SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: *therascreen* PIK3CA RGQ PCR Kit

Device Procode: OWD

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

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P190004

Date of FDA Notice of Approval: May 24, 2019

The current PMA was submitted for an indication for the *therascreen* PIK3CA RGQ PCR Kit to identify 11 mutations in phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) gene (Exon 7: C420R; Exon 9: E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and Exon 20: H1047L, H1047R, H1047Y) from circulating tumor DNA (ctDNA) isolated from K_2EDTA plasma from breast cancer patients who may be eligible for treatment with PIQRAY® (alpelisib).

Another PMA (P190001) for the same device using tissue specimens was also submitted for the qualitative detection of the same 11 mutations in the PIK3CA gene (Exon 7: C420R; Exon 9: E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and Exon 20: H1047L, H1047R, H1047Y) in DNA derived from formalin-fixed, paraffin-embedded (FFPE) breast tumor tissue for the treatment with PIQRAY (alpelisib). P190001 was also approved on May 24, 2019 in conjunction with P190004 approval.

The summary of safety and effectiveness data (SSED) to support the indication is available on the Center for Devices and Radiological Health (CDRH) website.

II. INDICATIONS FOR USE

The *therascreen* PIK3CA RGQ PCR Kit is a real-time qualitative PCR test for the detection of 11 mutations in the phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) gene (Exon 7: C420R; Exon 9: E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and Exon 20: H1047L, H1047R, H1047Y) using genomic DNA (gDNA) extracted from formalin-fixed, paraffin-embedded (FFPE)

breast tumor tissue or circulating tumor DNA (ctDNA) from plasma derived from K_2EDTA anticoagulated peripheral whole blood taken from patients with breast cancer.

The test is intended to aid clinicians in identifying breast cancer patients who may be eligible for treatment with PIQRAY® (alpelisib) based on a PIK3CA Mutation Detected result. Patients whose FFPE tissue or plasma specimen produce a positive *therascreen* PIK3CA RGQ PCR Kit test result for the presence of one or more PIK3CA mutations are eligible for treatment with PIQRAY (alpelisib). Patients whose plasma specimens produces a negative result using this test should be reflexed to testing with FFPE tumor tissue for the presence of PIK3CA mutations.

FFPE tumor specimens are processed using the QIAamp DSP DNA FFPE Tissue Kit for manual sample preparation. K_2EDTA anticoagulated whole peripheral venous blood plasma specimens are processed using the QIAamp DSP Circulating Nucleic Acid Kit for manual sample preparation. For both specimen types, the Rotor-Gene Q (RGQ) MDx (US) instrument is used for automated amplification and detection. The Kit is to be used by trained personnel in a professional laboratory environment.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the *therascreen* PIK3CA RGQ PCR Kit labeling.

V. DEVICE DESCRIPTION

The following components comprise the overall device for testing plasma specimens:

- QIAGEN QIAamp® DSP Circulating Nucleic Acid Kit
- QIAGEN *therascreen* PIK3CA RGQ PCR Kit
- QIAGEN Rotor-Gene Q MDx Instrument (RGQ)
- Automated data analysis and results interpretation using:
    - Rotor-Gene AssayManager® (RGAM) software version 2.1
    - Rotor-Gene AssayManager Gamma MDx plug-in version 1.0.0
    - *therascreen*_PIK3CA_Plasma Assay Profile v1.0.1

Specimen Preparation – ctDNA

Peripheral whole blood is collected from breast cancer patients using blood collection tubes containing di-potassium ethylenediamine tetraacetic acid (K_2EDTA) as an anticoagulant. The tubes are then centrifuged to pellet the cellular components of the blood before the plasma fraction is transferred to a fresh tube. ctDNA is manually extracted and purified

from the blood plasma using the QIAamp DSP Circulating Nucleic Acid Kit. QIAamp Mini columns can bind fragmented nucleic acids that are as short as 20 base pairs (bp), but the yield depends on the sample volume and the concentration of circulating nucleic acids in the sample (typically 1–100 ng/mL circulating nucleic acids in plasma). All assays in the *therascreen* PIK3CA RGQ PCR Kit are designed to amplify short DNA fragments with amplicons ranging from 75 bp to126 bp, however, the *therascreen* PIK3CA RGQ PCR Kit will not work with heavily fragmented DNA. If a sample fails the DNA sample assessment (Control Ct value is > 31.68), the purification process should be repeated with new samples.

The ctDNA purification process involves the following steps:

- Samples are lysed under denaturing conditions with proteinase K for 30 minutes at 60°C.
- The sample is passed through a silica-based membrane so that ctDNA binds to the membrane and contaminants are removed.
- The membrane is washed with buffers (using the QIAvac 24 Plus vacuum manifold to increase flow rates through the membrane).
- Purified ctDNA is eluted from the membrane using buffer.

Extracted ctDNA is ready for testing with the *therascreen* PIK3CA RGQ PCR Kit or can be stored at –30 to –15°C for up to 4 week before use.

The QIAamp DSP Circulating Nucleic Acid Kit provides reagents that enable standardized manual purification of cfDNA from blood plasma using a pre-defined protocol. The DNA extraction process should be followed as per kit instruction with the following *therascreen* PIK3CA RGQ PCR Kit custom steps:

- Instructions for sample volumes of 2 mL of plasma must be followed. Where 2 mL is not available, the volume must be adjusted to 2 mL using Phosphate-buffered Saline (PBS).
  Note: The extracted DNA should be within working control Ct range (24.69 ≥ and ≤ 31.68) for the sample to be valid.
- Proteinase K volume must be 250 µL.
- Purified ctDNA must be eluted in 70 µL of elution buffer (Buffer AVE) from the QIAamp DSP Circulating Nucleic Acid Kit.

PCR Amplification and Detection

The QIAGEN *therascreen* PIK3CA RGQ PCR Kit contains reagents for the detection of 11 individual mutations in exons 7, 9, and 20 of the phosphoinositide-3-kinase, catalytic, alpha (PIK3CA) gene, and a region in exon 15 of the PIK3CA gene used as a Control Reaction. The detection of the 11 individual mutations and region in exon 15 of the PIK3CA gene is achieved using six reaction mixes listed below:

Table 1: PIK3CA Mutations Detected by the *therascreen* PIK3CA RGQ PCR Kit

Reaction Mix	PIK3CA Mutation	Base change	Exon	COSMIC ID[1]
1	Control Reaction	N/A	15	N/A
2	E542K	1624 G>A	9	760
3	E545K E545D E545G	1633 G>A 1635 G>T 1634 A>G	9	763 765 764
4	E545A H1047Y Q546R	1634 A>C 3139 C>T 1637 A>G	9 20 9	12458 774 12459
5	C420R Q546E	1258 T>C 1636 C>G	9 7	757 6147
6	H1047L H1047R	3140 A>T 3140 A>G	20	776 775

[1]COSMIC IDs taken from the Catalogue of Somatic Mutations in Cancer: https://cancer.sanger.ac.uk/cosmic

The *therascreen* PIK3CA RGQ PCR Kit uses real-time PCR with the following technologies for PIK3CA sequence related amplification and detection: ARMS® Primers, LNA® Probes, TaqMan® Probes, Minor Groove Binding (MGB™) Probes, and PCR Clamps for Wild Type (WT) and pseudogene sequences.

PCR uses forward and reverse primers to hybridize to a specific DNA sequence to amplify it. The ARMS technique is based on the use of mutation sequence-specific PCR primers that allow amplification of test DNA only when the target allele is contained within the sample.

In addition to the primers, dye-linked oligonucleotides (i.e., probes; e.g., LNA, TaqMan and MGB) are contained in the reaction mixes. The probes, which are labeled with a 5' reporter dye (carboxyfluorescein [FAM™]) and a downstream, 3' dye-free quencher (i.e., BHQ1) which quenches the fluorescence of the reporter dye, also hybridize to the target sequence between the primers. When a probe is intact, the proximity of the reporter dye to the quencher results in suppression of the reporter fluorescence primarily by Förster-type energy transfer.

PCR clamp technology allows selective amplification of the mutant sequence. PCR clamps matched to WT or pseudogene sequence bind to the template and prevent amplification by interference with primer elongation. There are two types of PCR clamps used within the *therascreen* PIK3CA RGQ PCR Kit; peptide nucleic acids (PNAs) and 3' phosphate groups.

During PCR, forward and reverse primers and a probe bind to the target sequence. DNA polymerase extends the primers and the 5' to 3' exonuclease activity of the enzyme cleaves the probe between the reporter and the quencher leading to an increase in detectable reporter fluorescence. This process occurs in every PCR cycle. The increase in fluorescence is directly proportional to the target amplification during PCR.

The probes used in the mutation specific reaction mixes are labeled with carboxyfluorescein (FAM), hexachloro-fluorescein (HEX) and Cyanine (CY5.5) fluorescent reporter dyes, each with a distinct absorption and emission profile. The probe used in the Internal Control Reaction is labeled with Rhodamine (ROX).
FAM, HEX, ROX and CY5.5 absorb and fluoresce at different wavelengths:

- FAM: 6-carboxyfluorescein: A fluorophore that excites at a wavelength of 495nm and emits at a wavelength of 520nm. This fluoresces in the green RGQ channel.
- HEX: Hexachloro-fluorescein: A fluorophore that excites at a wavelength of 535nm and emits at a wavelength of 556nm. This fluoresces in the yellow RGQ channel.
- ROX: A fluorophore of the Rhodamine family that excites at 578nm and emits at 604nm. This fluoresces in the orange RGQ channel.
- CY5.5: A fluorophore of the Cyanine family that excites at 675nm and emits at 694nm. This fluoresces in the crimson RGQ channel.

The Control Reaction Mix contains a forward and reverse primer and labeled probe (detected in the Green Channel) to amplify a short sequence of exon 15 of the PIK3CA gene. The Control Reaction 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 Mix (Tube 1) 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 2. Any samples that do not give Ct values within this range are invalidated by the RGAM software.

Table 2: Control Reaction Working Range

Control Ct value	Interpretation	Action
> 31.68	Quantity of amplifiable ctDNA is not sufficient for mutation analysis	Additional samples should be extracted and tested
< 24.69	Quantity of amplifiable ctDNA is too high for mutation analysis	Dilute with the sample diluent water supplied in the kit
≥24.69 and ≤ 31.68	Quantity of amplifiable ctDNA is suitable for mutation analysis	No action required, sample is suitable

The PCR cycling parameters used for assessing the DNA sample with the control reaction mix are the same run parameters for mutation analysis using the mutation assays. 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 45 cycles of 95°C for 30 seconds, to denature, and 60°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 channels 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.

Test Controls

The *therascreen* PIK3CA RGQ PCR Kit contains three controls: An Internal Control (IC), a Positive Control (PC) and a No Template Control (NTC), which have been designed to detect fault conditions.

***Internal Control (IC)*:** Each PIK3CA reaction mix contains reagents (unlabeled primers, probe and oligonucleotide template) for an IC reaction designed to detect failure of the reaction, e.g. due to set up error, and confirms successful PCR reactions in every tube.

***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 mixture of plasmids representing one mutation for each of the mutation assays and the Control Assay. Detection of the targets within their acceptable ranges confirms the proper functioning of each of the reaction mixes in the kit.

Instrument(s) and Software

The *therascreen* PIK3CA RGQ PCR Kit is designed to be used with the 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 pass 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). Four of these channels; green, yellow, crimson and orange, are used with the *therascreen* PIK3CA RGQ PCR Kit.

Cycling parameters, data analysis and results interpretation for the *therascreen* PIK3CA RGQ PCR Kit are performed by the RGAM version 2.1 Software, Gamma MDx plug-in version 1.0.0 and *therascreen*_PIK3CA_Plasma Assay Profile v1.0.1. 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*_PIK3CA_Plasma 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 mainly intended to identify problems that occur during the real-time PCR amplification that potentially generate nontypical curve shapes due to saturation, noise, spikes, baseline dips, sloping curves related to the real-time PCR instrument parameters or due to a problem linked to the assay itself. The samples with 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 45.

Ct values generated by the Control and Mutation Reactions indicate the quantity of assay specific input DNA and are indirectly proportional to the input DNA levels. Low Ct values indicate higher input DNA levels and high Ct values indicate lower input DNA levels. The interpretation of the results obtained from the control reaction Ct is presented in Table 2.

