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: P190001

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 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 from breast cancer patients who may be eligible for treatment for treatment with PIQRAY® (alpelisib).

Another PMA (P190004) for the same device using plasma specimens was also submitted for the qualitative detection of same 11 mutations in 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 for the treatment with PIQRAY (alpelisib). P190004 was also approved on May 24, 2019 in conjunction with P190001 approval.

The summary of safety and effectivenеww 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 produces 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 specimen 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 FFPE tumor specimens.
:

- QIAGEN QIAamp DSP DNA FFPE Tissue Kit
- QIAGEN *therascreen* PIK3CA RGQ PCR Kit
- QIAGEN Rotor-Gene Q (RGQ) MDx Instrument with Automated data analysis and results interpretation using Rotor-Gene AssayManager® (RGAM) software version 2.1, Gamma MDx plug-in version 1.0.0, and *therascreen* PIK3CA MDx Assay Profile version 1.0.0 (therascreen_PIK3CA_FFPE_MDx)

Specimen Preparation

FFPE blocks from breast cancer patients are cut into 4-5µm sections and mounted onto glass slides. A hematoxylin and eosin (H&E) stained slide is used to confirm that there is tumor present. For extraction from resected tissue samples (RES), two non-stained tissue sections are scraped from the slide for Deoxyribonucleic Acid (DNA) extraction. For core needle biopsy (CNB) samples, between one and six sections are required for

extraction to obtain the total effective tumor area of at least 20 mm^2. If, however, 20 mm^2 is not achievable with 6 CNB sections, then extraction is performed with a maximum of 6 CNB sections. If a resected sample has a tumor content <10% in the region of interest (ROI), then the sample must be macrodissected.

DNA is manually extracted and purified using the QIAGEN QIAamp DSP DNA FFPE Tissue Kit. The FFPE sample is deparaffinized with xylene, the xylene supernatant is then removed, and any residual xylene is extracted with ethanol. The sample is lysed under denaturing conditions with proteinase K for one hour at 56°C. The sample is heated for one hour at 90°C to reverse formalin cross-linking of genomic DNA (gDNA). The sample is passed through a silica-based membrane so that the gDNA binds to the membrane and any contaminants are removed. The membrane is washed multiple times with buffers (using a centrifuge to increase flow rates through the membrane). Purified gDNA is eluted from the membrane using elution buffer (ATE) from the QIAamp DSP DNA FFPE Tissue Kit. Elution volumes vary by sample type (RES or CNB) to maintain the desired or expected DNA concentration. For RES, the gDNA samples must be eluted with 120µL of ATE, while for CNB, the gDNA samples must be eluted with 70µL of ATE.

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	1633 G>A	9	763
	E545D	1635 G>T		765
	E545G	1634 A>G		764
4	E545A	1634 A>C	9	12458
	H1047Y	3139 C>T	20	774
	Q546R	1637 A>G	9	12459
5	C420R	1258 T>C	9	757
	Q546E	1636 C>G	7	6147
6	H1047L	3140 A>T	20	776
	H1047R	3140 A>G		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
> 33.38	Quantity of amplifiable DNA is not sufficient for mutation analysis	Additional samples should be extracted and tested
< 23.23	Quantity of amplifiable DNA is too high for mutation analysis	Dilute with the sample diluent water supplied in the kit
Within 23.23 - 33.38	Quantity of amplifiable DNA is suitable for mutation analysis	No action required, sample is suitable

The PCR cycling parameters used for assessing the DNA sample 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 acceptable ranges confirms the proper functioning of each of the reaction mixes in the kit.

Instrument and Software

The *therascreen* PIK3CA RGQ PCR Kit is designed to be used with the Rotor-Gene Q MDx (hereafter referred to as 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 RGQ instrument 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_FFPE Assay Profile v1.0.0. 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_FFPE 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 non-typical 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 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. Low Ct values indicate higher input DNA levels and high Ct values indicate lower input DNA levels.

