Ct	**MT 1.5x LOD**	**Ct26**	**1**	**108**	**0**	**26.31**	0.0371	0.0782	0.1442	0.0000	0.0302	0.1026	0.1620
		Ct29	**1**	**108**	**0**	**29.31**	0.0000	0.0641	0.0611	0.0548	0.0692	0.1552	0.1857
	MT LOD	**Ct26**	**1**	**108**	**0**	**26.3**	0.0419	0.0857	0.1182	0.0000	0.0253	0.1120	0.1684
		Ct29	**1**	**108**	**0**	**29.31**	0.0000	0.1072	0.0763	0.0578	0.0342	0.1388	0.1822
	WT	**Ct26**	**1**	**108**	**0**	**26.19**	0.0315	0.0755	0.1628	0.0000	0.2130	0.1186	0.2528
		Ct29	**1**	**107**	**0**	**29**	0.0000	0.0343	0.0986	0.0000	0.3982	0.1385	0.3634
Orange Ct	**MT 1.5x LOD**	**Ct26**	**1**	**108**	**0**	**27**	0.0000	0.1815	0.9885	0.0000	0.0538	0.1870	0.7697
		Ct29	**1**	**108**	**0**	**30.07**	0.0000	0.0000	0.9030	0.0000	0.0596	0.4417	0.7805
		Ct26	**1**	**108**	**0**	**27.72**	0.1112	0.1349	1.2250	0.0000	0.0556	0.1878	0.9108

Analysis Variable	Template	Sam ple Green Ct	Site	Number of Amplified	Number of Non-Amplified	Mean	Between Day	Between Run Key Order	Between Instrument	Between Operator	Between Sample	Residual	#Total
							SD	SD	SD	SD	SD	SD	SD
	MT LOD												
		Ct29	1	108	0	31	0.0000	0.1335	1.0787	0.0618	0.0000	0.6344	1.0050

Table 12: Reproducibility - Variance Components in terms of SD

Analysis Variable	Sample	Sample Green Ct	Number of Amplified	Number of Non-Amplified	Mean	Between Site	Between Day Within Site	Between Run Key Order Within Site	Between Instrument Within Site	Between Operator Within Site	Between Sample Within Site	Residual	#Total
						SD	SD	SD	SD	SD	SD	SD	SD
Delta Ct	MT 1.5x LOD	Ct26	321	0	1.1	0.0000	0.0000	0.1810	0.6061	0.0000	0.0315	0.2269	0.6367
		Ct29	324	0	1.16	0.0000	0.0000	0.1918	0.5388	0.0000	0.1862	0.6527	0.8565
	MT LOD	Ct26	324	0	1.87	0.0000	0.0000	0.1681	0.7090	0.0000	0.1076	0.2430	0.7224
		Ct29	324	0	2.03	0.0000	0.0000	0.2575	0.5968	0.0000	0.0560	0.6753	0.9026
Green Ct	MT 1.5x LOD	Ct26	321	0	26.09	0.1742	0.0133	0.0773	0.0816	0.0000	0.0447	0.1080	0.2157
		Ct29	324	0	29.16	0.1171	0.0572	0.0578	0.0311	0.0349	0.0861	0.1712	0.2288
	MT LOD	Ct26	324	0	26.11	0.1521	0.0233	0.0665	0.0759	0.0000	0.0277	0.1112	0.1966
		Ct29	324	0	29.17	0.1108	0.0191	0.0876	0.0450	0.0557	0.0327	0.1679	0.2202
	WT	Ct26	324	0	25.9	0.1645	0.0316	0.0838	0.1093	0.0296	0.3000	0.1187	0.3617
		Ct29	323	0	28.82	0.0000	0.0654	0.0482	0.0656	0.0468	0.4373	0.1528	0.4477
Orange Ct	MT 1.5x LOD	Ct26	321	0	27.19	0.0000	0.0000	0.1470	0.4876	0.0000	0.0214	0.2333	0.5341
		Ct29	324	0	30.32	0.0000	0.0000	0.2418	0.4609	0.0000	0.1313	0.6602	0.8273
	MT LOD	Ct26	324	0	27.98	0.0000	0.0000	0.1493	0.6103	0.0000	0.1303	0.2435	0.6385
		Ct29	324	0	31.2	0.0000	0.0609	0.2534	0.5313	0.0000	0.0000	0.6558	0.8556

The repeatability and reproducibility were also performed for the PDGFRA Kit Positive Control and Non-template control (NTC). Each run performed contained a

positive control and NTC. None of the NTC samples included in the repeatability and reproducibility study amplified (i.e., no Ct values were generated). The results of the variance components for the positive control are below in Tables 13 and 14.

Table 13: Positive Control Repeatability - Variance Components in terms of SD

Analysis Variable	Template	Sample Ct	Site	Number of Amplified	Number of Non-Amplified	Mean	Between Day	Between Run Key Order	Between Instrument	Between Operator	Between Sample	Residual	#Total
							SD	SD	SD	SD	SD	SD	SD
Delta Ct	PC	Ct26	1	16	0	-1.87	0.0000	0.0000	0.9890	0.0546	0.0000	0.1197	0.7335
		Ct29	1	16	0	-1.86	0.1245	0.0000	0.8891	0.0000	0.0000	0.1599	0.6780
Green Ct	PC	Ct26	1	16	0	29.21	0.0000	0.0000	0.1707	0.1408	0.0000	0.3075	0.3523
		Ct29	1	16	0	28.98	0.0000	0.0000	0.1258	0.1324	0.0000	0.2630	0.2948
Orange Ct	PC	Ct26	1	16	0	27.34	0.0601	0.0000	0.7818	0.0000	0.0000	0.2660	0.6322
		Ct29	1	16	0	27.12	0.0000	0.0000	0.7285	0.0587	0.0000	0.2648	0.5958

Table 14: Positive Control Reproducibility - Variance Components in terms of SD

Analysis Variable	Template	Sample Ct	Number of Amplified	Number of Non-Amplified	Mean	Between Site	Between Day Within Site	Between Run Key Order Within Site	Between Instrument Within Site	Between Operator Within Site	Between Sample Within Site	Residual	#Total
						SD	SD	SD	SD	SD	SD	SD	SD
Delta Ct	PC	Ct26	40	0	-1.54	0.0000	0.0000	0.0000	0.5445	0.0000	0.0000	0.1310	0.5539
		Ct29	40	0	-1.57	0.0000	0.0691	0.0000	0.4733	0.0173	0.0000	0.1453	0.4969
Green Ct	PC	Ct26	40	0	29.04	0.1086	0.0000	0.0000	0.0897	0.1170	0.1008	0.2646	0.3166
		Ct29	40	0	28.88	0.0437	0.0000	0.0000	0.0574	0.0693	0.0415	0.2183	0.2398
Orange	PC	Ct26	40	0	27.49	0.0000	0.0000	0.0000	0.4072	0.0636	0.0000	0.2779	0.4892

Analysis Variable	Template	Sample Ct	Number of Amplified	Number of Non-Amplified	Mean	Between Site	Between Day Within Site	Between Run Key Order Within Site	Between Instrument Within Site	Between Operator Within Site	Between Sample Within Site	Residual	#Total
						SD	SD	SD	SD	SD	SD	SD	SD
		Ct29	40	0	27.31	0.0000	0.0000	0.0000	0.3746	0.0580	0.0000	0.2331	0.4399

7. **Specimen Handling – Extraction Reproducibility**

The objective of this study was to assess sample handling variability, specifically reproducibility of the DNA extraction as part of the *therascreen* PDGFRA RGQ PCR System process at three different sites (one located in the United Kingdom, and two in the USA). Two WT and two mutation positive clinical GIST FFPE specimens were used in this study. There were 34 FFPE sections required for each specimen; these FFPE sections were randomized and split into 17 extract sets. These extract sets were distributed evenly across the three testing sites, allowing for four extractions for each specimen per site. One QIAamp DSP DNA FFPE Tissue Kit lot was used for the extractions across all three sites. All DNA samples were then tested using a single kit lot of the PDGFRA Kit at the site in the United Kingdom.