Validity of controls and samples are determined based on the Ct values generated during a run.

Run Validity Criteria

For a *therascreen* PIK3CA 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. Each test run performed with the *therascreen* PIK3CA RGQ PCR Kit must meet all the validity criteria listed below (Table 3).

Table 3:Run, Sample Validity and Call Criteria

Sample	Reaction Mix	Target	RGQ Channel	Ct Range
Positive Control	1 and 5	Control	FAM	23.39 – 32.39
	2	E542K	FAM	22.42 – 31.42
	3	E545D	HEX	23.78 – 32.78
	3	E545G	CY5.5	22.61 – 31.61
	3	E545K	FAM	24.41 – 33.41
	4	E545A	FAM	22.5 – 31.5
	4	H1047Y	HEX	26.57 – 35.57
	4 and 5	Q546R	CY5.5	24.04 – 33.04
	5	C420R	HEX	23.31 – 34.31
	1 and 5	Q546E	FAM	24.72 – 35.72
	6	H1047L	CY5.5	24.02 – 33.02
	6	H1047R	HEX	23.33 – 32.33
NTC	All	All 6 reaction mixes	FAM	Has no value
IC	NTC	IC	ROX	25.52 – 36.51
	Test Sample	IC	ROX	25.51 – 36.51
	Positive Control	IC	ROX	25.51 – 36.51
Test Sample	1	Control	FAM	23.39 – 32.39

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

The individual sample results in each test run are accepted as valid, if the RGAM software obtains Ct values for the PIK3CA assay. If a sample fails to generate a Ct value for any PIK3CA mutant channel, then the RGAM software checks the Ct value obtained in the IC channel to ensure the qPCR reaction validity. If the RGAM fails to detect a signal within the validity criteria range in the IC, the sample is reported as invalid and no PIK3CA mutation status results for that sample are reported. For a *therascreen* PIK3CA 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.

Sample Validity Criteria and Control Assay Working Range

All samples must be tested with the Control Reaction mix to ensure that they give a Ct value within a specified range. This range is set to ensure that there is sufficient amplifiable DNA to proceed with analysis, but not so much as to overload the assay. The *therascreen* PIK3CA RGQ PCR Kit has been verified to work within a specific range (i.e. upper and lower Control Reaction Ct values) and any samples that do not give Ct values within this range are invalidated by the RGAM software, plug-in and associated assay profile.

Determination of Sample Status

If the Control Assay Ct falls within range, then the sample is analyzed for the presence of the mutation. The difference in Ct values (ΔCt) between the Control Reaction and the mutation-specific reaction (mutation assay) is a qualitative measure of PIK3CA mutation status and is calculated as:

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

Samples are classified as mutation positive if they give a ΔCt less than or equal to the cut-off ΔCt value for that assay. Above this value, the sample may either contain less than the percentage of mutation that can be detected by the *therascreen* PIK3CA RGQ PCR Kit (beyond the limit of detection of the assays), or the sample is mutation negative, both of which would be reported as "No Mutation Detected".

The Mutation Assay ΔCt Cut-offs are shown below in Table 4.

Table 4: Cut-off Values for Each Mutation in Each Assay

Reaction Mix	Mutation	Ct cut-off values
Tube 2	**E542K**	≤ 4.80
Tube 3	**E545K**	≤ 10.00
	E545D	≤ 7.00
	E545G	≤ 9.50
Tube 4	**E545A**	≤ 10.00
	H1047Y	≤ 6.20
	Q546R	≤7.00
Tube 5	**Q546E**	≤10.00

Reaction Mix	Mutation	Ct cut-off values
Tube 6	C420R	≤6.00
	H1047R	≤9.00
	H1047L	≤10.00

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 sample IC failed
- or if the Control Assay Ct was outside of the acceptance range

PIK3CA Mutation Detected:

- If all AUDAS checks passed
- and if all run control criteria were met
- and if the sample IC was within the defined acceptance range
- and if the Control Assay Ct was within the acceptable range
- and if any PIK3CA mutant signals were 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 sample IC was within the defined acceptance range
- and if the Control Assay Ct was within the acceptable range
- and if all mutant signals were above the predefined ΔCt cut-off

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA cleared or approved alternatives for the testing of plasma (or formalin-fixed, paraffin-embedded [FFPE] breast tumor tissue) for PIK3CA mutation status in the selection of patients who may be eligible for treatment with PIQRAY® (alpelisib).

VII. MARKETING HISTORY

The *therascreen* PIK3CA 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 PIK3CA mutation results, which could impact patient treatment decisions. A false positive test result may lead to inappropriate treatment and adverse effects associated with treatment with a targeted PIK3CA inhibitor rather than standard of care treatments. A false negative test result may prevent a patient from receiving alpelisib with potential to benefit from a targeted therapy.

For the specific adverse events that occurred in the clinical study, please see Section X below.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The specific performance characteristics of the *therascreen* PIK3CA RGQ PCR Kit were determined by studies using three types of test samples:

- Procured clinical plasma samples obtained from patients with advanced breast cancer.
- Contrived plasma samples comprising Healthy Donor (HD) EDTA plasma spiked with fragmented cell line DNA to mimic clinical plasma samples.
- Fragmented genomic DNA (gDNA) from cell line samples

Due to volume constraints of clinical plasma samples and due to the rarity of most of the mutations, contrived samples were utilized for some non-clinical studies. A contrived sample characterization study was conducted to demonstrate comparable performance of sheared cell line DNA diluted in HD plasma as compared to K_2EDTA plasma from clinical samples. Clinical specimens were used to confirm the estimated limit of detection (LoD), analytical accuracy and stability studies. Testing was performed using the final version of the *therascreen* PIK3CA RGQ PCR Kit, following established protocols. DNA was extracted and tested in accordance with the instructions for use.

1. Correlation with Reference Method

To demonstrate the analytical accuracy of the *therascreen* PIK3CA RGQ PCR Kit, results were compared with a validated Next Generation Sequencing (NGS)-based method using banked DNA from clinical plasma samples.

Plasma specimens were collected at baseline, prior to initiation of study treatment, from patients with breast cancer in the Novartis clinical study CBYL719C2301 (SOLAR-1, see section X). Samples were obtained from 554 of 572 total randomized patients enrolled into the SOLAR-1 clinical study. Eighteen (18) patients had no plasma samples available for testing. Of the 554, there were 552 samples with residual DNA available for use in this accuracy study. Of the 552 samples available, 5 samples were excluded from this analysis as the samples were collected after initiation of therapy, and an additional 5 did not have sufficient DNA for NGS analysis. The level of concordance

between the *therascreen* PIK3CA RGQ PCR Kit and the validated NGS method was demonstrated by comparing the results obtained from both methods.

Specimens were processed and tested by the *therascreen* PIK3CA RGQ PCR Kit according to the final product labeling. Invalid and indeterminate samples were retested according to the clinical test site protocol.

The data from these samples were used to determine the level of concordance between the two methods and the overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA) was reported with the corresponding two-sided Clopper-Pearson exact 95% confidence limits (Table 5).

Table 5: Agreement in Overall Mutation Status between *therascreen* PIK3CA RGQ PCR Kit and NGS

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	504/542	92.99	90.50	94.99
Positive Percent	149/153	97.39	93.44	99.28
Negative Percent	355/389	91.26	88.00	93.87

The data generated in this accuracy study has demonstrated that the observed PPA was 97.39%. The observed NPA was 91.26%.

In addition, the agreement was evaluated at the mutation level rather than at sample level. The results are shown in the table below (Table 6).

Table 6: PIK3CA Specific mutation status against NGS specific mutation status

Frequency		Next Generation Sequencing Mutation Status							
PIK3CA Plasma KitMutation Status	C420R	E542K	E542K, H1047R	E545G	E545K	H1047L	H1047R	Negative	Total
C420R	2	0	0	0	0	0	0	0	2
E542K	0	19	0	0	0	0	0	2	21
E542K, H1047R	0	0	1	0	0	0	0	0	1
E545G	0	0	0	2	0	0	0	1	3

E545K	0	0	0	0	38	0	0	8	46
H1047L	0	0	0	0	0	5	0	5	10
H1047R	0	0	0	0	0	0	82	18	100
Negative	0	2	0	0	0	0	2	355	359
Total	2	21	1	2	38	5	84	389	542

In this study 38 discordant results were observed out of 542 samples. Of these 38 samples, 4 were classified as mutation detected by the NGS method but mutation not detected by the *therascreen* PIK3CA RGQ PCR Kit using plasma (Plasma negative/NGS positive), and 34 were *therascreen* PIK3CA plasma positive but NGS plasma negative, including 29 *therascreen* PIK3CA tissue positive and 5 *therascreen* PIK3CA tissue negative [(i.e., out of 34, 29 were (PIK3CA Tissue+, Plasma+); and 5 were (PIK3CA Tissue-, Plasma +)].

There were five (5) mutations (E545A, E545D , Q546E, Q546R and H1047Y) out of 11 PIK3CA mutations that were not detected when plasma samples were tested by the *therascreen* PIK3CA RGQ PCR Kit and by the NGS method. These samples tested positive when the corresponding tissue samples were tested using the *therascreen* PIK3CA RGQ PCR Kit.

2. Contrived Sample Functional Characterization

The objective of this study was to determine whether the contrived samples consisiting of fragmented PIK3CA mutation cell-line DNA spiked into Healthy Donor (HD) plasma (contrived plasma samples) are equivalent to clinical PIK3CA mutation positive plasma samples derived from breast cancer patients.

The three most prevalent PIK3CA mutations, E545K, E542K and H1047R, were used to assess the performance of the kit using plasma specimens. Equivalency was assessed by comparison of "positivity rate" (mutation detected vs. mutation not detected) for the samples at the limit of detection (LoD). Fragmented DNA was spiked in from stocks at 50% mutation allele frequency (MAF). The DNA eluates were normalized to a control assay Ct of 30 in order to provide a challenging sample near the upper limit of the control working range. Four different mutant allele frequency (MAF%) levels (LoD to 0.35x LoD) were prepared for each mutation.

The prepared samples at different MAF levels were then tested with the control and corresponding mutation assays of the *therascreen* PIK3CA RGQ PCR Kit and subjected to statistical analysis of hit rate at the LoD MAF level. Based on these analyses, the results (Table 7) demonstrate that the performance of the *therascreen* PIK3CA RGQ PCR Kit is equivalent for both contrived plasma samples and clinical plasma samples. The 1X LoD values for the corresponding mutants are in bold in Table 7.

Table 7: Results of Contrived Sample Characterization Study

Grouping Variable(s)			Proportion		Two-Sided 95%	
Mutation	Sample Type	MAF[	Fraction	Percentage	Lower	Upper
E545K	Clinical	0.86	17 / 24	70.83%	48.91%	87.38%
		1.22	23 / 24	95.83%	78.88%	99.89%
		1.72	24 / 24	100.00%	85.75%	100.00%
		2.42	**24 / 24**	**100.00%**	**85.75%**	**100.00%**
	Contrived	0.86	22 / 24	91.67%	73.00%	98.97%
		1.22	23 / 24	95.83%	78.88%	99.89%
		1.72	24 / 24	100.00%	85.75%	100.00%
		2.42	**24 / 24**	**100.00**	**85.75**	**100.00%**
H1047R	Clinical	0.71	17 / 24	70.83%	48.91	87.38%
		1	22 / 24	91.67%	73.00	98.97%
		1.4	23 / 24	95.83%	78.88	99.89%
		1.98	**24 / 24**	**100.00**	**85.75**	**100.00%**
	Contrived	0.71	19 / 24	79.17%	57.85	92.87%
		1	24 / 24	100.00	85.75	100.00%
		1.4	24 / 24	100.00	85.75	100.00%
		1.98	**24 / 24**	**100.00**	**85.75**	**100.00%**
E542K	Clinical	1.804	11 / 24	45.83%	25.55	67.18%
		2.544	15 / 24	62.50%	40.59	81.20%
		3.587	23 / 24	95.83%	78.88	99.89%
		5.058	**24 / 24**	**100.00**	**85.75**	**100.00%**
	Contrived	1.804	11 / 24	45.83%	25.55	67.18%
		2.544	19 / 24	79.17%	57.85	92.87%
		3.587	22 / 24	91.67%	73.00	98.97%
		5.058	**24 / 24**	**100.00**	**85.75**	**100.00%**

3. Analytical Sensitivity

(i) Limit of Blank (LoB)

The LoB is defined in CLSI guideline EP17-A2 as "the highest measurement result that is likely to be observed (with a stated probability) for a blank sample". For the *therascreen* PIK3CA RGQ PCR Kit this is the data point that corresponds to the upper 95% percentile in the blank samples.