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

Run Validity Criteria

For *therascreen* PIK3CA RGQ PCR Kit test runs to be accepted as valid, the RGAM software requires run data for PC and NTC to meet criteria specified in the PIK3CA MDx Assay Profile v1.0.0 in accordance with the analysis rules of the Gamma MDx Plug-in v1.0.0. The PC and NTC validity criteria are shown below. Each test run performed with the *therascreen* PIK3CA RGQ PCR Kit must meet all the validity criteria listed below.

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

Sample	Reaction Mix	Target	RGQ Channel	Ct Range
	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 working 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 classed 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 name	Ct Cut-off values
Tube 2	E542K	4.8
Tube 3	E545K	6.5
	E545D	7.5
	E545G	9.5
Tube 4	E545A	10.0
	H1047Y	6.2
	Q546R	7.0
Tube 5	Q546E	10.0
	C420R	6.0
Tube 6	H1047R	7.0
	H1047L	10.0

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 FFPE breast tumor tissue for PIK3CA mutation status in the selection of patients who are eligible for treatment with 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 (henceforth referred to as PIK3CA Kit) were determined by studies using formalin-fixed, paraffin-embedded (FFPE) tissue specimens obtained from patients with advanced breast cancer, FFPE human cell lines, and gDNA pre-extracted from human cell lines. FFPE cell lines were sectioned and processed similar to FFPE patient specimens. Cell line DNA was extracted and tested in accordance with the instructions for surgical resections.

Mutation status of specimens was confirmed by Digital Droplet PCR (ddPCR) and/or bi-directional Sanger sequencing. The similarity between FFPE cell lines and FFPE clinical specimens was assessed for high prevalence mutations (E542K, H1047R, and E545K) by comparing the differences in mean ΔCt between the two sample types and by comparing the positivity rates and probit models for the two sample types. The results of the evaluation demonstrated that evaluation of FFPE cell lines does not lead to overestimation of assay performance.

1. Correlation with Reference Method

The accuracy of the PIK3CA Kit was demonstrated relative to a validated Next Generation Sequencing (NGS) assay using FFPE clinical specimens from the breast

cancer patients randomized in the SOLAR-1 trial from for which there was sufficient quantity and quality of specimen available for testing with the NGS comparator assay. 453 of 572 randomized patient samples had available FFPE slides for testing. Of these 453 clinical specimens, 385 met the NGS comparator sample requirements for tissue volume and tumor content, and 379 yielded a valid result for NGS. The baseline clinical and demographic characteristics of the patients whose specimens were available for this retrospective testing were in general comparable to those of otherwise eligible patients whose specimens were not available for retesting. The overall results are shown in Table 5.

Table 5: PIK3CA Kit Compared to NGS – SOLAR-1 Clinical Trial Specimens

PIK3CA Kit Call	NGS Mutation Status																			
	C420R	E542K	E545A	E545D	E545G	E545K	H1047L	H1047R	Q546E	Q546R	C420R & H1047L	C420R & H1047R	E542K & E545K	E542K & H1047R	E545A & H1047R	E545D & H1047R	E545G & H1047R	E545K & H1047R	NMD	Total
C420R	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
E542K	0	26	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	6	33
E545A	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
E545D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
E545G	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4
E545K	0	0	0	0	0	46	0	0	0	0	0	0	0	0	0	0	0	0	2	48
H1047L	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	2	13
H1047R	0	0	0	0	0	0	0	95	0	0	0	0	0	0	0	0	0	0	3	98
Q546E	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Q546R	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2
C420R & H1047L	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
C420R & H1047R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
E542K & E545K	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
E542K & H1047R	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
E545A & H1047R	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	2
E545D & H1047R	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
E545G & H1047R	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
E545K & H1047R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
NMD	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	162	164
Total	3	26	1	0	3	48	11	100	1	2	1	0	0	1	1	0	0	1	180	379

Samples with both NGS and PIK3CA Kit valid results were analyzed to assess overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA) based on the agreements between the two methods for mutation status. The percentages, together with the corresponding two-sided exact 95% CI are summarized below.