When comparing the results of the samples across all three sites, the percentage of correct mutation calls for mutation positive and WT samples was 100.00% (Table 15).

Table 15: Proportion of Correct Calls Based on Overall Mutation Status

Grouping Variable(s)	Proportion		Two-Sided 95% Confidence Limit	
Sample Type	Fraction	Percentage	Lower	Upper
MT	24 / 24	100.00%	85.75%	100.00%
WT	23 / 23	100.00%	85.18%	100.00%

8. **Lot-to-Lot Interchangeability**

The objective of this study was to demonstrate lot-to-lot interchangeability and to demonstrate consistency of the mutation status across the QIAamp DSP DNA FFPE Tissue Kit and the PDGFRA Kit. The study utilized three lots of the FFPE Extraction Kit and three lots of the PDGFRA Kit to test five mutation positive and five WT clinical GIST FFPE specimens. Both sample type sets were comprised of high, medium, and low DNA input levels. The mutant samples also covered a range of natural %MAF levels, from approximately 2x LOD to approximately 4x

LOD. Each sample was extracted with three different lots of QIAamp DSP DNA FFPE Tissue Kit. Two replicate extractions were carried out per QIAamp DSP DNA FFPE Tissue Kit to give a total of six extractions per sample. All extracts were tested with three different lots of the *therascreen* PDGFRA RGQ PCR Kit for a total of 180 data points.

The overall percentage of correct mutation status calls across lots for all mutation positive and wild type samples tested was 100% (Tables 16 and 17). The study showed that lot-to-lot interchangeability has no impact on assay performance.

Table 16: Proportion of Correct Mutation call

Grouping Variable(s)	Proportion		Two-Sided 95% Confidence Limit	
Template	**Fraction**	**Percentage**	**Lower**	**Upper**
MT	90 / 90	100.00%	95.98%	100.00%
WT	90 / 90	100.00%	95.98%	100.00%

Table 17: Variance Components Reported In Terms of Standard Deviation

Analysis Variable	Template	Number of Amplified	Number of Non-Amplified	Mean	Between Kit	Between Extraction Kit	Between Run Key Order	Between Sample	Residual	#Total
					SD	SD	SD	SD	SD	SD
Green Ct	**MT**	**90**	**0**	**25.78**	0.0000	0.3508	1.0442	0.8241	0.1423	1.3511
	WT	**90**	**0**	**26.48**	0.0000	0.0000	2.6394	1.4193	0.5644	2.8959
Orange Ct	**MT**	**90**	**0**	**26.71**	0.0000	0.0000	0.7880	1.5311	0.7326	1.8773
Delta Ct	**MT**	**90**	**0**	**0.93**	0.0000	0.0000	0.6111	1.1464	0.6593	1.4407

9. Guardbanding

The objective of the guardbanding studies was to establish the robustness of the PDGFRA Kit. The following studies were conducted to: 1) determine the effect of varying reagent volume on the mutation status of samples called by the PDGFRA Kit, 2) determine the effect of varying reagent mixing, 3) determine the effect of varying the thaw time and set-up time of the PDGFRA Kit reagents and samples, and 4) assess the tolerance of the PDGFRA Kit to temperature variations in the annealing step during PCR that could be introduced by the RGQ instrument. For all

guardband studies, DNA samples extracted from one WT clinical GIST FFPE specimen and one D842V mutation positive clinical GIST FFPE specimen were assessed at low DNA input (green channel (control) Ct 29), and at 1.5x LoD (13.5% MAF) for mutation positive samples.

a. Volumetric Guardband

The objective of this study was to determine the effect of varying reagent volume on the mutation status of samples called by the PDGFRA Kit. The standard volumes as stated in the instructions for use are 19.8 µl reaction mix, 0.20 µl *Taq*, and 5 µl of sample. The volumetric tolerance was tested by varying the volume of each individual component while keeping the volume of the other components constant. Each component volume was varied by ±6%. This represents the total error that can be introduced by pipetting calculated by relative accuracy and precision stated in the pipette specifications.

The WT and mutation positive samples were tested with three replicates per run over four runs for each condition. A total of 12 replicates were generated per test condition. The study demonstrated that the differences in the mean ΔCt values for the mutation positive samples, and the mean Ct values of the Control reaction for the WT samples between each test condition and the nominal condition were within ±2x SD (standard deviation). The overall correct calls under the multiple conditions was 100% for both WT and mutation positive samples.

b. Mixing Guardband

The objective of this study was to determine the effect of varying reagent mixing conditions on the mutation status called by the PDGFRA Kit. The standard mixing conditions as stated in the instructions for use are to mix the reaction mix, positive control, and master mix by vortexing for 3-5 seconds. The mixing of *Taq* DNA polymerase is done by inverting the tube 10 times. The mixing conditions that were tested for the reaction mix, *Taq* DNA polymerase, positive control, samples and master mix is shown in the Table 18 below. The WT and mutation positive samples each had a total of 12 replicates generated per condition.

Table 18: Mixing Conditions

Mixing Conditions				
Condition	**Description**	**Taq**	**Reaction Mix/ PC/Samples**	**Master Mix***
1	Vortex (Standard condition)	Invert 10 times followed by brief centrifugation	Vortex 5 seconds followed by brief centrifugation	Vortex 5 seconds followed by brief centrifugation

2	Inverting	Invert 10 times followed by brief centrifugation	Invert 10 times followed by brief centrifugation	Invert 10 times followed by brief centrifugation
3	No-Mixing	No action	No action, brief centrifugation	No action, brief centrifugation

* Performed after *Taq* has been added to Reaction mix.

Mixing the reagents by inversion had no impact on the PDGFRA Kit. For the mutation positive samples, the mean ΔCt values between the test condition and the standard condition (vortexing) were within ±2x SD, and for the WT samples, the difference in the mean Ct value of the control reaction between the test condition and standard condition was within ±2x SD.

Not mixing the reagents did have a negative impact on the PDGFRA Kit performance. The mean ΔCt value for mutant samples, and the mean Control reaction Ct values for wild-type samples was greater than ±2x SD between the test condition (no-mixing) and the standard condition (vortexing).

c. Thawing and Set-up Time Guardband

The objective of this study was to determine the tolerance of the PDGFRA Kit to variations in thawing time and set-up time that can be introduced by the user.