The LoB was confirmed by testing 60 unique HD plasma samples. The 60 samples were spiked with serially diluted fragmented Wild Type PIK3CA DNA at six DNA input levels to produce control assay Ct values spanning the *therascreen* PIK3CA RGQ PCR Kit (for plasma specimens) control assay working range (24.69 – 31.68). For each sample one replicate was tested with each of the three kit lots to give a total

of 180 data points per assay. The lowest value of the three LoB estimates (one from each *therascreen* PIK3CA RGQ PCR Kit lot) was determined to be the LoB value.

The LoB values for each of the mutation assays (in terms of ΔCt) detected by the *therascreen* PIK3CA RGQ PCR Kit were verified to be above the ΔCt cut-off values determined for each of the assays as summarized in Table 8. The false positive call rates were also calculated as summarized in Table 8.

(ii) ΔCt Analytical Cut-off Validation

For the ΔCt cut-off verification the following acceptance criteria were set: For each mutation (i.e. each tube and each channel), using only the WT samples and using the final assay cut-off value in terms of ΔCt, the estimated false positive rate must be less than 3%. Using the WT sample data, the false positive fraction was calculated using the cutoffs set in development. As the proportion of false positive calls in this study was found to be 2/180 (1.11%) across all 11 mutation assays, meeting the acceptance criteria, these cut-offs were used as the final assay cutoffs.

Table 8: LoB and false positive rate for each Mutation Assay

Reaction Mix	Assay	LoB (ΔCt)	False positive rate (%)
Tube 2	**E542K**	8.32	0.00%
Tube 3	**E545K**	15.74	0.00%
	E545D	9.13	0.00%
	E545G	13.39	0.00%
Tube 4	**E545A**	15.82	0.00%
	H1047Y	9.89	0.00%
	Q546R	10.19	0.56%
Tube 5	**Q546E**	15.82	0.00%
	C420R	11.15	0.00%
Tube 6	**H1047R**	11.93	0.00%
	H1047L	15.55	0.56%

(iii) Limit of Detection (LoD)

The design of the study was guided by the CLSI guideline EP17-A2. The LoD for each mutation assay in the *therascreen* PIK3CA RGQ PCR Kit was estimated using contrived plasma samples. For the *therascreen* PIK3CA RGQ PCR Kot LoD is 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. A 5-level MAF% dilution series was made by serially diluting the original sample in a WT background. These dilution series were then tested using the *therascreen* PIK3CA RGQ PCR Kit for each of the mutations. The evaluation was performed using 3 different *therascreen* PIK3CA RGQ PCR Kit lots with 24 replicates tested per kit lot per level. The LoD value was determined as the highest value (in terms of MAF%) across all kit lots (Table 9).

Table 9: LoD for the 11 PIK3CA Mutations

Mutation Name	LoD (MAF%)
C420R	4.46*
E542K	5.06†
E545A	1.82*
E545D	3.21*
E545G	1.94†
E545K	2.42†
H1047L	2.37†
H1047R	1.98†
H1047Y	7.07*
Q546E	5.31*
Q546R	4.22*

* LoD values were established using cell line samples
† LoD values were verified using intended use clinical plasma samples

LoD confirmation with Clinical Samples

A combined precision and LoD study was performed such that the LoD for the *therascreen* PIK3CA RGQ PCR Kit was confirmed using clinical PIK3CA mutation positive and WT clinical plasma samples derived from the intended use population. Clinical samples, positive for the most prevalent mutations, H1047R, E545K, E542K and H1047L, were used in this study A sample with a rare mutation (E545G) was also included in this study.

Extracted mutation positive DNA was normalized to a control assay Ct of 30.00 and diluted in clinical WT DNA to the MAF% equivalent to the estimated LoD. Prepared samples at LoD were tested using 2 lots of the *therascreen* PIK3CA PCR RGQ Kit wth 2 operators, 1 run per operator per day for 2 non-consecutive days at 3 sites, with a total of 24 replicates (8 replicates per site).

The positive call rates for all variants were above 95%, as shown in Table 10. Thus, the previously determined LoDs were confirmed for the tested mutations using clinical plasma samples.

Table 10: LoD confirmation with clinical samples

Mutation	Frequency of Positive Mutation Calls	Proportion of Positive Mutation Calls	Two-sided lower 95% CI	Two-sided lower 95% CI
E542K	24/24	100%	85.75%	100%
E545G	23/24	95.83%	78.88%	99.89%
E545K	24/24	100%	85.75%	100%
H1047L	23/23	100%	85.15%	100%
H1047R	21/22	95.45%	77.16%	99.88%

(iv) Effect of DNA Input on ΔCt (Linearity Studies)

The purpose of this study was to demonstrate that the performance of the *therascreen* PIK3CA RGQ PCR Kit is consistent across the DNA input range (control assay working range, 24.69 – 31.68 Ct); showing a consistent ΔCt for a given MAF irrespective of the DNA input level. Genomic DNA (gDNA) was used for all 11 PIK3CA mutations in this study. The linear range of the *therascreen* PIK3CA RGQ PCR Kit was determined for each of the11 mutation assays by testing across 8 DNA input levels with the upper level being outside of the control assay Ct working range (24.69 – 31.68 Ct). Each mutation sample was diluted to a constant MAF level representing 3X LoD and normalized to different target dilution control assay Ct values. The evaluation was performed using one *therascreen* PIK3CA RGQ PCR Kit lot with 2 replicates tested per DNA level.

The data was analyzed using regression analysis to determine the linear range. For the assay to be determined as linear across a DNA input, there should be no change across the range in ΔCt, i.e., there is no statistically significant linear, quadratic or cubic effect (i.e. $p > 0.05$). A consistent linear ΔCt range was observed across the full tested DNA input range for E542K, E545K, E545D, E545A, H1047L, H1047Y, Q546R, C420R and H1047R assays. The E545G and Q546E assays did not show a linear ΔCt range across the full tested DNA input range. Removal of dilution 8 from the analysis of E545G and Q546E assays removed the non-linear effects such that the assays were linear across dilutions 1 to 7 which span the control assay working range. The linear range of the assays is shown below (Table 11).

Table 11: Linear Range of the Assay

Assay	Linear Range based on observed Mean Control assay Ct
E542K	25.21 to 33.35
E545K	24.98 to 33.45

Assay	Linear Range based on observed Mean Control assay Ct
E545D	24.92 to 33.35
E545G	24.76 to 31.43
E545A	24.74 to 33.89
H1047Y	24.90 to 34.03
Q546R	24.76 to 32.12
Q546E	24.90 to 32.68
C420R	24.79 to 34.11
H1047R	25.00 to 33.41
H1047L	25.11 to 34.72

4. Analytical Specificity

(i) Cross Reactivity and Primer and Probe Specificity

This study aimed to assess whether cross reactivity between mutations detected by the assay had been correctly accounted for in the setting of the analytical ΔCt cut-off values of the *therascreen* PIK3CA RGQ PCR Kit. The cross reactivity of the six optimized reaction mixes was assessed by testing each tube against the 11 mutations detected by the *therascreen* PIK3CA RGQ PCR Kit. Samples were tested at low DNA input and low MAF% as well as high DNA input and high MAF%. Contrived plasma samples were used for all mutations. For each sample, two replicates were tested with 3 *therascreen* PIK3CA RGQ PCR Kit lots (generating 239 data points total). Overall, 1/239 (0.42%) data points showed mutant non-specific amplification. The single data point showing mutant non-specific (where a E542K low MAF sample was classified as Q546R) amplification was inconsistent with other replicates from the same sample, including the high DNA input, high MAF sample, which did not show the same mutant non-specific amplification. This result was therefore not considered to be a result of cross reactivity and was attributed to non-specific amplification.

Known PCR cross reactivity was observed during feasibility studies between H1047L and H1047R . This cross reactivity is uni-directional, i.e., if a double H1047L and H1047R sample is seen this will be reported as H1047R Mutation Detected. This rule is incorporated into the automated *therascreen*_PIK3CA_Plasma MDx Assay Profile algorithm.

(ii) Cross Contamination

To evaluate the potential occurrence of cross contamination during the DNA extraction and subsequent *therascreen* PIK3CA RGQ PCR Kit testing procedures, this study was performed with H1047R (the highest prevalent mutation) and Wild Type contrived plasma samples. Samples were extracted following a pre-defined extraction matrix and mutation status was assessed across ten PCR runs. Eighteen extractions were carried out for the mutation positive (H1047R) samples and 42 extractions were carried out for the WT samples.

The observed percentage of correct mutation calls for Wild Type samples was 99.49%, (194 out of 195 replicates), demonstrating no significant cross contamination of the Wild Type samples by mutant samples sharing the same DNA extraction and run setup procedure.

(iii) Interfering Substances

The effect of potential interfering substances from both endogenous and exogenous substances was measured by comparison of ΔCt between interferent spiked and control spiked extracts of each mutant (3X LoD) used within the study. The assessment of interfering substances for WT samples was made by evaluating all reaction mixes to demonstrate correct WT calls. Thirteen potential interfering substances were tested:

- Endogenous interfering substances that could be present in a clinical plasma sample: hemoglobin (2 g/L), triglycerides (37 mmol/L), EDTA (3.4 μmol/L), Caffeine (308 μmol/L), Albumin (30 mg/mL), Conjugated Bilirubin (342 μmol/L) and Unconjugated Bilirubin (342 μmol/L).

- Exogenous substances that could be introduced into a sample during the DNA extraction process: ethanol, proteinase K, buffer ACL, buffer ACB, buffer ACW1 and buffer ACW2, tested based on the highest carryover volume during extraction.

Contrived plasma samples were used for representative mutations for each reaction mix in the *therascreen* PIK3CA RGQ PCR Kit (E542K, E545K, H1047R, Q546R and C420R) and for WT sample. The evaluation was performed using one *therascreen* PIK3CA RGQ PCR Kit lot with 6 replicates tested per interferent. All results in both mutant and WT samples were as expected. Where a statistically significant difference was observed between spiked and control samples, this was within acceptable intermediate precision of the assay and was therefore within the inherent variability of the assay.

In a further analysis of the interfering substances data, the difference in absolute Ct for the mutant assays rather than ΔCt was examined. Should a potential interferent have a proportionate effect on both the control and mutant Ct values (i.e., Ct values

are shifted by a similar value due to the interferent), the resulting ΔCt could remain comparable to the interferent free control despite a Ct shift. Therefore, this could mask the effect of the interferent which may result in a false negative call in samples close to the assay's LoD.

The acceptance criteria were kept consistent for the analysis of the absolute Ct values, that is for an interferent to be classed as having no impact, the p-value for the estimate should be >0.05 or if the p-value <0.05 then the difference in absolute Ct must be within acceptable intermediate precision of the corresponding assay. The results showed that interferents tested had no impact on the absolute mutant Ct value. Based on the results, none of the interferents tested had any impact on kit performance.

(iv) <u>Repeatability and Reproducibility</u>

The objective of this study was to demonstrate the repeatability and reproducibility of the *therascreen* PIK3CA RGQ PCR Kit (including intermediate precision).

The reproducibility of the *therascreen* PIK3CA RGQ PCR Kit for the detection of mutations in the PIK3CA gene was evaluated in two studies. The first study used WT and 11 contrived cell line DNA samples carrying the PIK3CA mutations spiked into HD plasma. All extractions were carried out using two lots of QIAamp DSP Circulating Nucleic Acid Kit. Extracted DNA was normalized to a control assay Ct value of 30 and diluted to the MAF levels of 1X LoD and 3X LoD.

To demonstrate repeatability, two runs per day, by three operators and using three instruments, were carried out across 20 non-consecutive days to give a total of 120 runs at Site 1. The proportion of correct mutation calls and the lower one-sided exact 95% confidence intervals (95% CI) were reported in Table 12 below.