The results demonstrate point estimates of PPA, NPA and OPA of 99.0%, 90.0% and 94.7% respectively (Table 6).

Table 6: PIK3CA Kit vs. NGS Concordance in the SOLAR-1 Trial Specimens

Measure of Agreement	Percent Agreement (N)	95% CI*
PPA	99.0% (197/199)	(96.4%, 99.9%)
NPA	90.0% (162/180)	(84.7%, 94.0%)
OPA	94.7% (359/379)	(92.0%, 96.7%)

* The 95%CI calculated using the Clopper-Pearson Exact method

For the 20 overall mutation status discordant results, 2 samples with PIK3CA Kit negative results had NGS positive results, while 18 samples with PIK3CA Kit positive results gave NGS negative results. Of the 2 samples with PIK3CA Kit negative results that had NGS positive results, both were detected by the NGS at mutant allele frequencies levels below the PIK3CA Kit limit of detection. Of the18 samples, determined positive by the PIK3CA Kit and negative by NGS, eleven were low positive (within 1 ΔCt of the cut-off using the PIK3CA Kit and therefore low positive samples). One case was detected as H1047L by the PIK3CA Kit but detected as H1047I by the NGS assay. The underlying cause for the 6 remaining discordant results was not identified.

Additional agreement analyses were also conducted for each specimen type (RES vs. CNB) and for each of the mutation assays in the PIK3CA Kit except for H1047Y as this mutation was not represented in the study. For CNB samples the point estimates of OPA, PPA and NPA were 98.72% (77/78), 97.73% (43/44) and 100% (34/34), respectively. For RES the point estimates of OPA, PPA and NPA were 93.69% (282/301), 99.35% (154/155) and 87.67% (128/146), respectively.

2. **Analytical Sensitivity**

a. Analytical Sensitivity – Limit of Blank (LoB)

The LoB of the PIK3CA Kit was established by testing DNA extracted from 56 individual clinical wild type (WT) FFPE samples (30 WT RES samples and 26 WT CNB samples) with two replicates per sample for each of three PIK3CA Kit lots (generating 336 data points total). The LoB was defined as the highest measurement result that corresponds to the upper 95th percentile in the WT samples. The lowest value of the three LoB estimates (one from each *therascreen* PIK3CA Kit lot), across both RES and CNB sample types was determined to be the LoB value.

The LoB values for each of the mutation assays (in terms of ΔCt) detected by the PIK3CA Kit were verified to be above the ΔCt cut-off values determined

for each of the assays and are summarized below in Table 7 along with the false positive call rates obtained.

Table 7: LoB Values and False Positive Call Rate for all 11 Mutation Assays

Mutation	LoB (ΔCt)	False Positive Call Rate (Percent)
E542K	5.09	1.88
E545K	6.74	1.57
E545D	9.19	0.31
E545G	13.03	0.00
E545A	13.03	0.00
H1047Y	7.61	0.63
Q546R	8.72	0.00
Q546E	13.03	0.00
C420R	7.57	0.94
H1047R	9.80	1.25
H1047L	12.63	0.94

b. Analytical Sensitivity – Limit of Detection (LoD)

The PIK3CA Kit does not use a specific concentration of DNA as determined by spectrophotometry. DNA input is based on the Control Reaction Ct result which is used to indicate that there is sufficient amplifiable DNA present in the sample. The stated DNA input for the assay is defined by the Control Ct prespecified range of 23.23 to 33.38, as shown in Table 2 above.