The standard set-up and thawing conditions for the PDGFRA Kit reaction mix, *Taq* polymerase, water for NTC and DNA templates (including the positive control) is one hour at ambient temperature followed by an immediate set-up of the PCR run in the RGQ instrument. To determine the effect of variability that may be introduced by the end user, different thawing and RGQ set-up scenarios were evaluated, including storage for prolonged periods of time at room temperature or in a refrigerator (2-8°C). The PDGFRA Kit was tested across nine different set-up and thawing conditions, including eight variations of the standard condition and the standard condition itself for comparison (Table 19 below).

Table 19: Conditions tested for thawing and set-up time guardband

Condition	Thawing (hours)	Set-up Time
1***	1	0*
2	1	1.5
3	1	4.5
4	1	7.5
5	1	1.5+16hrs**

6	4.5	0*
7	4.5	1.5
8	4.5	4.5
9	4.5	1.5+16hrs**

* indicates that the run will be started on the RGQ immediately following set-up.
**16-hour set-up and left for 1.5 hours at room temperature followed by 16 hours stored in a refrigerator at 4–8°C.
***indicates the standard condition.

Two runs were performed per condition. The standard condition runs included 12 sample replicates for each sample (24 replicates total per sample, i.e., 12 replicates x 2 runs). The other condition runs included six sample replicates for each sample (12 replicates total per sample). The difference in mean ΔCt and Control reaction (green channel) Ct values between the test conditions and the standard condition were within ±2x SD. In addition, all conditions produced 100% correct mutation calls for both WT and mutation positive samples.

d. PCR cycling Guardband

The study was designed to determine the tolerance of the PDGFRA Kit to temperature variations of the annealing step during PCR that could be introduced by the RGQ instrument.

The standard cycling conditions for the PDGFRA Kit are denaturation at 95°C for 30 seconds and annealing at 61°C for 60 seconds. Annealing temperature was tested across a seven-point range 61°C ± 2°C. The 60°C to 62°C temperature range was selected to represent temperatures within the RGQ dynamic temperature specification, and two conditions, 59°C and 63°C, were selected to represent temperatures outside the RGQ specification range. A total of 24 replicates were tested per temperature condition per sample.

When the PCR annealing temperature was varied by ± 1°C, there was no impact on sample status called and all mutation positive samples were correctly identified. When the PCR annealing temperature was varied by ± 2°C, there was a statistically significant difference (≥ ±2 SD) observed in the WT samples between each test condition and the standard condition. Despite the observed significant difference, the mutation status call on all but one (1/120) WT sample replicate were correct (99.17%) within the RGQ specification range.

10. Stability Studies

a. Kit Stability

The *therascreen* PDGFRA RGQ PCR Kit was assessed for:

- Real-time stability (shelf-life, closed bottle, post-transport simulation)
- In-use stability (including freeze-thaw cycles and open vial, post-transport simulation)
- Transport simulation study (integrated in Real-time and In-use stability study)

For all real-time and in-use stability test timepoints (TTP), the same batch of clinical D842V mutation positive samples at 1.5x LoD (13.5% MAF) with two different DNA input levels (Control reaction (green channel) Ct 26 and Ct 29) and a clinical WT sample at DNA input level of Ct 26 were used. Real-time and In-use stability were assessed using the same three lots of the PDGFRA Kit.

i. Real-time Stability Testing

In the real-time stability study, a minimum of nine replicates of WT sample and nine replicates of each mutation positive sample were tested at each TTP. Testing was performed at eleven testing timepoints up to 25 months (TTP0, TTP2, TTP3+1 [three months plus one week], TTP6+1 [six months plus one week], TTP10, TTP12, TTP13, TTP18, TTP19, TTP24 and TTP25).

The real-time timepoints for WT and mutation positive samples showed 100% correct calls. The real-time stability study supported stability of the PDFGRA Kit for 24 months at -30°C to -15°C.

ii. In-Use Stability Testing

In the in-use stability study, a minimum of six replicates of WT and six replicates of each mutation positive sample were tested at each TTP. Additionally, the in-use stability study used three lots of the PDGFRA Kit to test samples over six timepoints up to 25 months (TTP0, TTP3, TTP6, TTP12, TTP18, and TTP25).

For all in-use timepoints evaluated, the percentage of correct mutation status calls was 100%. The in-use stability claim for the PDGFRA Kit is for five freeze/thaw cycles (N-1) for 24 months of storage at -30°C to -15°C.

iii. Transport Simulation Study

The stability study kits were exposed to conditions designed to simulate the extremes of environmental factors that may be experienced during the distribution from the manufacturing site to the customer. To ensure the worst-case scenario was simulated, three cycles of transport conditions were applied. The first and second cycle simulate the transportation to the warehouses and a subsequent interim storage, and the third cycle represents the transport to the

customer and the storage at their site. The transport conditions are shown below in Table 20.

Table 20: Transport Simulation Conditions

Cycle	Duration	Place	Temperature
1	5 days ± 2 hours	On dry ice	N/A
	2 days ± 2 hours	Freezer	-30°C to -15°C
2	5 days ± 2 hours	On dry ice	N/A
	2 days ± 2 hours	Freezer	-30°C to -15°C
3	5 days ± 2 hours	On dry ice	N/A
	2 days ± 2 hours	Freezer	-30°C to -15°C
	Storage	Freezer	-30°C to -15°C

Kits subjected to transport conditions were also used for Real-time and In-use stability studies. For the real-time stability study, one kit lot was subjected to simulated transport cycles in the final packaging after timepoint zero. For the in-use stability study, all three kit lots were subjected to transport conditions before timepoint zero to reflect the customer use. Thus, the transport stability was incorporated into the overall product stability claims based on real-time and in-use stability data. The samples evaluated using the kit lots that underwent transport simulation demonstrated 100% correct mutation calls. The transport simulation claim is 24 months at -30°C to -15°C.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY

QIAGEN GmbH (QIAGEN) performed a clinical performance study to establish a reasonable assurance of safety and effectiveness of *therascreen* PDGFRA RGQ PCR Kit for the detection of D842V somatic mutations in exon 18 of the *PDFGRA* gene using genomic DNA extracted from a patient with GIST's FFPE tumor tissue to select patients with GIST for whom AYVAKIT (avapritinib) was indicated in the US under NDA 212608. Data from this clinical study and the bridging study between the clinical trial assays (CTAs) and the *therascreen* PDGFRA RGQ PCR Kit were the clinical basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