Table 12: Repeatability – Proportion of Correct Calls for PIK3CA Mutation Tested

Mutation	Template	Fractional Proportion	Percentage	Two-Sided Lower 95% Confidence Limit
C402R	LOD	120/120	100.00%	96.97%
	3xLOD	120/120	100.00%	96.97%
E542K	LOD	120/120	100.00%	96.97%
	3xLOD	120/120	100.00%	96.97%
E542A	LOD	119/120	99.17%	95.44%
	3xLOD	120/120	100.00%	96.97%
E545D	LOD	120/120	100.00%	96.97%
	3xLOD	120/120	100.00%	96.97%
E545G	LOD	119/120	99.17%	95.44%
	3xLOD	120/120	100.00%	96.97%

Mutation	Template	Fractional Proportion	Percentage	Two-Sided Lower 95% Confidence Limit
E545K	LOD	111/120	92.50%	86.24%
	LOD*	120/120	100.00%	96.97%
H1047L	LOD	120/120	100.00%	96.97%
	3xLOD	120/120	100.00%	96.97%
H1047R	LOD*	110/120	91.67%	85.21%
	3xLOD*	120/120	100.00%	96.97%
H1047Y	LOD	120/120	100.00%	96.97%
	3xLOD	120/120	100.00%	96.97%
Q546E	LOD	120/120	100.00%	96.97%
	3xLOD	120/120	100.00%	96.97%
Q546R	LOD	115/120	95.83%	90.54%
	3xLOD	120/120	100.00%	96.97%
WT	Ct30	114/120	95.00%	89.43%

*For E545K and H1047R the LoD used were 1.99 and 1.44, respectively. The LoD was readjusted and confirmed in a subsequent study. The readjusted LoD was used in the subsequent study (see Table 13 below).

Reproducibility was assessed over three sites Site 1, Site 2 and Site 3 (Table 13). Two runs a day were performed per three operators and three instruments per site by the two external sites (Sites 2 and 3) over 10 non-consecutive days to give an additional 60 runs per external site. Three unique lots of reagents were included in the study where each lot was tested at two sites. Three sets of single use aliquots were prepared for each of the samples; one set was kept at Site 1 for testing while the remaining two sets and were shipped, on dry ice, to the two external sites for testing.

Proportion of correct calls with two-sided lower 95% confidence level and variance component analysis in terms of standard deviation (SD) of ΔCt are included in Table 14 and Table 15.

Table 13: Reproducibility - Proportion of Correct Calls for PIK3CA Mutations Tested Across All Sites

Mutation	Template	Fractional Proportion of Valid Results	Percentage	Two-Sided Lower 95% Confidence Limit
C420R	LoD	237/238	99.58%	97.68%
	3x LoD	238/238	100.00%	98.46%
E542K	LOD	237/240	98.75%	96.39%
	3xLOD	240/240	100.00%	98.47%
E545A	LOD	239/240	99.58%	97.70%

Mutation	Template	Fractional Proportion of Valid Results	Percentage	Two-Sided Lower 95% Confidence Limit
	3xLOD	240/240	100.00%	98.47%
E545D	LOD	240/240	100.00%	98.47%
	3xLOD	240/240	100.00%	98.47%
E545G	LOD	237/240	98.75%	96.39%
	3xLOD	239/239	100.00%	98.47%
E545K	LOD*	432/432	100.00%	99.15
	3xLOD	240/240	10.000%	89.47%
H1047L	LOD	236/238	99.16%	97.00%
	3xLOD	238/238	100.00%	98.46%
H1047R	LOD*	430/432	99.54%	98.34%
	3xLOD	236/236	100.00%	98.45%
H1047Y	LOD	239/240	99.58%	97.70%
	3xLOD	240/240	100.00%	98.47%
Q546E	LOD	238/238	100.00%	98.46%
	3xLOD	238/238	100.00%	98.46%
Q546R	LOD	232/240	96.67%	93.54%
	3xLOD	240/240	100.00%	98.47%
WT	Ct30	223/238	93.70	89.82

*Samples at revised LoD with E545K and H1047R (as per Table 6) were evaluated for 6 days accross 3 sites, by 3 operators, with 2 runs and 4 replicates for a total of 144 measurements per site, 432 total in 3 sites.

All mutant samples demonstrated 100% agreement at 3X LoD. In addition, at 1X LoD, all 11 mutants showed > 99% agreement except Q546R at 1X LoD.

In the repeatability study the WT sample correct call rate was 95.00% (data not shown), with a lower two-sided exact 95% confidence limit of 89.43% . Similarly, in the reproducibility study, the percentage correct calls for the WT samples was 93.70% (data not shown), resulting in a lower two-sided 95% confidence limit of 89.82%. An additional Reproducibility and Repeatability will be performed to evaluate WT and mutation positive samples per the Instructions for Use, that is, by testing all 6 reactions mixes per sample (see CDRH decision below).

The second reproducibility study includes the LoD confirmation study with 5 clinical samples which is described in Section 3 (iv), above.

The WT sample evaluated in the repeatability and reproducibility study did not meet the acceptance criteria in relation to proportion of correct calls. An investigation indicated that the WT sample had an additional mutation with low allelic frequency

as determined by an NGS-based method. A new repeatability and reproducibility study will be conducted postmarket with an appropriately qualified WT sample. Refer to section XIV.

A variance component analysis was used to estimate the standard deviation for between-run, between-day, between-lot, between-operator, between-instrument, and between-day variability for the reproducibility study (Table 14).

Table 14: Repeatability - Standard Deviation of Variance Components

Mutation	Level	Number	Mean	Between Kit Lot SD	Between Run SD	Between Operator SD	Between Day SD	Between Instrument SD	Within Run SD	Total SD
E542K	3xLOD	**120**	**1.06**	0.09	0.18	0.07	0.00	0.21	0.21	0.33
	LOD	**120**	**3.28**	0.02	0.10	0.00	0.00	0.24	0.46	0.51
E545D	3xLOD	**120**	**2.97**	0.000	0.10	0.00	0.000	0.07	0.30	0.32
	LOD	**120**	**4.1**	0.09	0.00	0.00	0.12	0.07	0.48	0.50
E545G	3xLOD	**120**	**1.9**	0.01	0.17	0.00	0.00	0.00	0.32	0.36
	LOD	**120**	**5.72**	0.00	0.24	0.00	0.00	0.27	1.06	1.1
E545K	3xLOD	**120**	**4.57**	0.09	0.07	0.00	0.00	0.22	0.58	0.62
	LOD	**144**	**5.28**	0.13	0.086	0.19	0.00	0.42	0.66	0.77
E545A	3xLOD	**120**	**0.85**	0.05	0.17	0.00	0.00	0.05	0.30	0.35
	LOD	**120**	**3.66**	0.00	0.37	0.00	0.40	0.26	1.21	1.3
H1047Y	3xLOD	**120**	**3.45**	0.06	0.12	0.02	0.00	0.09	0.25	0.29
	LOD	**120**	**5.05**	0.00	0.00	0.02	0.00	0.13	0.41	0.43
Q546R	3xLOD	**120**	**1.91**	0.057	0.26	0.00	0.00	0.00	0.36	0.44
	LOD	**120**	**4.74**	0.29	0.11	0.16	0.34	0.180	1.07	1.16
C420R	3xLOD	**120**	**1.45**	0.04	0.06	0.04	0.08	0.20	0.30	0.36
	LOD	**120**	**3.23**	0.000	0.09	0.00	0.21	0.18	0.65	0.71
Q546E	3xLOD	**120**	**1.14**	0.06	0.11	0.00	0.00	0.00	0.21	0.24

Mutation	Level	Number	Mean	Between Kit Lot SD	Between Run SD	Between Operator SD	Between Day SD	Between Instrument SD	Within Run SD	Total SD
	LOD	**120**	**5.38**	0.21	0.00	0.123	0.21	0.17	0.83	0.88
H1047L	3xLOD	**120**	**2.18**	0.00	0.14	0.03	0.10	0.11	0.22	0.30
	LOD	**120**	**5.18**	0.20	0.00	0.00	0.00	0.00	0.63	0.64
H1047R	3xLOD	**120**	**3.68**	0.07	0.19	0.03	0.06	0.03	0.52	0.56
	LOD	**143**	**4.49**	0.00	0.20	0.00	0.00	0.16	0.75	0.77

Table 15: Reproducibility - Standard Deviation of Variance Components

Mutation	Level	Nimber	Mean	Between Site (SD)	Between Run Within Site (SD)	Between Operator (SD)	Between Instrument (SD)	Between Day (SD)	Between Lot (SD)	Within Site (SD)	Within Run (SD)	Total (SD)
E542K	3xLOD	**240**	**1.07**	0.14	0.20	0.03	0.25	0.00	0.06	0.41	0.24	0.40
	LOD	**240**	**3.24**	0.13	0.24	0.00	0.32	0.00	0.00	0.63	0.48	0.61
E545D	3xLOD	**240**	**3.00**	0.08	0.16	0.00	0.09	0.00	0.00	0.37	0.32	0.40
	LOD	**240**	**4.14**	0.11	0.12	0.00	0.17	0.00	0.00	0.55	0.51	0.55
E545G	3xLOD	**239**	**1.88**	0.00	0.08	0.00	0.00	0.00	0.00	0.36	0.35	0.36
	LOD	**239**	**5.82**	0.39	0.10	0.00	0.26	0.16	0.14	1.16	1.10	1.18
E545K	3xLOD	**240**	**4.74**	0.19	0.00	0.00	0.29	0.00	0.00	0.72	0.66	0.72
E545K	LOD	**432**	**4.99**	0.34	0.21	0.10	0.28	0.13	0.07	0.75	0.63	0.79
E545A	3xLOD	**240**	**0.91**	0.00	0.12	0.01	0.11	0.00	0.00	0.39	0.35	0.39
	LOD	**240**	**3.67**	0.00	0.34	0.00	0.19	0.07	0.00	1.18	1.09	1.18

Mutation	Level	Nimber	Mean	Between Site (SD)	Between Run Within Site (SD)	Between Operator (SD)	Between Instrument (SD)	Between Day (SD)	Between Lot (SD)	Within Site (SD)	Within Run (SD)	Total (SD)
H1047Y	3xLOD	**240**	**3.42**	0.08	0.13	0.00	0.14	0.00	0.07	0.32	0.25	0.32
	LOD	**240**	**5.02**	0.02	0.05	0.00	0.15	0.00	0.01	0.44	0.41	0.44
Q546R	3xLOD	**240**	**1.87**	0.06	0.22	0.00	0.03	0.00	0.03	0.44	0.38	0.44
	LOD	**240**	**4.66**	0.10	0.16	0.16	0.35	0.00	0.18	1.15	1.06	1.13
C420R	3xLOD	**238**	**1.36**	0.06	0.12	0.00	0.17	0.04	0.05	0.38	0.31	0.38
	LOD	**238**	**3.18**	0.00	0.19	0.00	0.12	0.11	0.05	0.70	0.65	0.70
Q546E	3xLOD	**238**	**1.11**	0.00	0.08	0.00	0.00	0.03	0.07	0.25	0.22	0.24
	LOD	**238**	**5.36**	0.00	0.17	0.00	0.07	0.12	0.00	0.90	0.87	0.90
H1047L	3xLOD	**238**	**2.08**	0.08	0.14	0.02	0.09	0.02	0.10	0.30	0.23	0.30
H1047L	LOD	**238**	**5.18**	0.00	0.00	0.13	0.00	0.11	0.00	0.99	0.97	0.99
H1047R	3xLOD	**236**	**3.67**	0.00	0.18	0.00	0.10	0.00	0.00	0.64	0.61	0.64
H1047R	LOD	**430**	**4.41**	0.17	0.21	0.00	0.08	0.07	0.09	0.82	0.77	0.82

(v) <u>Lot-to-Lot Reproducibility</u>

To demonstrate lot-to-lot interchangeability, three lots of the Plasma Extraction Kit and one lot of the *therascreen* PIK3CA RGQ PCR Kit were used to test contrived plasma samples for all 11 mutations and HD plasma as the WT sample Each sample was extracted with three different lots of the QIAamp DSP Circulating Nucleic Acid Kit. The ages of the three different DNA Extraction Kit lots used for the lot-to-lot study were 21.6 months, 10.5 months, and 9.6 months. Six replicate extractions were carried out per sample per extraction kit lot. The correct overall percentage mutation result was reported across all samples and kit lots, along with the corresponding two-sided exact 95% confidence limits. For each *therascreen* PIK3CA RGQ PCR Kit lot and Plasma Extraction Kit lot combination, a total of 216 valid data points (12 targets x 6 replicates x 3 extraction kit lots) were collected. The percentage of correct overall mutation call was 100% (216/216) across all samples (11 mutants and the wild type) and extraction kit lots, with the corresponding two

sided exact 95% confidence limits of 98.31% and 100%. Additionally no statistically significant difference was observed in ΔCt values for any mutation positive samples between extraction kits lots.