For the PIK3CA Kit 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. The LoDs for the 11 mutation assays (C420R, E542K, E545A, E545D E545G, E545K, H1047L, H1047R, H1047Y, Q546E and Q546R) in the PIK3CA Kit were assessed using a selection of breast cancer FFPE clinical specimens or FFPE cell lines for the low prevalence mutations. The percent mutant DNA or mutant allele frequency (MAF) of each individual sample was determined previously by using Sanger sequencing for clinical samples and from information available in Cancer Cell line Encyclopedia (CCLE) for the cell lines. Five or six point MAF dilution series were made by serially diluting mutant samples in a FFPE clinical WT background. For each PIK3CA mutation, the percentage of correct calls was assessed across dilution levels using three different PIK3CA Kit lots with 24 replicates tested per kit lot per level. The LoD was determined at low DNA input (Control Ct of ~ 30.00). The hit rates for each mutant concentration, per kit lot, were obtained and a probit model was fitted to the data. A plot was produced of hit rate against log2 mutation level and the LoD for each mutation was determined as the concentration which gave a 95% estimated probability of a positive call. The final LoD value for each

mutation was determined as the highest value (in terms of MAF%) across all kit lots.

Table 8: Determined LoD for the 11 Mutations Detected by the PIK3CA Kit

Mutation Name	Mutation Prevalence	LoD (MAF%)
C420R	<1%	2.41*
E542K	11%	5.47†
E545A	1.50%	3.54*
E545D	<1%	2.69†
E545G	<1%	4.98†
E545K	20%	4.13†
H1047L	5%	2.56†
H1047R	55%	3.13†
H1047Y	<1%	14.04*
Q546E	<1%	4.50*
Q546R	<1%	6.08†

* LoD values were established using FFPE cell lines samples
† LoD values were established using breast cancer FFPE clinical specimens

c. <u>Analytical Sensitivity – Control Ct Range, RFI Validation and ΔCt Cut-offs</u>

i. Control Ct Ranges

The objective of this study was to set an appropriate Control Reaction Ct working range for use in assessing DNA sample validity. The Control Reaction Ct working range defines the amount of total amplifiable DNA in a sample and was determined using a total of 20 WT FFPE clinical samples generating 107 data points. The WT status of samples was previously assessed using an independent reference method. To determine the Control Ct working range, only the data gained from the Control Ct produced by the WT samples was used. Different confidence levels were calculated to allow the best range where 95% of the population would fit. Parametric and non-parametric tolerance intervals were calculated for Control Ct values. The Control working range was set based on the non-parametric tolerance intervals since the data was not normally distributed.

The final Control Reaction Ct working range was set at a Ct value of 23.23 to 33.38. This interval provides limits for which 95% of the population falls with a given confidence level of 97.3%. The final Control Reaction Ct working range allows mutation analysis on small amounts of input DNA such as that from CNBs.

ii. Relative Fluorescence Increase (RFI) Validation

During development data from the positive control (PC), gDNA from positive cell lines and non-template control (NTC) were used to calculate appropriate fluorescence threshold values for the Green, Yellow, and Crimson RGQ channels used in PIK3CA Kit. Assessment of signal to noise ratio (SNR) was conducted to ensure appropriate fluorescence thresholds were set for each RGQ MDx channel used in the *therascreen* PIK3CA RGQ PCR System. The fluorescence threshold selected was required to be suitable for all assays detected in any one channel.
The fluorescence threshold for the Orange channel, which is used for the detection of the IC, could not be calculated following the same methodology described for the Green, Yellow and Crimson RGQ channels. Since the IC is present in all tubes and is designed to amplify consistently within defined specifications, it was not possible to compare this positive signal to a negative sample. However, since the IC contains a standardized single concentration of synthetic DNA, the fluorescence threshold selected was not required to factor in assay performance across a range of different DNA inputs and as such the difference between signal and noise is not critical for this assay. The Orange channel threshold was selected such that its position was at the start of the exponential phase of the amplification plot. This was determined to be 0.0600 and therefore selected as the threshold for the Orange channel, which is used for the IC. The fluorescence thresholds for the Green, Yellow and Crimson channel were determined and are given as 0.0510, 0.475 and 0.625 respectively.

iii. ΔCt Cut-offs

Assay ΔCt cut-offs were established during development using clinical, WT, and mutation-positive FFPE clinical specimens, FFPE cell line FFPE and cell line gDNA. The cut-offs were determined in terms of ΔCt values and were chosen with respect to the following parameters: false positive fraction, false negative fraction and assay sensitivity. In addition to statistical analysis of ΔCt values, design requirements for false positive rates considering false negative rates were used to define an acceptable target range for cut-off values.