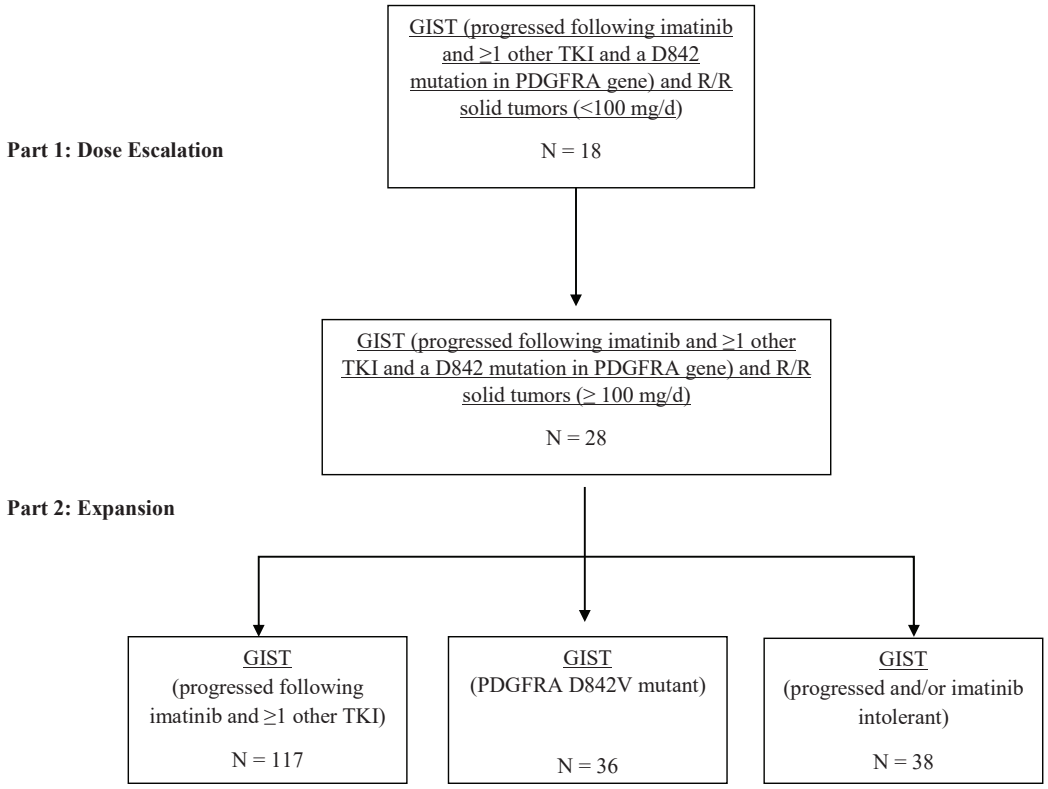
BLU-285-1101 (NAVIGATOR, NCT02508532) was an open-label, multicenter study of avapritinib (AYVAKIT) in adult patients with unresectable or metastatic GIST or

other relapsed or refractory solid tumors. The primary objective of the trial was to determine maximum tolerated dose, recommended Phase 2 dose, ORR of different patient groups, and overall safety and tolerability of avapritinib.

The first patient was enrolled on October 07, 2015, and the last patient was enrolled in November 2018. The database for this PMA reflected data collected through November 16, 2018 and included 237 patients. There were 19 investigational sites of which 16 enrolled at least 1 patient.

The study was initiated as a Phase 1, first-in-human study of avapritinib but was expanded with registrational intent in advanced GIST based on initial efficacy observed in dose escalation (Figure 1).

Figure 1: Study Schema BLU-285-1101



The study included a dose escalation part (Part 1, n = 46, 13 of whom were treated with either 300 or 400 mg quaque die [QD; once daily]) to determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) and an expansion part (Part 2, N = 191, who were all treated with either 300 or 400 mg QD) to further evaluate the safety and tolerability, and to assess the clinical efficacy of AYVAKIT (avapritinib) at the MTD/RP2D. In Part 2, patients were initially treated at a dose of 400 mg QD (MTD). Based on the emerging safety data, the dose utilized for Part 2 was reduced to 300 mg QD (RP2D).

In Part 2, two groups of patients with unresectable GIST with the following characteristics were enrolled and treated with AYVAKIT (avapritinib):

- Group 1 (n = 117): Patients with unresectable GIST that had progressed following treatment with imatinib and at least 1 of the following: sunitinib, regorafenib, sorafenib, dasatinib, pazopanib, or an experimental kinase inhibitor therapy and who did not have a D842V mutation in PDGFRA.

- Group 2 (n = 36): Patients with unresectable GIST harboring a D842V mutation in the *PDGFRA* gene.

A further group (Part 2, Group 3) of patients (n = 38) was defined with unresectable GIST that had progressed and/or those who had experienced intolerance following treatment with imatinib (including in the adjuvant setting) and who had not received additional kinase inhibitor therapy and did not have a known D842V mutation in PDGFRA.

Based on the clinical activity and tolerability of AYVAKIT (avapritinib) in Parts 1 and 2, the focus of the efficacy analysis shifted to the following two high medical need patient populations:

- Patients with unresectable or metastatic GIST who have been treated with at least 3 prior lines of tyrosine kinase inhibitor (TKI) therapy (4L+ population);

- Patients with unresectable or metastatic GIST who harbor PDGFRA exon 18 mutations, including PDGFRA D842V mutation, regardless of prior therapy (PDGFRA exon 18 population).

The 4L+ population reflects the majority of patients enrolled in Study BLU-285-1101 (n=121) and consists of a patient population with an unmet medical need. The D842V mutation is the most common PDGFRA exon 18 mutation occurring in patients with GIST. The remaining exon 18 mutations are a diverse subset of mutations and occur at very low incidence rates.

1. **Tumor Specimens and Testing**
   Specimens for PDGFRA testing were collected at screening. The PDGFRA mutation was identified by local or central assessment, either in an archival tissue sample or a new tumor biopsy obtained prior to treatment with avapritinib.

2. **Clinical Inclusion and Exclusion Criteria**
   An abbreviated list of the study inclusion/exclusion criteria is provided below. Enrollment in the BLU-285-1101 study was limited to patients who met the following inclusion criteria:
   a. Patient was ≥ 18 years of age.

b. For Part 1: Histologically- or cytologically-confirmed diagnosis of unresectable GIST or another advanced solid tumor. Patients with unresectable GIST must have had disease that had progressed following imatinib and at least 1 of the following: sunitinib, regorafenib, sorafenib, dasatinib, pazopanib or an experimental kinase-inhibitor agent, or disease with a D842 mutation in the *PDGFRA* gene. Patients with an advanced solid tumor other than GIST must have had relapsed or refractory disease without an available effective therapy.
   - At daily doses < 100 mg QD patients could have had the diagnosis of either GIST or a relapsed or refractory solid tumor.
   - At daily doses ≥ 100 mg QD, at least 2 patients in a cohort (4 patients if the cohort was expanded) must have had the diagnosis of GIST.

c. For Part 2:
   - Group 1: Patients must have had a confirmed diagnosis of unresectable GIST that had progressed following imatinib and at least 1 of the following: sunitinib, regorafenib, sorafenib, dasatinib, pazopanib, or an experimental kinase-inhibitor agent, and the patient did not have a D842V mutation in PDGFRA.
   - Group 2: Patients must have had a confirmed diagnosis of unresectable GIST with a D842V mutation in the *PDGFRA* gene. The PDGFRA mutation should have been identified by local or central assessment, either in an archival tissue sample or a new tumor biopsy obtained prior to treatment with avapritinib.
   - Group 3: Patients must have had a confirmed diagnosis of unresectable GIST that had progressed and/or patients must have experienced intolerance to imatinib and not have received additional kinase-inhibitor therapy. Patients must not have had a known D842V mutation in PDGFRA.
   - Groups 1, 2, and 3: At least 1 measurable lesion defined by the modified Response Evaluation Criteria in Solid Tumors (mRECIST) version 1.1 for patients with GIST.
   - Groups 1 and 2: A tumor sample (archival tissue or a new tumor biopsy) had been submitted for mutational testing.

d. Patient had Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2.
e. Patient or legal guardian, if permitted by local regulatory authorities, provided informed consent to participate in the study.