(vi) Sample Handling

Patient samples were collected at the SOLAR-1 drug investigational sites in accordance with the SOLAR-1 protocol and supporting documents. Patient blood samples were collected at cycle one day one after enrollment into the trial and prior to start of SOLAR-1 study treatment. Blood was collected in 10 mL EDTA tubes and within 2 hours plasma was separated by double centrifugation (10 minutes at room temperature at 1,600 g and 3,000 g, respectively). The plasma was aliquoted at approximately 1 mL into 2 mL plain cap pre-labeled cryogenic vials and stored at -70°C or colder.

The frozen plasma samples were shipped on dry ice to for central storage at -70°C or colder. Prior to testing, frozen samples were shipped on dry ice from the central storage to the sample testing site.. At the testing site, frozen samples were received and stored at -70°C to -90°C until further testing.

To demonstrate that different laboratories produce acceptable results when starting from the same plasma sample, extractions were performed across 3 different sites. Contrived samples consisting of fragmented cell line DNA spiked into HD plasma for all 11 mutations as well as a WT sample were used in this study. There were 18 x 2 mL aliquots required for each sample; these aliquots were randomized and split into 18 extract sets. These extract sets were then distributed evenly across the three testing sites, six extracts per study site. Testing of the extracts using the *therascreen* PIK3CA RGQ PCR Kit was performed at Site 1. When comparing the results of each sample across all 3 sites, the percentage of correct mutation calls for mutation positive and WT samples was 100%.

(vii) Guardbanding

Four guardbanding studies (PCR annealing temperature, Reagent volume, Thawing and PCR Set-Up) were designed to assess the performance of the PCR kit which is shared by both the Tissue and Plasma indications (see P190001 SSED),

A guardband study was conducted for the QIAamp DSP Circulating Nucleic Acid Kit (specific for Plasma) using WT and mutation positive contrived plasma samples for E542K, E545K, Q546R, C420R and H1047R) that could be detected by each of the reaction mixes within the kit normalized to a Ct value of 30 and diluted to a MAF% of 3X LoD. MAF% dilutions were prepared in a background of WT DNA. DNA was extracted using QIAamp DSP Circulating Nucleic Acid Kit. During the extraction process the following conditions were varied:

- Proteinase K incubation time (Time 1, 30min at standard conditions ±10min) and temperature (Temp. 1, 60ºC at standard conditions ±5 ºC)

- Membrane drying time (Time 2, 10min at standard conditions ±5min) and temperature (Temp. 2, 56ºC at standard conditions ±5 ºC)

Twenty unique combinations of the varied conditions including standard conditions were tested so that the main and second order polynomial effects could be estimated for all possible combinations. To determine the effect of varying incubation temperature (Incubation 1: 55ºC to 65ºC, Incubation 2: 51ºC to 61ºC) and incubation time (Incubation 1: 20 mins to 40 mins, Incubation 2: 5 mins to 15 mins) during the DNA extraction process, the temperature and duration of two incubations were varied while keeping all other factors constant.

For WT samples the correct overall mutation call was determined across all tested conditions; 100% correct mutation call rate ("No mutation detected") was observed. Therefore, varying the incubation time and temperature from the standard condition had no impact on the WT correct call rate.

For each mutation positive sample, separate ANOVA models were fitted with ΔCt as the response variable, centralized (to the standard condition) incubation temperatures and times along with their corresponding second order interactions and power terms as continuous explanatory variables. For each of the mutant samples, the estimated differences to the nominal guardband condition were generated.

The acceptable extraction time and temperature tolerances, which have been demonstrated to have no significant impact on the performance of the *therascreen* PIK3CA RGQ PCR Kit are shown in Table 16.

Table 16: Acceptable Plasma Extraction Parameters

Step	Description	Handbook condition	Acceptable variation
8 (Temp 1)	Incubation with Proteinase K	60 ºC	±2°C
8 (Time 1)		30 min	±4 mins
24 (Temp 2)	Drying Membrane	56 ºC	±3°C
24 (Time 2)		10 min	±4 mins

Correct mutation call was observed for all WT and mutation positive samples under all conditions tested. Acceptable time and temperature tolerances were established.

(viii) <u>Blood Collection Tube Validation</u>

This study was designed to determine the impact of blood to plasma separation time on plasma sample quality and subsequent results when tested with the *therascreen*

PIK3CA RGQ PCR Kit. The time up to four hours between blood collection and plasma separation was assessed.

Contrived blood samples for H1047R (the most prevalent mutation) and whole blood samples as WT samples were used in this study. Blood samples were collected into 10 mL K_2EDTA tubes from four donors (eight tubes per donor). Contrived blood samples were generated by spiking H1047R fragmented cell line DNA into blood tubes from two donors after collection. Blood samples were separated into plasma at 1h, 2h, 3h and 4h timepoints post collection. DNA was extracted from the plasma samples using theQIAamp DSP Circulating Nucleic Acid Kit and each target was tested with the *therascreen* PIK3CA RGQ PCR Kit in 16 replicates per each timepoint.

The percentage of correct calls based on the overall mutation status (i.e. Mutation Detected or No Mutation Detected) was 100% for all samples across all donors at each timepoint. There was no statistically significant drift in ΔCt observed for the H1047R sample. This study demonstrated that there is no impact of the blood to plasma separation time, if processed within four hours, on the *therascreen* PIK3CA RGQ PCR Kit.

(ix) <u>Stability Studies</u>

(a) Stability of Specimens (Plasma)

Long term stability of two mutant positive clinical plasma samples (E545K and H1047R) is currently being assessed when stored at -80°C. Samples will be extracted and tested with the *therascreen* PIK3CA RGQ PCR Kit at the following time points: baseline (T0), 7 months (T1), 12 months (T2), 19 months (T3), 24 months (T4) and 25 months (T5).

So far the interim stability data includes time points T0, T1and T2 (up to 12 months). Three clinical samples have been used in this study; E545K and H1047R mutation positive samples (around 2X LoD) in addition to a WT plasma sample. A 100% correct call rate was achieved across all samples at all time points (6 replicates per sample per time point). The data has demonstrated that plasma samples are stable when stored at -80°C for ≤ 11 months.

Stability of plasma samples stored at -80˚C will be tested over a 25 month time frame to assess long term storage.

Plasma sample stability in simulated shipping conditions have also been assessed up to eight days using clinical mutation positive and WT samples stored on dry ice. 100% correct call rate was achieved at the tested time points demonstrating plasma samples are stable when shipped on dry ice for ≤ 7 days.

(b) Stability of Specimens (DNA)

One clinical sample and five contrived plasma samples, generated by spiking fragmented cell line DNA into HD plasma, for E542K, E545K, Q546R, C420R and H1047R were used in this study. DNA from contrived plasma samples was extracted using the QIAamp DSP Circulating Nucleic Acid Kit and DNA eluates were normalized to the control assay Ct of 30. All contrived samples used in this study were normalized to an MAF level corresponding to 1.5X LoD. The clinical sample was adjusted to 3X LoD.

To assess the stability of extracted DNA when stored at -35°C to -15°C for the *therascreen* PIK3CA RGQ PCR Kit, the stability of DNA extracted was tested (6 replicates per sample per time point) against temporal and freeze-thaw conditions:

- DNA was stored at -15°C to -35C for 2, 4 and 5 weeks before testing with the *therascreen* PIK3CA RGQ PCR Kit
- DNA was subjected to 1 – 5 freeze-thaw cycles over the course of five weeks before testing with the *therascreen* PIK3CA RGQ PCR Kit. Samples were stored at -15°C to -35˚C between freeze-thaw cycles.

All tested samples at all time points were valid and the correct call rate was 100% in both temporal and freeze-thaw cycle stability studies. Results from this study show that DNA extracted from plasma specimens can be stored at -20ºC (range: -35 to -15ºC) for up to four weeks and undergo up to four freeze-thaw cycles.

(c) Kit Stability

The kit stability study for both tissue and plasma was done simultaneously using the same protocol with the exception that the PIK3CA mutant used for the study was different. For the plasma test, E545K was used and for tissue, H1047R was used.

The *therascreen* PIK3CA RGQ PCR Kit was assessed for:

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

To evaluate real-time and in-use stability all test time points assessed the same batch of extracted plasma DNA from mutant (E545K) positive clinical samples

at 2X LoD and WT, as well as cell line mutant sample for E545K at 3X LoD level. Transport Simulation and In-Use stability were assessed using one *therascreen* PIK3CA RGQ PCR Kit. Real-Time stability was assessed using three *therascreen* PIK3CA RGQ PCR Kit lots. Six replicates were tested at each time point for Real-Time stability. Two replicates were tested at each time point for In-Use stability. At time point 13 months, 100% correct calls were observed for all samples and test conditions tested (Real-Time, In-Use and Transport stability). A linear regression for ΔCt values (for the Cell Line H1047R mutation positive sample) also showed no significant shift in ΔCt over the time tested.

The real time stability data generated during this study supports a shelf-life claim of 12 months the *therascreen* PIK3CA RGQ PCR Kit when stored at -30°C to -15°C.

The in-use data generated during this study for the *therascreen* PIK3CA RGQ PCR Kit at all time points up to 13 months confirms the stability after 5 freeze-thaw cycles.

A post market study will be conducted using representative mutation positive samples from each reaction tube at 2X LoD to validate and support the reagent stability of all the components of the *therascreen* PIK3CA RGQ PCR Kit. Refer for section XIV.

(d) Plasma DNA Extraction Kit Stability

The preliminary stability of the QIAamp® DSP Circulating Nucleic Acid Kit was provided to allow a minimum stability claim for 6 months. A post market study based on an approved protocol will be conducted using representative mutation positive to demonstrate real-time stability of the QIAamp® DSP Circulating Nucleic Acid Kit. Refer for section XIV.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY

The QIAGEN GmbH (QIAGEN) performed a clinical performance study to establish a reasonable assurance of safety and effectiveness of the *therascreen* PIK3CA RGQ PCR Kit as an aid to clinicians in identifying breast cancer patients who may be eligible for treatment with PIQRAY® (alpelisib), based on a PIK3CA mutation detected result in plasma specimens. The study was originally initiated under Investigational Device Exemption (IDE) G160122 when the patients were selected and randomized based on the results from the patients tissue specimens using a Clinical Trial Assay (CTA) and the *therascreen*

PIK3CA RGQ PCR Kit. The results from the corcordance study between the tissue and plasma test, and the clinical efficacy from the clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

Clinical performance of the Tissue test using the *therascreen* PIK3CA RGQ PCR Kit (PIK3CA tissue) was demonstrated (see SSED for P190001) using tumor biopsies from patients enrolled in study CBYL719C2301 (SOLAR-1). SOLAR-1 is a pivotal registrational trial for alpelisib, an α-specific phosphatidylinositol-3-kinase (PI3K) inhibitor. For detailed description of SOLAR-1 study design, see SSED for P190001.

A summary of the study conducted to support an alpelisib selection claim is presented below.

A. Study Design

For a complete SOLAR-1 study design see SSED for P190001. The study was initiated on July 23, 2015. The cut-off date for efficacy analysis in PIK3CA mutant cohort and safety for both cohorts is June 12, 2018. The database for this PMA reflected data collected through June 12, 2018 for the PIK3CA mutant cohort and December 23, 2016 for the PIK3CA non-mutant cohort and included 572 patients.

The study was a pivotal Phase III, randomized, double-blind, placebo controlled, international, multicenter study of alpelisib in combination with fulvestrant in men and postmenopausal women with hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) locally advanced breast cancer whose disease had progressed or recurred on or after an aromatase inhibitor based treatment (with or without CDK4/6 combination).