A study was conducted to verify the ΔCt cut-offs for each mutation (refer to Section V for the assays cut-off values) established during development using WT FFPE clinical samples, mutation positive FFPE clinical samples and cell-line FFPE samples. The ΔCt cut-off values were verified by testing three low-input (Ct 30) / low-positive samples (low MAF%). The results of the study demonstrated that each individual mutation assay had a false positive rate below 3%, while the false positive percentage for the overall PIK3CA Kit was 7.21%.

3. **Linearity – Effect of DNA input on ΔCt**

To demonstrate that the performance of the PIK3CA Kit is consistent across the DNA input range a series of nine dilutions with varying DNA input levels with the upper and lower levels being outside of the Control Reaction Ct working range (23.23 - 33.38 Ct), were evaluated with mutation positive samples covering all the 11 variants detected by the PIK3CA Kit. Three different sample types were used in this study: clinical FFPE resection samples, cell line FFPE samples, and gDNA pre-extracted from cell lines. The mutant allele frequencies were held constant while DNA input was varied; the target Ct values for the lowest and highest concentrations were 23.00 Ct and 33.50 Ct, respectively.

The evaluation was performed using one PIK3CA Kit lot with three 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 the DNA input range, there should be no change across the range in ΔCt, i.e., there is no statistically significant linear, quadratic or cubic effect. The E542K, E545D, E545G, E545A, H1047Y, Q546E, C420R and H1047R assays did not show a statistically significant ($p>0.05$) first, second or third order terms for all models tested. These assays show no change in ΔCt across the tested DNA input range.

The E545K assay was statistically significant for the first order and second order polynomial in the linear ($p=0.006$) and quadratic ($p=0.002$) models, respectively. The Q546R assay was statistically significant for the first order and third order polynomial in the linear ($p<0.001$) and cubic ($p=0.001$) models, respectively. The H1047L assay was statistically significant for the first order and third order polynomial in the linear ($p<0.001$) and cubic ($p<0.001$) models, respectively. The E545K, Q546R and H1047L assays are not linear for ΔCt across the tested DNA input range. E545K showed statistically significant values for only low DNA input samples. Q546R and H1047L showed statistically significant values for both low and high DNA input samples. An investigation determined that the non-linear effects had no effect on the performance of the E545K and H1047L assays. However, an effect on the Q546R assay performance was determined; samples at LoD may be called false negative when the DNA input is high (approximately Control Ct 23), however, the probability of this occurring is extremely low, approximately 0.0052%.

The ranges in which the assays are linear are shown below (Table 9).

Table 9: Linear range for the PIK3CA Kit

Mutation	Linear Range based on observed Mean Ct
E542K	24.42 to 33.77
E545K	24.08 to 31.02
E545D	23.02 to 34.99
E545G	22.83 to 35.71
E545A	23.18 to 34.31
H1047Y	23.24 to 34.64

Q546R	24.28 to 32.69
Q546E	23.18 to 34.65
C420R	23.26 to 34.11
H1047R	23.76 to 33.49
H1047L	25.74 to 31.61

The data was also analyzed to assess the amplification efficiency for each of the assays. Amplification efficiencies ranged from 82.15 to 100.83% for all assays.

4. **Analytical Specificity**

a. Cross Reactivity/Exclusivity

The PIK3CA Kit is comprised of 6 separate reaction mixes: one single Control Reaction that detects a region in exon 15 of the PIK3CA gene and eleven mutation assays that detect PIK3CA mutations (Table 1). There is no reaction that specifically measures the wild-type PIK3CA sequence at exons 7, 9 or 20. The PIK3CA Kit "No Mutation Detected" result is determined from the absence of any positive mutation results.