Patients were not permitted to enroll in the BLU-285-1101 study if they met any of the following exclusion criteria:

a. Patient had any of the following within 14 days prior to the first dose of study drug:
   i. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) >3× upper limit of normal (ULN) if no hepatic metastases were present; >5× ULN if hepatic metastases were present.

ii. Total bilirubin >1.5× ULN; >3× ULN with direct bilirubin >1.5× ULN in the presence of Gilbert's Disease.
iii. Estimated (Cockcroft-Gault formula) or measured creatinine clearance <40 mL/min.
iv. Platelet count $<90\times10^9$/L.
v. Absolute neutrophil count (ANC) $<1.0\times10^9$/L.
vi. Hemoglobin (Hgb) <9 g/dL. Transfusion and erythropoietin may have been used to reach at least 9 g/dL, but must have been administered at least 2 weeks prior to the first dose of study drug.

b. Patient received a prior anti-cancer drug less than 5 half-lives or 14 days (whichever was shorter) prior to the first dose of study drug.
c. Patient had received neutrophil growth factor support within 14 days of the first dose of study drug.
d. Group 3: Patients known to be *KIT* gene wild type.
e. Patient required therapy with a concomitant medication that was a strong inhibitor or strong inducer of cytochrome P450 (CYP) 3A4.
f. Patient had a major surgical procedure (minor surgical procedures such as central venous catheter placement, tumor needle biopsy, and feeding tube placement were not considered major surgical procedures) within 14 days of the first dose of study drug.
g. Patient had a history of another primary malignancy that had been diagnosed or required therapy within 1 year prior to the first dose of study drug. (The following were exempt from the 1-year limit: completely resected basal cell and squamous cell skin cancer, curatively treated localized prostate cancer, and completely resected carcinoma in situ of any site.)
h. Patient had a QT interval corrected using Fridericia's formula (QTcF) > 450 milliseconds.
i. Patient had a history of a seizure disorder (e.g., epilepsy) or requirement for anti-seizure medication.
j. Patient had a history of a cerebrovascular accident or transient ischemic attacks within 1 year prior to the first dose of study drug.
k. Patient had a known risk of intracranial bleeding, such as a brain aneurysm or history of subdural or subarachnoid bleeding.
l. Patient had a primary brain malignancy or metastases to the brain.
m. Patient had clinically significant, uncontrolled, cardiovascular disease, including congestive heart failure Grades II, III or IV according to the New York Heart Association classification, myocardial infarction or unstable angina within the previous 6 months, or poorly controlled hypertension.
n. Patient had a known diagnosis of human immunodeficiency virus infection or active viral hepatitis; viral testing was not required.
o. Patient had a prior or ongoing clinically significant illness, medical condition, surgical history, physical finding, or laboratory abnormality that, in the Investigator's opinion, could have affected the safety of the patient, alter the absorption, distribution, metabolism or excretion of the study drug, or impair the assessment of study results.

3. **Follow-up Schedule**

All patients attended an End of Treatment (EOT) visit within 14 (±7) days after the last dose of study drug. A safety Follow-up was conducted by telephone contact for resolution of any residual AE within 30 days (+7 days) after the last dose of study drug. Thereafter, patients were followed for disease assessment, subsequent antineoplastic therapy and survival approximately every 3 months until death, withdrawal of consent, or closure of the study by the Sponsor.

4. **Clinical Endpoints**

The primary endpoints of Part 1 of this study were: 1) determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) of avapritinib, and 2) to determine overall safety and tolerability of avapritinib.

The primary endpoints of Part 2 of this study were: 1) to determine the overall response rate (ORR) by mRECIST version 1.1 criteria at the MTD/RP2D of avapritinib in patients with GIST who had a D842V mutation in PDGFRA, 2) to determine the ORR by mRECIST version 1.1 criteria at the MTD/RP2D of avapritinib in patients with GIST that had progressed following treatment with imatinib and at least one other kinase inhibitor, and who are not known to have a D842V mutation in PDGFRA, 3) to determine the ORR by mRECIST version 1.1 criteria at the MTD/R2PD of avapritinib in patients with GIST who had progressed or who experienced intolerance to imatinib, including in the adjuvant setting, and who had not received additional kinase inhibitor therapy and did not have a known D842V mutation in PDGFRA, and 4) to determine the overall safety and tolerability of avapritinib.

5. **Clinical Bridging Study**

A non-interventional, retrospective clinical performance bridging study, for testing DNA extracted from patients' GIST FFPE tumor tissue biopsy samples using the *therascreen* PDGFRA RGQ PCR Kit in comparison with the Clinical Trial Assays (CTAs) used in clinical protocol BLU-285-1101 was conducted using extracted DNA samples obtained as part of the BLU-285-1101 study.

The primary objective of this study was to evaluate the concordance of the CTAs and the PDGFRA Kit and to assess the clinical efficacy of the PDGFRA Kit. The primary objective of the clinical efficacy analysis was to estimate the overall response rate (ORR) of AYVAKIT (avapritinib) in PDGFRA D842V mutation positive and PDGFRA D842V mutation negative patients (separately), when using the CDx to aid clinicians in identification of patients with GIST who may be eligible for treatment with avapritinib.

   a. **Sample Selection/Inclusion Criteria**

   Remnant DNA samples from all patients who provided consent, under the clinical trial BLU-285-1101, were included in the device study.

The disposition of samples from the clinical trial through to bridging study results is illustrated in "Accountability of PMA Cohort" section below.

B. Accountability of PMA Cohort

A total of 257 patients were screened for participation in the BLU-285-1101 trial using the CTAs. PDGFRA test records indicate39 patients were not enrolled due to screen failures (19 patients), inclusion/exclusion criteria not being met (18 patients), a serious adverse event (SAE) (1 patient) or withdrawal of consent prior to C1D1 (1 patient). Of the 257 patients tested by the CTAs, 237 patients were enrolled. Of the 237 patients enrolled, 236 produced valid CTA results (180 patients were PDGFRA D842V negative and 56 patients were positive).

Of 237 subjects who completed full study screening, 38 patients from Part 2 Group 3 were out of scope of the bridging study and one patient was not eligible due to CTA invalid results or inadequate sample, yielding 198 patient samples for evaluation.

From the 198 patient samples, remnant DNA samples from 166 GIST FFPE tumor biopsies were available for re-testing in the Bridging Study, evaluating the PDGFRA Kit efficacy and concordance.

The Primary Efficacy Population included only patients treated with a starting dose of 300 or 400 mg QD in Part 1 and Part 2 Groups 1 and 2. From the 166 patient samples available for the bridging study, some patients from Part 1 dose escalation phase were excluded, yielding 139 samples for the primary efficacy analysis. From the 139 samples, seven patients had an invalid result when testing with the PDGFRA Kit, yielding 132 evaluable samples for the Primary efficacy analysis (31 positive and 101 negative samples, as detected by the PDGFRA Kit).