A total of 572 breast cancer patients were enrolled into two cohorts, with or without a PIK3CA mutation. Patients and investigators were blinded to mutational status. Patients were randomized to receive either alpelisib 300 mg plus fulvestrant or placebo plus fulvestrant in a 1:1 ratio. Randomization was stratified by presence of lung and/or liver metastasis and previous treatment with Cyclin-Dependent Kinases 4 and 6 (CDK4/6) inhibitor(s). Blood samples for plasma ctDNA were collected from randomized patients on Cycle 1 Day 1 prior to initiation of study treatment.

During the randomized treatment phase, alpelisib 300 mg or placebo was administered orally once daily on a continuous basis. Fulvestrant 500 mg was administered intramuscularly on Cycle 1 Day 1 and 15 and then at Day 1 of a 28-day cycle during treatment phase (administration +/- 3 days). Patients were not allowed to cross over from placebo to alpelisib during the study or after disease progression.

The primary endpoint for the SOLAR-1 clinical study was progression free survival (PFS) using Response Evaluation Criteria in Solid Tumors (RECIST v1.1), based on investigator assessment in advanced breast cancer patients enrolled with a PIK3CA mutation as determined in FFPE tumor tissue. A secondary endpoint was PFS for

patients without PIK3CA mutation as determined in tumor tissue, and PFS for patients with and without PIK3CA mutations as determined in plasma at baseline.

For the plasma testing, the plasma samples collected prior to study treatment were retrospectively tested to determine PIK3CA mutation status using the *therascreen* PIK3CA RGQ PCR Kit (hereafter refer to as *therascreen* PIK3CA RGQ PCR Kit using plasma). The patients had been previously tested for PIK3CA mutation status with the *therascreen* PIK3CA RGQ PCR Kit using tissue biopsy samples. Clinical efficacy of alpelisib in combination with fulvestrant was estimated for patients with a PIK3CA mutation as determined using plasma samples. Agreement of PIK3CA mutation results from plasma samples was compared to results from tissue samples for the SOLAR-1 randomized population. Secondary analyses were performed to estimate efficacy in the plasma negative population and in the plasma/tissue subpopulations.

Informed consent for the use of blood samples was obtained from patients in accordance with the Novartis CBYL719C2301 clinical protocol.

1. Key Inclusion and Exclusion Criteria

Enrollment in the SOLAR-1 study was limited to patients who met the following key inclusion criteria:

- Adults (female postmenopausal or men) ≥ 18 years old
- Radiological or objective evidence of recurrence or
- progression during or after Aromatase Inhibitor (AI) therapy
- Histologically and/or cytologically confirmed diagnosis of estrogen receptors (ER)-positive and/or Progesterone receptor positive breast cancer
- Has HER2- breast cancer
- Identified PIK3CA status (mutant or non-mutant; determined by a Novartis designated laboratory).
- Subject either had
    - Measurable disease, i.e. at least one measurable lesion per RECIST 1.1 or
    - If no measurable disease was present, then at least one predominantly lytic bone lesion must be present
- Subject had advanced (locoregionally recurrent not amenable to curative therapy or metastatic) breast cancer
- Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1
- Adequate bone marrow and organ function as described in protocol

Patients were not permitted to enroll in the SOLAR-1 study if they met any of the following key exclusion criteria:

- Had inflammatory breast cancer or uncontrolled central nervous system metastases.
- Had concurrent malignancy or malignancy within 3 years of randomization, except for adequately treated, basal or squamous cell carcinoma, non-melanomatous skin cancer, or curatively resected cervical cancer.
- Received prior treatment with chemotherapy in the advanced setting or prior therapy with fulvestrant or any PI3K, AKT or mTOR inhibitor
- Had Type 1 or uncontrolled Type 2 diabetes mellitus
- Had documented pneumonitis at time of screening

2. **Follow-up Schedule**

Safety follow-up
Subjects who discontinued study treatment were followed for safety, until 30 days after last study treatment administration, except in the case of death, loss to follow-up, or withdrawal of consent.

Efficacy follow-up
Subjects who discontinued study treatment for reasons other than disease progression, death, loss to follow-up, or withdrawal of consent, were followed every eight weeks ± one week for efficacy during the first 18 months and every 12 weeks ± one week thereafter until disease progression, death, loss to follow-up, or withdrawal of consent.

Survival follow-up
All subjects were followed for survival every 12 weeks until death, loss to follow-up or withdrawal of consent until the final number of OS events was reached or if the study had stopped for other reasons. During the survival follow-up, data relaed to subsequent antineoplastic therapies initiated after study treatment discontinuation were collected along with the start/end date and date of disease progression on
subsequent therapies.

1. Clinical Endpoints

Primary Efficacy Endpoint: PFS in PIK3CA mutant cohort
The primary endpoint for the study was PFS using RECIST v1.1 criteria, based on investigator assessment in advanced breast cancer patients enrolled with a PIK3CA mutation based on tissue tumor samples.

Safety Endpoints
Safety and tolerability were evaluated by assessment of type, frequency and severity of adverse events and laboratory toxicities per Common Terminology Criteria for Adverse Events (CTCAE) v4.03.

2. Plasma Diagnostic Study Objective and Design

The primary objectives of the studyusing Plasma specimens were

- to demonstrate the clinical utility of the *therascreen* PIK3CA RGQ PCR Kit using plasma in identifying breast cancer patients with a PIK3CA mutation for treatment with alpelisib in combination with fulvestrant, and
- to evaluate the concordance of PIK3CA mutation status in plasma compared to tissue samples.

The secondary objectives of the *therascreen* PIK3CA RGQ PCR Kit using plasma were

- to evaluate the clinical efficacy of PIK3CA mutation negative patients identified by the *therascreen* PIK3CA RGQ PCR Kit for treatment with alpelisib in combination with fulvestrant against placebo in combination with fulvestrant and
- to evaluate the clinical efficacy of patients identified as plasma PIK3CA mutation negative by the *therascreen* PIK3CA RGQ PCR Kit using plasma and tissue PIK3CA mutation positive by the *therascreen* PIK3CA RGQ PCR Kit using tissue, for treatment with alpelisib in combination with fulvestrant against placebo in combination with fulvestrant
- to evaluate the clinical efficacy of plasma PIK3CA mutation positive patients identified by the *therascreen* PIK3CA RGQ PCR Kit using plasma and tissue PIK3CA mutation positive by the *therascreen* PIK3CA RGQ PCR Kit using tissue for treatment with alpelisib in combination with fulvestrant against placebo in combination with fulvestrant
- to evaluate the clinical efficacy of patients harboringplasma PIK3CA mutation tested by the *therascreen* PIK3CA RGQ PCR Kit using plasma and tissue PIK3CA mutation negative tested by the *therascreen* PIK3CA RGQ PCR Kit using tissue for treatment with alpelisib in combination with fulvestrant against placebo in combination with fulvestrant
- to evaluate the clinical efficacy of patients identified as plasma PIK3CA mutation negative by the *therascreen* PIK3CA RGQ PCR Kit using plasma and tissue PIK3CA mutation negative tested by the *therascreen* PIK3CA RGQ PCR Kit using tissue for treatment with alpelisib in combination with fulvestrant against placebo in combination with fulvestrant

Diagnostic device study design

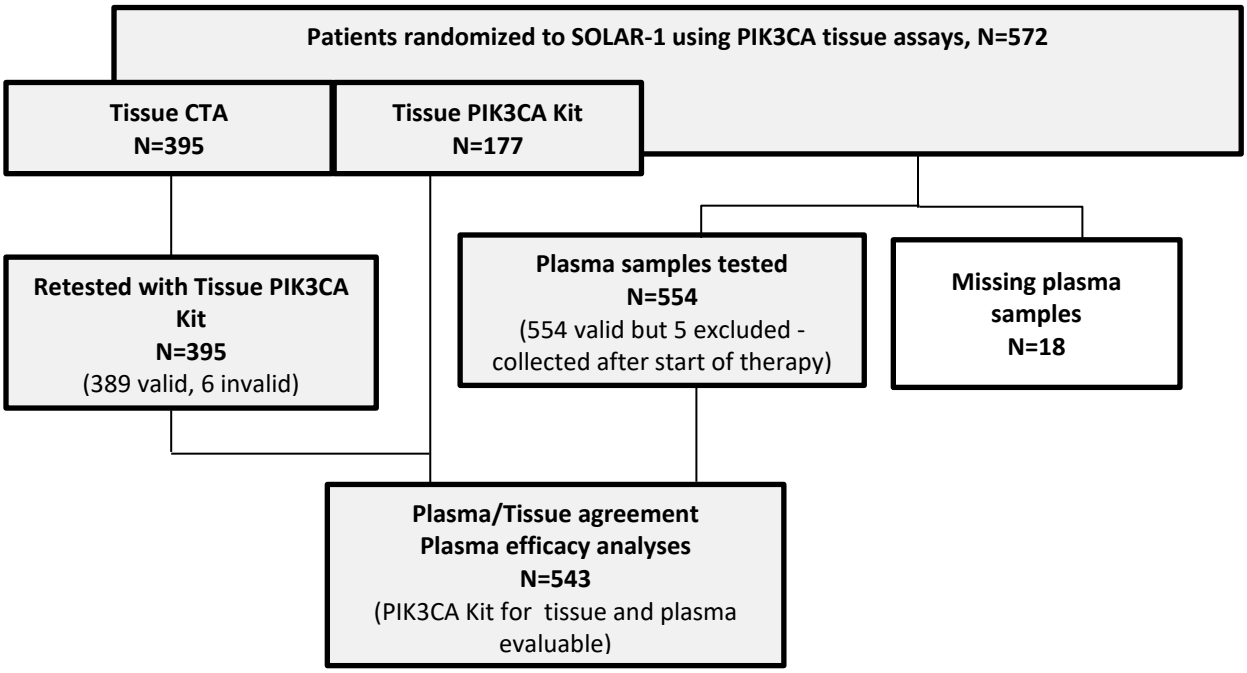
The device study retrospectively tested plasma samples from patients with advanced breast cancer randomized in the SOLAR-1 clinical study, to demonstrate safety and effectiveness of the *therascreen* PIK3CA RGQ PCR Kit for identification of HR+, HER2- advanced breast cancer patients with PIK3CA mutations eligible for treatment with alpelisib.

B. Accountability of PMA Cohort

Of the 1442 patients screened for SOLAR-1 with regards to PIK3CA testing of tissue samples, a total of 572 mutation positive and negative patients were randomized to SOLAR-1 based upon PIK3CA mutation status, determined using FFPE tissue (For detail, see SSED for P190001). Full disposition of the patient samples from SOLAR-1 and those used for clinical study with plasma specimens is shown in Figure 1.

Blood samples were collected from randomized patients prior to initiation of study treatment, processed to plasma and stored frozen until retrospective analysis using the *therascreen* PIK3CA RGQ PCR Kit using plasma. Of the 572 total randomized patients in SOLAR 1, plasma samples were available for 554 and unavailable for 18 randomized patients. Of the 554 samples tested, 5 were excluded from analysis due to plasma samples being collected after initiation of the SOLAR-1 study treatment. In total there were 543 *therascreen* PIK3CA RGQ PCR Kit-evaluable patients with both valid PIK3CA tissue results and PIK3CA plasma results. The 29 *therascreen* PIK3CA RGQ PCR Kit-unevaluable patients were due to 6 missing PIK3CA tissue results and 23 missing PIK3CA plasma results. The tissue and plasma sample accountability for the SOLAR-1 randomized patients is shown in Figure 1.

Figure 1: Specimen Accountability



Tissue samples from the 395 CTA-enrolled patients were retested with the PIK3CA Tissue and yielded 389 valid results with 6 missing due to invalid results. The total PIK3CA Tissue evaluable population was 566 patients. Within the PIK3CA tissue evaluable population there were 347 mutation positive patients (172 *therascreen* PIK3CA RGQ PCR Kit-enrolled and 175 from retrospective retesting of CTA-enrolled

patients) and 219 mutation negative patients (5 *therascreen* PIK3CA RGQ PCR Kit using tissue-enrolled and 214 from retrospective retesting of the CTA-enrolled patients).