The objective of this study was to assess whether cross reactivity has been correctly accounted for in the setting of the analytical ΔCt cut-off values. The cross reactivity of the six optimized reaction mixes was assessed by testing each mutation assay against the 11 mutants detected by the PIK3CA Kit. Samples were tested at low DNA input and low MAF% and high DNA input and high MAF%. Mutation positive clinical FFPE resection samples were used for the four most prevalent mutations (E542K, E545K, H1047L and H1047R), while cell line FFPE samples were used for the less prevalent mutations (C420R, E545A, E545D, E545G, H1047Y, Q546E and Q546R). For each sample, two replicates were tested for each of three PIK3CA Kit lots (generating 240 data points total).
Within this study, there was one instance of cross reactivity with E545D and H1047R, and one instance with C420R and H1047R. There were also four instances of mutant non-specific amplification between the high MAF sample E545A and H1047L. Overall 6/240 data points showed mutant non-specific amplification. The six data points showing mutant non-specific amplification were sporadic and inconsistent with other replicates from the same sample. These results were therefore not considered to be a result of cross reactivity.

PCR cross reactivity was observed between H1047L and H1047R. This cross reactivity is uni-directional i.e., if a double H1047R and H1047L sample is seen this will be reported as H1047R Mutation Detected. This rule is incorporated into the automated therascreen_PIK3CA_FFPE Assay Profile algorithm.

b. Interference – Effect of Necrotic Tissue

To evaluate the potential interference of necrotic tissue content in breast cancer FFPE specimens on the performance of the PIK3CA Kit, FFPE clinical specimens from SOLAR-1 with both PIK3CA Kit and NGS results were analyzed. A total of 180 specimens determined PIK3CA mutant negative by NGS and 199 specimens determined PIK3CA mutant positive by NGS were evaluated. The specimens included both CNB and RES specimens. Percent necrosis, as identified by a pathologist varied from 0 to 10% for mutant negative and 0 to 20% for mutant positive samples.

For both mutant positive and mutant negative FFPE specimens, all except 20 samples have PIK3CA Kit results that matched the expected NGS results. The 20 mismatched results were from 17 mutant negative and 2 mutant positive samples with less than 5% necrotic content; and 1 mutant negative sample with less than 10% necrotic content; thus, it is unlikely that necrosis was the reason for the discordant results. The results support the use of the PIK3CA Kit with breast cancer FFPE specimen with necrotic tissue content up to 20%.

c. <u>Interference – Exogenous Substances and Hemoglobin</u>

The effect of potential interfering substances introduced from the FFPE Extraction Kit (an exogenous substance) or from the sample itself (hemoglobin) on assay performance were measured by comparison of ΔCt between interferent spiked and control spiked extracts for each mutant and comparison of the correct calls for WT DNA samples. The exogenous substances tested were (1) paraffin wax, (2) ethanol, (3) xylene, (4) extraction buffers (AW1, ATL, AW2 and AL) and (5) proteinase K. Clinical FFPE resection samples were evaluated for mutations E542K, E545K, Q546R, C420R, H1047R and for WT, while FFPE cell line samples were used to evaluate the Q546R and C420R mutations.

Samples that were spiked with exogenous interferents were first normalized to Ct 30.00 and then diluted with WT (also normalized to Ct 30.00) to give the ΔCt expected at a MAF representing 3x LoD. Samples spiked with hemoglobin (endogenous interferent) during the extraction process were not normalized to Ct 30.00 or diluted to 3x LoD prior to mutation assessment but used immediately following extraction. This was to avoid removing any variability which may have been introduced by the interferent.