The Concordance and Representativeness Analysis Population included patients treated with any dose. From the 166 patient samples available for the bridging study, patients from Part 2 Group 3 were excluded leaving 154 samples for the concordance analysis. From the 154 samples, seven patients had an invalid result when testing with the PDGFRA Kit, yielding 147 evaluable samples for the Concordance and Representativeness analysis.

C. Study Population Demographics and Baseline Parameters

The demographics of population enrolled in the study was consistent with the expected population of patients with GIST.

Demographics and baseline characteristics of the 237 patients in the safety population are summarized in Table 21 below.

Among the 237 patients in the safety population, most were male (145 patients; 61%), white (173 patients, 73%), and <65 years of age (144 patients; 61%). The median age of the patient population was 62 years and ranged from 25 to 90 years. Median BMI was 25.1 kg/m^2 and ranged from 15.3 to 55.6 kg/m^2. Most patients (229 patients,

97%) had an ECOG performance status of 0 or 1 at baseline. Overall, 112 (47%) patients were treated at study sites in Europe, 108 (46%) patients were treated at study sites in the US, and 17 (7%) patients were treated at study sites in Asia.

No meaningful differences were noted across the avapritinib starting dose groups for demographic or baseline characteristics.

Table 21: Overall Demographic and Baseline Characteristics (Safety Population)

Parameter	Avapritinib Starting Dose (QD)				All Doses[a] (N=237)
	<300 mg[b] (N=30)	300 mg (N=154)	400 mg (N=50)	300/400 mg[c] (N=204)	
Age (years), n	30	154	50	204	237
Mean (StdDev)	60.4 (9.46)	59.8 (11.30)	58.7 (10.39)	59.5 (11.06)	59.5 (11.03)
Median	60.5	62.0	60.5	62.0	62.0
Min, Max	41, 77	29, 90	35, 85	29, 90	25, 90
Age Group, n (%)					
<65 years	20 (66.7)	91 (59.1)	31 (62.0)	122 (59.8)	144 (60.8)
≥65 years	10 (33.3)	63 (40.9)	19 (38.0)	82 (40.2)	93 (39.2)
Sex, n (%)					
Female	11 (36.7)	60 (39.0)	20 (40.0)	80 (39.2)	92 (38.8)
Male	19 (63.3)	94 (61.0)	30 (60.0)	124 (60.8)	145 (61.2)
Race, n (%)					
American Indian or Alaska Native	0	1 (<1)	1 (2.0)	2 (<1)	2 (<1)
Asian	0	21 (13.6)	0	21 (10.3)	21 (8.9)
Black or African American	2 (6.7)	7 (4.5)	1 (2.0)	8 (3.9)	10 (4.2)
White	24 (80.0)	106 (68.8)	40 (80.0)	146 (71.6)	173 (73.0)
Unknown	4 (13.3)	14 (9.1)	8 (16.0)	22 (10.8)	26 (11.0)
Other	0	5 (3.2)	0	5 (2.5)	5 (2.1)
Ethnicity, n (%)					
Hispanic or Latino	0	5 (3.2)	1 (2.0)	6 (2.9)	6 (2.5)
Not Hispanic or Latino	24 (80.0)	132 (85.7)	40 (80.0)	172 (84.3)	199 (84.0)
Not Reported	3 (10.0)	10 (6.5)	4 (8.0)	14 (6.9)	17 (7.2)
Unknown	3 (10.0)	7 (4.5)	5 (10.0)	12 (5.9)	15 (6.3)
Region, n (%)					
US	14 (46.7)	71 (46.1)	21 (42.0)	92 (45.1)	108 (45.6)
Europe	16 (53.3)	66 (42.9)	29 (58.0)	95 (46.6)	112 (47.3)
Asia	0	17 (11.0)	0	17 (8.3)	17 (7.2)
Weight (kg), n	30	154	50	204	237
Mean (StdDev)	83.31 (20.740)	75.74 (20.988)	76.98 (21.199)	76.04 (20.994)	77.04 (21.045)
Median	79.80	73.70	75.60	74.10	75.00
Min, Max	46.1, 131.1	42.0, 156.3	39.5, 125.3	39.5, 156.3	39.5, 156.3
BMI (kg/mg^2), n	27	139	46	185	215
Mean (StdDev)	26.83 (5.872)	25.77 (6.443)	26.23 (5.935)	25.88 (6.308)	26.02 (6.221)
Median	26.26	24.03	25.83	24.62	25.06
Min, Max	18.0, 41.1	15.6, 55.6	15.3, 42.0	15.3, 55.6	15.3, 55.6

Parameter	Avapritinib Starting Dose (QD)				All Doses[a] (N=237)
	<300 mg[b] (N=30)	300 mg (N=154)	400 mg (N=50)	300/400 mg[c] (N=204)	
ECOG Performance Status, n (%)					
0	15 (50.0)	58 (37.7)	19 (38.0)	77 (37.7)	93 (39.2)
1	14 (46.7)	91 (59.1)	29 (58.0)	120 (58.8)	136 (57.4)
2	1 (3.3)	5 (3.2)	2 (4.0)	7 (3.4)	8 (3.4)

Abbreviations: BMI=body mass index; ECOG=Eastern Cooperative Oncology Group; Max=maximum; Min=minimum; StdDev=standard deviation; QD=once daily; US=United States.

[a] Includes 3 patients who received 600 mg avapritinib

[b] Includes patients who received avapritinib at starting dose levels of 30 mg, 60 mg, 90 mg, 135 mg, or 200 mg

[c] Includes patients who received a starting dose of either 300 mg or 400 mg avapritinib

For the 139 4L+ patients, males comprised 56% of the population and most were white (73%) and <65 years of age (66%) with a median age of 58 years, ranging from 33 to 80 years. ECOG performance status was 0 or 1 at baseline in 97% of patients.

The demographics and baseline disease characteristics of the patients with the PDGFRA D842V mutation in the safety population are summarized in Table 22 below. Within the safety population, 56 patients with PDGFRA D842V mutations, detected by CTAs, were enrolled in the clinical trial. Of the 56 patients enrolled, 38 patients were included in the efficacy population. Of the 38 patients considered efficacy evaluable, 31 patients were identified to contain the PDGFRA D842V mutation, as detected by the CDx, i.e., the PDGFRA Kit.