Concordance of Plasma and Tissue Tests using *therascreen* PIK3CA RGQ PCR Kit

Concordance of the PIK3CA mutation results of *therascreen* PIK3CA RGQ PCR Kit using plasma to the *therascreen* PIK3CA RGQ PCR Kit using tissue results is shown in Table 17. Of the 328 PIK3CA tissue positive patients, 179 were plasma PIK3CA positive. Of the 215 PIK3CA tissue negative patients, 209 were plasma PIK3CA negative. There were no invalid plasma results. Note that H1047Y, Q546R, Q546E, E545D, and E545A were not detected by *therascreen* PIK3CA RGQ PCR Kit using plasma from clinical samples.

Table 17: Contingency Table Between PIK3CA Tissue Results and PIK3CA Plasma Results (Full Analysis Set)

PIK3CA plasma	PIK3CA tissue			
	Positive	Negative	Invalid	Total
Positive	179	6	1	186
Negative	149	209	5	363
Invalid	0	0	0	0
Total	328	215	6	549
Samples not tested due to missing or collected after start of therapy are excluded from this table.				

Agreement (PPA, NPA and OPA) between the PIK3CA plasma and PIK3CA tissue results was calculated using the PIK3CA tissue results as reference (Table 18). The point estimates of PPA, NPA and OPA were 54.6%, 97.2% and 71.5% respectively. Because plasma test failed to detect approximately 46% of the mutation detected in tissue, a reflex testing using tissue specimens will be required, if plasma test is negative.

Table 18: Agreement Between PIK3CA Plasma Results and PIK3CA Tissue Results using the PIK3CA Tissue Results as Reference (Full Analysis Set)

Measure of	Percent agreement (N)	95% CI*
PPA	54.6% (179/328)	(49.0%, 60.1%)
NPA	97.2% (209/215)	(94.0%, 99.0%)
OPA	71.5% (388/543)	(67.5%, 75.2%)
* The 95% Confidence Interval (CI) calculated using the Clopper-Pearson Exact method.		

Confirmatory Testing of Plasma Samples with a Reference Assay

As part of the analytical validation using plasma, all SOLAR-1 plasma samples with residual ctDNA were tested using a validated NGS method. The NGS plasma results were also used to investigate discordance between the PIK3CA plasma and PIK3CA tissue results. Of the 543 patients in the plasma/tissue concordance study, NGS plasma

results were available for 536 patients with 7 missing due to insufficient DNA. The NGS plasma results confirmed the PIK3CA plasma results for 495 of the 543 patients (91%) with 7 missing NGS results.

In the plasma/tissue concordance analysis, a total of 155 of the 543 plasma samples showed discordant PIK3CA mutation status between the PIK3CA plasma results and PIK3CA tissue results (Table 17).

Of the 6 PIK3CA positive samples (Table 17) that were tissue PIK3CA negative:

- 5 cases were confirmed plasma positive by NGS.
- 1 case was plasma negative by NGS and concordant with the tissue PIK3CA negative result.

Of the 149 plasma PIK3CA negative samples that were PIK3CA tissue positive:

- 119 cases were confirmed plasma negative by NGS.
- 28 cases were negative by NGS due to the locked NGS assay cut-off being set at a value that called these "low positive" samples negative.
- 2 cases had missing NGS results due to insufficient residual DNA.

Of the 388 cases that were concordant for PIK3CA plasma results and PIK3CA tissue results, NGS confirmed the PIK3CA plasma results in 371 cases (96%), NGS plasma results were discordant with PIK3CA plasma in 12 cases, and 5 cases had missing NGS results due to insufficient residual DNA.

C. Study Population Demographics and Baseline Parameters

Tissue and plasma sample characteristics for the plasma positive patients compared to plasma negative patients

Within the SOLAR-1 tissue mutant cohort, 92% of patients were screened for PIK3CA status using archival tumor biopsies, and 78% of all tumor biopsies were from primary breast tumor. Tissue sample characteristics were similar for the tissue non-mutant cohort (90% archival and 76% from primary breast tumors). For the plasma positive patients, 90% of the corresponding tissue samples were archival and 77% were from primary breast tumor with similar characteristics for the plasma negative patients (92% of the corresponding tissue samples were archival and 77% from primary breast tumor).

Mean time from plasma sample collection to retrospective testing for the tissue positive patients was 9 months (range 1 to 22 months) versus 14 months (range 6 to 22 months) for the plasma from tissue negative patients. The difference in the age of the plasma samples is predominantly due to the starting date of the retrospective testing in June 2017. The PIK3CA non-mutant cohort of SOLAR-1 completed enrollment (and hence collection of baseline plasma samples fromtissue negative

patients) in December 2016, whereas collection of baseline plasma samples from tissue positive patients continued for the PIK3CA mutant cohort until July 2017.

Baseline demographics and disease characteristics for the plasma positive compared to plasma negative patients

Baseline demographics and disease characteristics for the plasma positive patients were similar, except with regards to ECOG performance status and extent of disease. At study entry:

- ECOG status: 38% of plasma positive patients had ECOG status of 1 (versus 0) compared to 29% of plasma negative patients
- Bone involvement: 81% of plasma positive patients had bone involvement compared to 70% of plasma negative patients
- Liver involvement: 37% of plasma positive patients had liver involvement compared to 28% of plasma negative patients.
- Lung involvement: 32% of plasma positive patients had lung involvement compared to 41% of plasma negative patients

PIK3CA mutation positive populations

The baseline demographics of the 186 plasma positive patients were similar to the 341 tissue positive patients in the SOLAR-1 mutant cohort. The plasma positive patients were distributed equally across the two treatment arms with 92 patients in the alpelisib plus fulvestrant arm and 94 patients in the placebo plus fulvestrant arm. Demographics were generally well-balanced across treatment arms. Of the 186 plasma positive patients, approximately 51% were from Europe, 20% from Asia, 14% from North America and 5% from Latin America.

Baseline disease characteristics including extent and location of disease and prior therapies were similar for the plasma PIK3CA positive patients compared to the SOLAR-1 tissue mutant cohort, except with regards to bone involvement. In both the plasma positive and tissue positive populations, 98% of patients had Stage IV disease, 57% of patients had visceral involvement and 23% of patients had bone only disease at study entry. However, the proportion of plasma positive patients with bone involvement (81%) was higher than observed for tissue positive patients (74%).

PIK3CA mutation negative populations

The baseline demographics and disease characteristics for the 363 plasma negative patients were similar to the 231 tissue negative patients in the SOLAR-1 non-mutant cohort. The plasma negative patients were distributed equally across the two treatment arms with 181 patients in the alpelisib plus fulvestrant arm and 182 patients in the placebo plus fulvestrant arm.

Of the 363 plasma negative patients, 53% were from Europe, 23% from Asia, 10% from North America and 8% from Latin America. At study entry, 58% of patients had visceral involvement and 21% had bone only disease. In the tissue negative

population, characteristics were generally balanced across treatment arms except where ≥ 10% differences were observed in race (71% white in the alpelisib plus fulvestrant arm versus 60% in the placebo plus fulvestrant arm), prior tamoxifen use (32% in the alpelisib plus fulvestrant arm versus 43% in the placebo plus fulvestrant arm), first line endocrine resistance (50% in the alpelisib plus fulvestrant arm versus 36% in the placebo plus fulvestrant arm), 2nd line progression following metastatic treatment (13% in the alpelisib plus fulvestrant arm versus 26% in the placebo plus fulvestrant arm) and lung involvement (32% in the alpelisib plus fulvestrant arm versus 47% in the placebo plus fulvestrant arm).

In the plasma negative population, characteristics were generally balanced across treatment arms except where ≥ 10% differences were observed for race and lung involvement with differences equivalent to those observed in the tissue negative population.

Mutation prevalence in the PIK3CA positive populations

The PIK3CA tissue positive population consisted of 347 patients (172 *therascreen* PIK3CA RGQ PCR Kit-enrolled and 175 from retrospective retesting of CTA-enrolled patients). All 11 of the targeted PIK3CA mutations were present in the PIK3CA tissue positive population. The most prevalent variants were H1047R, E545K, E542K and H1047L.

The plasma PIK3CA positive population consisted of 186 patients. The most prevalent PIK3CA tissue variants (H1047R, E545K, E542K and H1047L) and two low frequency variants (C420R, E545G) were present in the plasma PIK3CA positive population.

Similar to the tissue PIK3CA positive patients, the most prevalent PIK3CA mutations in the plasma PIK3CA positive patients were H1074R, E545K, E542K and H1047L. In the plasma PIK3CA positive population the E542K prevalence was lower (12%) and the H1047R prevalence was higher (55%) compared to the E542K and H1047R prevalence (19% and 46%, respectively) in the PIK3CA tissue positive population.

D. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with alpelisib is not comprehensively addressed in the SSED for the therascreen PIK3CA RGQ PCR Kit. The evaluation of safety was based on the analysis of adverse events (AEs), clinical laboratory evaluations, physical examinations, and vital signs. Refer to PIQRAY® (alpelisib) label for more information.

The most common adverse reactions including laboratory abnormalities (all grades, incidence ≥ 20%) were glucose increased, creatinine increased, diarrhea, rash, lymphocyte count decreased, GGT increased, nausea, ALT increased, fatigue,

hemoglobin decreased, lipase increased, decreased appetite, stomatitis, vomiting, weight decreased, calcium decreased, glucose decreased, aPTT prolonged, and alopecia.

Serious adverse reactions occurred in 35% of patients receiving PIQRAY® plus fulvestrant. Serious adverse reactions in > 2% of patients receiving PIQRAY® plus fulvestrant included hyperglycemia (10%), rash (3.5%), diarrhea (2.8%), acute kidney injury (2.5%), abdominal pain (2.1%), and anemia (2.1%).

The median duration of exposure to alpelisib plus fulvestrant was 8.2 months with 59% of patients exposed for > 6 months. Dose reductions due to ARs occurred in 55% of patients receiving PIQRAY® plus fulvestrant. The most frequent ARs leading to dose reduction in > 2% patients receiving PIQRAY® plus fulvestrant were hyperglycemia (29%), rash (9%), diarrhea (6%), stomatitis (3.5%) and mucosal inflammation (2.1%). Among patients receiving PIQRAY® plus fulvestrant, 4.6% permanently discontinued both PIQRAY® and fulvestrant and 21% permanently discontinued PIQRAY® alone, due to ARs. The most frequent ARs leading to treatment discontinuation of PIQRAY® in > 2% patients receiving PIQRAY® plus fulvestrant were hyperglycemia (6%), rash (4.2%), diarrhea (2.8%), and fatigue (2.5%).

Safety Results - Adverse device effects in connection with the PMA clinical study

The testing for the plasma using *therascreen* PIK3CA RGQ PCR Kit was conducted retrospectively on banked study specimens and the test results were not reported to patients. No patient treatment decisions were made as a result of any of the test results; therefore, as expected there were no Unanticipated Adverse Device Effect (UADEs) related to the study products or procedures to patients or operators.

2. <u>Effectiveness Results</u>

A detailed description of the *therascreen* PIK3CA RGQ PCR Kit using tissue and the clinical study conducted to demonstrate safety and effectiveness as the companion diagnostic for PIQRAY® (alpelisib) is provided in SSED for P190001.

Efficacy analyses by PFS, based on local investigator assessment per RECIST 1.1, were performed for patients determined to be plasma PIK3CA positive using Cox regression analysis in the *therascreen* PIK3CA RGQ PCR Kit-evaluable population. Analyses were performed for the total plasma PIK3CA positive population and the subpopulations [PIK3CA tissue(+), plasma PIK3CA (+)] and [(tissue PIK3CA (-), plasma PIK3CA (+)].

The clinically relevant covariates identified from the covariates analysis were included in the stratified Cox model when estimating the hazard ratio (HR) for the [tissue PIK3CA (+), plasma PIK3CA (+)] patients. The unstratified Cox model

without adjusting for the clinically relevant covariates was used when estimating the hazard ratio for the [tissue PIK3CA (-), plasma PIK3CA (+)] patients due to the small population (N=6) and the limited number of events (N=3). Clinical efficacy in the plasma PIK3CA positive population was estimated by pooling the hazard ratios for the [tissue PIK3CA (+), plasma PIK3CA (+)] and [tissue PIK3CA (-), plasma PIK3CA (+)] patients.

Plasma PIK3CA Clinical Validation Primary Efficacy Analysis: PFS analysis in the Plasma PIK3CA Positive Ppatients

Clinical efficacy of alpelisib in combination with fulvestrant for the plasma PIK3CA positive population (N=185) was demonstrated with an estimated 46% risk reduction in disease progression or death in the alpelisib plus fulvestrant arm compared to the placebo plus fulvestrant arm (HR = 0.54, 95% CI: 0.33, 0.88) calculated through bridging study (Table 19). In comparison, the PFS HR in the PIK3CA tissue mutation positive population was 0.64 (95% CI: 0.48, 0.85) and 0.65 (95% CI: 0.50, 0.85) in the SOLAR-1 PIK3CA mutant cohort as determined by the enrolling tissue assay.

Table 19: Primary Efficacy Analysis: Progression Free Survival Analysis in the Plasma PIK3CA Positive Patients (Full Analysis Set)

Progression free survival	**HR (95% CI) PIQRAY 300mg qd + Fulv / Placebo qd + Fulv [(1)]**
plasma positive (N=185)	0.54 (0.33, 0.88)
[(1)] Hazard ratio and the 95% CI calculated using enrichment adjustment. qd = Once daily, Fulv = Fulvestrant	

Efficacy in the Plasma PIK3CA Positive Subpopulation

In the plasma PIK3CA positive subpopulation:

- The PFS HR for the 179 tissue PIK3CA positive, plasma PIK3CA positive patients was 0.53 (95% CI: 0.33, 0.84). Median PFS was 10.9 months for the alpelisib plus fulvestrant arm vs. 3.6 months for the placebo plus fulvestrant arm (Table 20, Figure 2).

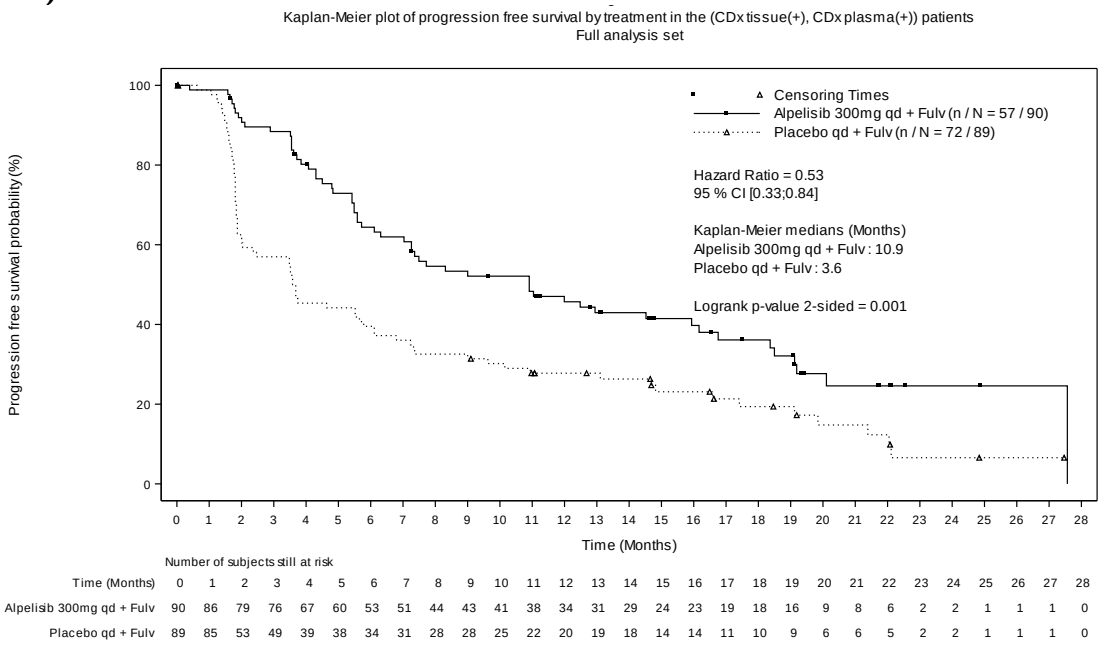
Table 20: Progression Free Survival (months) in the Tissue PIK3CA Positive, Plasma PIK3CA Positive Patients (Full Analysis Set)

Progression free survival	**PIQRAY 300mg qd + Fulv N=90**	**Placebo qd + Fulv N=89**	**HR (95% CI)[1] PIQRAY 300mg qd + Fulv/Placebo qd + Fulv**
No of events (%)	57 (63.3)	72 (80.9)	0.53 (0.33, 0.84)
Events: PD (%)	55 (61.1)	67 (75.3)	
Events: Death (%)	2 (2.2)	5 (5.6)	

No of censored (%)	33 (36.7)	17 (19.1)	
Median PFS months (95% CI)	10.9 (7.0,	3.6 (2.0, 5.8)	
p-value[2]	0.001		

[1] Cox regression model stratified by the presence of lung and/or liver metastases, and previous treatment with any CDK4/6 inhibitor.
[2] p-value obtained from 2-sided stratified logrank test.

Figure 2: Kaplan-Meier Plot of Progression Free Survival by Treatment in the Tissue PIK3CA Positive, Plasma PIK3CA Positive Patients (Full Analysis Set)



Hazard ratio obtained with stratified Cox model adjusted for clinically relevant covariates using strata defined by (i) prior CDK 4/6 inhibitor use (ii) presence of liver and/or lung metastases.

- Median PFS for the 6 tissue PIK3CA negative, plasma PIK3CA positive patients was not estimable for the PIQRAY plus fulvestrant arm due to no events. The PFS HR for the 6 tissue PIK3CA negative, plasma PIK3CA positive patients (2 patients on PIQRAY 300mg qd+Fulv and 4 patients on Placebo qd+Fulv) was 0.68 (95% CI: 0.02, 28.54).

3. Subgroup Analyses

No subgroup analyses were performed.

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 322 investigators. 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. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not Applicable.

XII. 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.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical effectiveness of the *therascreen* PIK3CA RGQ PCR assay using plasma specimens was demonstrated in Study SOLAR-1. The results are summarized below:

For the primary analyses:

- Clinical efficacy of PIQRAY in combination with fulvestrant for the plasma PIK3CA mutation positive population was demonstrated with an estimated 46% risk reduction in disease progression or death compared to placebo plus fulvestrant (HR=0.54, 95% CI: 0.33, 0.88). This compares favorably with PFS results in the PIK3CA tissue mutation positive population (HR = 0.64, 95% CI: 0.48, 0.85) and in the SOLAR-1 PIK3CA mutant cohort as determined by the enrolling tissue assays (HR = 0.65; 95% CI: 0.50, 0.85).

- The point estimates of PPA, NPA and OPA between the plasma test using the *therascreen* PIK3CA RGQ PCR Kit using plasma and tissue test using the

therascreen PIK3CA RGQ PCR Kit using tissue and the corresponding 95% confidence intervals were:

- PPA 54.6% (95% CI: 49.0%, 60.1%)
- NPA 97.2% (95% CI: 94.0%, 99.0%)
- OPA 71.5% (95% CI: 67.5%, 75.2%)

For the secondary analyses:

- The PFS hazard ratio of alpelisib in combination with fulvestrant versus placebo in combination with fulvestrant for the plasma PIK3CA mutation negative population was 0.67 (95% CI: 0.45, 0.99).

- PFS hazard ratios for alpelisib in combination with fulvestrant versus placebo in combination with fulvestrant for the subpopulations were:

  - PIK3CA plasma positive, PIK3CA tissue positive patients (N=179): HR = 0.53 (95% CI: 0.33, 0.84)
  - PIK3CA plasma positive, PIK3CA tissue negative patients (N=6): HR = 0.68 (95% CI: 0.02, 28.54)
  - PIK3CA plasma negative, PIK3CA tissue positive patients (N=149): HR = 0.63 (95% CI: 0.38, 1.05)
  - PIK3CA plasma negative, PIK3CA tissue negative patients (N=209): HR = 0.68 (95% CI: 0.41, 1.13)
  - PIK3CA plasma negative (N= 358): HR = 0.67 (95% CI: 0.45, 0.99)

In conclusion, the Plasma test using the *therascreen* PIK3CA RGQ PCR Kit device results for clinical efficacy based on PFS support the use of the *therascreen* PIK3CA RGQ PCR Kit to aid clinicians in identifying breast cancer patients with PIK3CA mutations who may be eligible for treatment with alpelisib.

B. Safety Conclusions

As a diagnostic test, the *therascreen* PIK3CA RGQ PCR Kit involves testing on plasma isolated from EDTA anti-coagulated peripheral blood collected from breast cancer patients. The probable risks of the *therascreen* PIK3CA RGQ PCR Kit are associated with the potential mismanagement of patients 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 PIK3CA test results, and consequently improper patient management decisions in brease cancer treatment. Since a patient with a negative result (including a false negative result) from the *therascreen* PIK3CA RGQ PCR Kit will be reflexed to having their PIK3CA status determined from their FFPE tissue specimen, the risks of the *therascreen* PIK3CA RGQ PCR Kit are largely associated with a false positive result in a patient, who may then undergo treatment with alpelisib with inappropriate expectation of therapeutic benefit and experience side effects.

C. Benefit-Risk Determination

The probable benefits of the use of the *therascreen* PIK3CA RGQ PCR Kit are based on data collected in the pivotal registrational trial of alpelisib (PIQRAY), CBYL719C2301 (SOLAR-1), showing improved progression-free survival in a defined population of patients with recurrent breast cancer. Clinical efficacy of PIQRAY in combination with fulvestrant for the plasma PIK3CA mutation positive population was demonstrated with an estimated 46% risk reduction in disease progression or death compared to placebo plus fulvestrant (HR=0.54, 95% CI: 0.33, 0.88). This compares favorably with PFS results in the PIK3CA tissue mutation positive population (HR = 0.64, 95% CI: 0.48, 0.85) and in the SOLAR-1 PIK3CA mutant cohort as determined by the enrolling tissue assays (HR = 0.65; 95% CI: 0.50, 0.85).

The risks of the use of the *therascreen* PIK3CA RGQ PCR Kit relate to false positive results, which may lead to subject a patient to the toxicity of alpelisib while providing only limited expected benefit; and to false negative results, which could potentially result in the patient forgoing potentially beneficial treatment with alpelisib. Due to the plasma test missing a substantial portion of tissue positive patients, reflex tissue testing should be performed for plasma negative patients, to mitigate this risk. The toxicity profile of alpelisib in this clinical setting is judged to be acceptable.

In conclusion, given the available information above, the data support that the use of the *therascreen*® PIK3CA RGQ PCR Kit in breast cancer patients with specifically defined characteristics, the probable benefits outweigh the probable risks.

Patient Perspectives

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

In conclusion, given the available information above, the data support that for the probable benefits outweigh the probable risks.

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 the Phase III SOLAR-1 clinical study support the utility of the *therascreen* PIK3CA RGQ PCR Kit as an aid in selecting patients with advanced breast cancer for whom alpelisib (PIQRAY), is indicated. Alpelisib (PIQRAY) demonstrated clinical benefit in terms of PFS that appears to be robust and of a magnitude to reasonably predict clinical benefit for alpelisib (PIQRAY) in patients identified with the *therascreen* PIK3CA RGQ PCR Kit.

XIV. CDRH DECISION

CDRH issued an approval order on May 24, 2019. Additional studies are requested as conditions of approval cited in the approval order are described below.

1. Obtain additional repeatability and reproducibility data using representative PIK3CA mutation positive and wild type specimens according to the agreed upon study proposal and the *therascreen* PIK3CA RGQ PCR Kit Instructions for Use.

2. Obtain additional *therascreen* PIK3CA RGQ PCR Kit reagent stability data with representative mutation positive specimens from each reaction tube according to the agreed upon study proposal.

3. Obtain additional QIAamp DSP Circulating Nucleic Acid Kit stability data with representative mutation positive specimens according to the agreed upon study proposal.

4. Conduct an accuracy study with mutation positive clinical plasma samples for the following mutations H1047Y, Q546R, Q546E, E545D, and E545A to demonstrate that the *therascreen* PIK3CA RGQ PCR Kit can detect these mutations accurately using intended use clinical specimens

The final study data, study conclusions, and labeling revisions should be submitted within 1 years of the PMA approval date for the items 1 and 4, and 2 years of the PMA approval date for the items 2 and 3.

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

XV. 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. Refer to the drug label for alpelisib (PIQRAY) for additional information related to use of the drug.

Post-approval Requirements and Restrictions: See approval order.