Table 22: Demographics and Baseline Disease Characteristics (Safety Population) – PDGFRα D842V Patients

Parameter	PDGFRα D842V Patients[a]		
	CDx positive[b,c] (N=31)	Efficacy Evaluable[b,d] (N=38)	Total Enrolled[e] (N=56)
Age (years), n	31	38	56
Mean (StdDev)	60.5 (12.94)	61.4 (12.16)	61.7 (12.35)
Median	63.0	63.5	64.0
Min, Max	29, 90	29, 90	25, 90
Age Group, n (%)			
<65 years	19 (61.3)	22 (57.9)	31 (55.4)
≥65 years	12 (38.7)	16 (42.1)	25 (44.6)
Sex, n (%)			
Female	13 (41.9)	13 (34.2)	17 (30.4)
Male	18 (58.1)	25 (65.8)	39 (69.6)
Race, n (%)			
American Indian or Alaska Native	0	0	0
Asian	6 (19.4)	6 (15.8)	6 (10.7)

Parameter	PDGFRα D842V Patients[a]		
	CDx positive[b,c] (N=31)	Efficacy Evaluable[b,d] (N=38)	Total Enrolled[e] (N=56)
Black or African American	3 (9.7)	3 (7.9)	4 (7.1)
White	20 (64.4)	25 (65.8)	39 (69.6)
Unknown	1 (3.2)	3 (7.9)	6 (10.7)
Other	1 (3.2)	1 (2.6)	1 (1.8)
Ethnicity, n (%)			
Hispanic or Latino	1 (3.2)	1 (2.6)	1 (1.8)
Not Hispanic or Latino	29 (93.5)	34 (89.5)	48 (85.7)
Not Reported	1 (3.2)	3 (7.9)	4 (7.1)
Unknown	0	0	3 (5.4)
Region, n (%)			
US	9 (29.0)	11 (28.9)	17 (30.4)
Europe	17 (54.8)	22 (57.9)	34 (60.7)
Asia	5 (16.1)	5 (13.2)	5 (8.9)
Weight (kg), n	31	38	56
Mean (StdDev)	75.65 (22.714)	77.04 (22.029)	79.44 (21.315)
Median	75.50	74.75	75.25
Min, Max	42.2, 156.3	42.2, 156.3	42.2, 156.3
BMI (kg/mg^2), n	29	36	54
Mean (StdDev)	27.01 (7.112)	27.16 (6.975)	27.36 (6.482)
Median	25.78	25.71	25.95
Min, Max	15.3, 53.1	15.3, 53.1	15.3, 53.1
ECOG Performance Status, n (%)			
0	10 (32.3)	13 (34.2)	21 (37.5)
1	19 (61.3)	23 (60.5)	32 (57.1)
2	2 (6.5)	2 (5.3)	3 (5.4)

Abbreviations: BMI=body mass index; ECOG=Eastern Cooperative Oncology Group; Max=maximum; Min=minimum; StdDev=standard deviation; US=United States.

[a] Patients with PDGFRA D842V mutation as determined by CTAs

[b] Includes 300mg/400mg avapritinib starting dose once daily (QD)

[c] Patients with PDGFRA D842V mutation as determined by the CDx

[d] Patients included in the efficacy analysis population for avapritinib

[e] Includes all doses

D. Safety and Effectiveness Results

1. Safety Results

The safety of the *therascreen* PDGFRA RGQ PCR Kit is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks. The safety with respect to treatment with avapritinib is not comprehensively addressed in the SSED for the *therascreen* PDGFRA RGQ PCR Kit. The evaluation of safety was addressed during review of the NDA and is based on the analysis of adverse events (AEs), clinical laboratory evaluations,

physical examinations, and vital signs. Refer to AYVAKIT (avapritinib) label at Drugs@FDA for complete safety information AYVAKIT (avapritinib).

a. Adverse effects that occurred in the PMA clinical study:
No adverse events were reported in connection with the studies used to support this PMA with the final market ready *therascreen* PDGFRA RGQ PCR Kit.

2. Effectiveness Results

The analysis of effectiveness was based on the clinical trial Primary Efficacy endpoints of Overall Response Rate (ORR) in 38 evaluable PDGFRA D842V mutation positive patients, as detected by CTAs, at the database cut-off of November 16, 2018.

a. Overall Response Rate in BLU-285-1101/NAVIGATOR Study
23. Of the 38 patients in the efficacy evaluable population, 2 patients were enrolled in Part 1 and 36 patients were enrolled in Part 2 Group 2.

Table 23: Efficacy Results for Patients with GIST Harboring PDGFRA Exon 18 D842V Mutations in NAVIGATOR

Efficacy Parameter	**PDGFRA D842V N = 38**
Overall Response Rate (95% CI)	89% (75%, 97%)
Complete Response, n (%)	3 (8%)
Partial Response, n (%)	31 (82%)
Duration of Response	n=34
Median in months (range)	NR (1.9+, 20.3+)
Patients with DOR ≥ 6- months, n (%)*	20 (59%)

Abbreviations: CI=confidence interval; NR=not reached; NE=not estimable
+ Denotes ongoing response
* 11 patients with an ongoing response were followed < 6 months from onset of response.

b. Overall Response Rate in CDx Efficacy Evaluable Population
Of the 38 evaluable patients, 31 patients in the 300/400 mg starting dose group had PDGFRA D842V mutations identified by the PDGFRA Kit. Of the remaining seven patients, five patient samples were not testable by the PDGFRA Kit, 1 patient sample was invalid, and one patient sample did not have the PDGFRA D842V mutation detected by the PDGFRA Kit. Of these 31 patients identified with PDGFRA D842V mutations by the PDGFRA Kit, 29 had confirmed complete response (CR) or partial response (PR) based on central radiology review for an ORR of 94% (95% CI: 78.6%, 99.2%). Best response

for these 31 patients was CR in 2 patients, PR in 27 patients, and stable disease (SD) in 2 patients, for a clinical benefit rate (CBR) of 100%. Median duration of response (DOR) was not reached in the 300/400 mg dose group; 22 patients (76%) were censored at the time of the data cut-off based on FDA censoring rules with 64% and 51% estimated to be in response at 12 months and 18 months, respectively.

The observed ORR in avapritinib treated subjects with a positive PDGFRA Kit result was calculated as a proportion of subjects with complete or partial response in all treated subjects with a positive PDGFRA Kit result. As shown below in Table 24, the ORR in the positive PDGFRA Kit subjects was 0.94 (95% CI: 0.79, 0.99).

Table 24: Overall Response Rate in CDx Evaluable Patients from the Primary Efficacy Analysis Population

CTA and CDx Result Group**	Frequencies	ORR (95% CI)*
CDx+	29 / 31	0.94 (0.79, 0.99)
CTA+, CDx+	29 / 31	0.94 (0.79, 0.99)
CTA-, CDx+	-	-
CDx-	18 / 101	0.18 (0.11, 0.27)
CTA-, CDx-	17 / 100	0.17 (0.10, 0.26)
CTA+, CDx-	1 / 1	1.00 (0.03, 1.00)

*Clopper-Pearson (Exact) Binomial two-sided 95% Confidence Interval
**No CTA-, CDx+ subjects have been observed.

c. Sensitivity Analysis

To assess the impact of missing data on the estimate of the overall weighted ORR, a multiple imputation analyses was performed using the Primary Efficacy Analysis Population. A logistic regression model was used to impute CDx results based on the CTA results, the ORR was then calculated for those patients with CDx mutation detected results (including imputed results). The ORR as given in Table 25 was estimated to be 0.94, which was in accordance with the results observed in the primary efficacy analysis, therefore, there was no impact of missing data on the efficacy results.

Table 25: Multiple Imputation Analysis to Assess the Impact of Missing Data on Overall Response Rate, CDx Positive Patients (including any Imputed CDx Positive Patients) from the Primary Efficacy Analysis Population

Number of Imputed Datasets	ORR Estimate	Std Error	Lower Confidence Limit	Upper Confidence Limit	Logit Transformed Estimate	Logit Transformed Std Error	Transformed Lower Confidence Limit	Transformed Upper Confidence Limit	Back Transformed Lower Confidence Limit	Back Transformed Upper Confidence Limit
50	0.94	0.043041	0.852778	1.021496	2.70187	0.73061	1.27	4.13	0.78	0.98

d. Clinical Concordance

In the BLU-285-1101 study, the D842V mutation status for screening and enrollment of patients was determined by CTA. The concordance between the *therascreen* PDGFRA RGQ PCR Kit (CDx) and the CTA was assessed. The measures of agreement including the respective Clopper-Pearson exact two-sided 95% confidence intervals are provided in tables 26 and 27.

i. Concordance Analysis Based on CDx Evaluable Patients

All CDx evaluable patients in the Bridging Study testing population were included in a concordance analysis, with CTA as reference. For the 147 subjects that had valid PDGFRA Kit results, the estimated Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and OPA between the PDGFRA Kit and the CTAs, with CTA as the reference method, were 95.24% (95% CI: 83.84, 99.42), 100.00% (95% CI: 96.55, 100.00) and 98.64% (95% CI: 95.17, 99.83) respectively (detailed in Table 26, below).

Table 16: Concordance Between CDx and CTA; CDx Evaluable Patients from the Primary Concordance/Representativeness Analysis Population

Measure Of Agreement	Frequencies	Percent Agreement	Clopper-Pearson (Exact) Binomial Lower Two-sided 95% Confidence Limit	Clopper-Pearson (Exact) Binomial Upper Two-sided 95% Confidence Limit
Overall Percent Agreement	145/147	98.64	95.17	99.83
Positive Percent Agreement	40/42	95.24	83.84	99.42
Negative Percent Agreement	105/105	100.00	96.55	100.00

ii. Concordance Analysis Based on CDx Testable Patients

For the 154 subjects that were testable with the PDGFRA Kit, the estimated PPA, NPA and OPA between the PDGFRA Kit and the CTA (with CTA as

the reference method) were 93.02% (95% CI: 80.94, 98.54), 94.59% (95% CI: 88.61, 97.99) and 94.16% (95% CI: 89.20, 97.29), respectively (refer to Table 27 below). Seven subjects with invalid PDGFRA Kit results were included as discordant results in the analysis.

Table 27: Concordance Between CDx and CTA; CDx Testable Patients from the Primary Concordance/Representativeness Analysis Population

Measure Of Agreement	Frequencies	Percent Agreement	Clopper-Pearson (Exact) Binomial Lower Two-sided 95% Confidence Limit	Clopper-Pearson (Exact) Binomial Upper Two-sided 95% Confidence Limit
Overall Percent Agreement	145/154	94.16	89.20	97.29
Positive Percent Agreement	40/43	93.02	80.94	98.54
Negative Percent Agreement	105/111	94.59	88.61	97.99

3. Subgroup Analyses

The following preoperative characteristics were evaluated for potential association with outcomes: age (<65 years, ≥65 years), gender (male, female), region (US, Europe, Asian) and race (white, non-white) and size of target lesion. There were no meaningful differences in avapritinib efficacy across the patient subgroups based on age, gender, race, region, or largest target lesion.

4. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 1 investigator. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and

recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Effectiveness of treatment with AYVAKIT (avapritinib), when used with the therascreen PDGFRA RGQ PCR Kit, is shown in the bridging study using FFPE specimens from patients screened for enrollment into the Navigator trial and demonstrating that the primary efficacy based on ORR is supported. The ORR for PDGFRA D842V Mutation positive patients when using the CDx was 0.94 (95% CI: (0.79, 0.99)), corresponding to 94%.

B. Safety Conclusions

The *therascreen* PDGFRA RGQ PCR Kit is not expected to directly cause actual or potential adverse effects, but test results directly impact patient treatment. The risks of the *therascreen* PDGFRA RGQ PCR Kit are associated with the potential mismanagement of patient treatment resulting from false results of the test. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect PDGFRA test results, and consequently improper patient management decisions.

A patient with a false positive result may undergo treatment with inappropriate expectation of therapeutic benefit. A patient with a false negative result may be treated without effective drugs, and not experience the potential benefit. The data from the analytical validation studies including the high accuracy when compared to a validated orthogonal sequencing method, support the reasonable assurance of safety of the PDGFRA assay when used in accordance with the indications for use.

C. Benefit-Risk Determination

The probable benefits of the *therascreen* PDGFRA RGQ PCR Kit are based on data collected in the BLU-285-1101 of AYVAKIT (avapritinib) which were then reanalyzed in the bridging study. For the subjects that had valid *therascreen* PDGFRA RGQ PCR Kit results, the estimated Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and Overall Percent Agreement (OPA) between the QIAGEN *therascreen* PDGFRA RGQ PCR Kit and the CTAs, with CTA as the reference method, were 95.24% (95% CI: 83.84, 99.42), 100.00% (95% CI: 96.55, 100.00) and 98.64% (95% CI: 95.17, 99.83) respectively . Treatment with AYVAKIT (avapritinib) provides meaningful clinical benefit to PDGFRA D842V mutant GIST patients, as measured by ORR. The ORR for PDGFRA D842V Mutation positive patients when using the CDx was 0.94 (95% CI: (0.79, 0.99)), corresponding to 94%, which is a clinically meaningful response rate, given the context of this disease; also, the efficacy in the NDA ITT population, which had an ORR of 89% (95% CI: 75%-97%), was maintained This device has probable benefit for the identification of PDGFRA D842V patients, for treatment with AYVAKIT (avapritinib).

The potential risks of the *therascreen* PDGFRA RGQ PCR Kit are associated with the potential mismanagement of patients' treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug combination that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen.

The likelihood of false results was assessed and showed acceptable analytical performance with comparison to NGS in an analytical accuracy study. The *therascreen* PDGFRA RGQ PCR Kit produced a PPA of 98.44% (95% CI: (92.80%, 99.92%)) and an NPA of 100.00% (95% CI: (97.72%, 100.00%), with NGS as the reference method, in the analytical accuracy study.

Given the available information, the data supports the conclusion that the *therascreen* PDGFRA RGQ PCR Kit has probable benefit that outweighs probable risks in selecting patients with PDGFRA D842V mutation positive GIST for treatment with AYVAKIT (avapritinib).

Patient Perspective

This submission did not include specific information on patient perspectives for this device.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from BLU-285-1101 support the utility of *therascreen* PDGFRA RGQ PCR Kit as an aid in selecting patients with GIST with PDGFRA D842V mutation for whom AYVAKIT (avapritinib) is indicated. The BLU-285-1101 Study met its primary objective, demonstrating treatment with AYVAKIT™ (avapritinib) provides meaningful clinical benefit to patients with PDGFRA D842V mutant GIST, as measured by ORR. Overall, data from the analytical and clinical validation studies provided in this application support the use of *therascreen* PDGFRA RGQ PCR Kit to aid clinicians in identification of patients with GIST who may be eligible for treatment with AYVAKIT (avapritinib) based on the detection of D842V somatic mutation in the PDGFRA gene using genomic DNA extracted from GIST patient's FFPE tumor tissue.

XIII. CDRH DECISION

CDRH issued an approval order on June 29, 2023.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.