The study required the preparation of a test sample set and a blank sample set (ATE for exogenous substances and water for hemoglobin). The test sample set included all mutant and WT samples spiked with an interferent. The blank sample set included mutant and WT samples spiked with an appropriate control substance. Samples tested with hemoglobin were spiked during the extraction process to reflect what would be introduced via the FFPE sample. The test concentration of hemoglobin and the estimated tissue volume used in the extraction process were based on CLSI guidelines (CLSI

EP7-A2, Appendix D, 2005, Interference Testing in Clinical Chemistry; Approved Guideline). The recommended testing concentration of hemoglobin given in EP07-A, Appendix D, 2005 is 2mg/ml. Samples tested with potential exogenous interferents were spiked following normalization to Ct 30.00 and dilution to 3x LoD at a concentration representing the highest (worst-case) feasible level of the interfering substance carry over into a sample (10x concentration). In total, six replicates of each sample/interferent combination were tested with one PIK3CA Kit lot. All mutation calls in both mutant and WT samples were as expected. Where a significant difference was observed between the spiked and control samples, this was within acceptable intermediate precision of the assay and was, therefore within the inherent variability of the assay. In conclusion, none of the interferents tested had any impact on kit performance.

5. **Repeatability and Reproducibility**

The repeatability and reproducibility of the PIK3CA Kit was investigated by testing DNA extracted from breast cancer FFPE clinical specimens for mutations E542K, E545G, E545K, H1047L, H1047R and Q546R, and Cell Line FFPE samples for mutations C420R, E545A, E545D, H1047Y, Q546E and Q546R. A WT FFPE clinical specimen was also included in the study. To demonstrate repeatability, samples at two mutation levels (LoD and 3x LoD) were tested in duplicate with two (2) runs per day, by three (3) operators across 20 non-consecutive days resulting in 120 data points at one site (located in the United Kingdom) except for samples at LoD with E545A and Q546R PIK3CA mutations. Samples with E545A and Q546R mutations at LoD were evaluated for six (6) days at one site by three (3) operators, with two (2) runs and four (4) replicates for a total of 144 measurements to demonstrate repeatability. For reproducibility two (2) runs a day were performed per operator (3 operators per site) by two additional sites (both located in the USA) over 10 days to give additional 60 data points for each additional site except for samples with at LoD with E545A and Q546R PIK3CA mutations. Samples at LoD with E545A and Q546R PIK3CA mutations were evaluated for six (6) days for two (2) more sites, by three (3) operators, with two (2) runs and four (4) replicates for a total of 144 measurements per site, 432 in total across three (3) sites. At each site, samples were tested using two (2) PIK3CA Kit lots (3 lots across 3 sites). One to two lots of QIAamp® DSP DNA FFPE Tissue Kit were used to extract DNA from FFPE samples. Samples were prepared to have low DNA input levels where a control Ct value of approximately 30 was targeted.

Mutation positive samples were only run with the Control Reaction mix and the relevant reaction mix specific to the tube of the mutation of interest. WT samples were run with all reaction mixes per the PIK3CA Kit Instructions For Use (IFU).

A new repeatability and reproducibility study will be conducted by running samples with all the 11 PIK3CA mutations using 6 reaction tubes in accordance with the PIK3CA Kit IFU; refer to section XIII.

For each sample, the proportion of correct mutation calls along with the corresponding two-sided exact 95% confidence limit are reported in Table 10 below, for repeatability.

Table 10: Assay Repeatability – Proportion of Correct Calls for PIK3CA Mutation Tested

Mutation	Template	Fractional Proportion of Valid Results	Percentage	Two-Sided Lower 95% Confidence Limit
C420R	3x LoD	120 / 120	100.00%	96.97%
	LoD	120 / 120	100.00%	96.97%
E542K	3x LoD	120 / 120	100.00%	96.97%
	LoD	119 / 119	100.00%	96.95%
E545A	3x LoD	120 / 120	100.00%	96.97%
	LoD*	144 / 144	100.00%	97.47%
E545D	3x LoD	120 / 120	100.00%	96.97%
	LoD	120 / 120	100.00%	96.97%
E545G	3x LoD	120 / 120	100.00%	96.97%
	LoD	120 / 120	100.00%	96.97%
E545K	3x LoD	120 / 120	100.00%	96.97%
	LoD	118 / 120	98.33%	94.11%
H1047L	3x LoD	120 / 120	100.00%	96.97%
	LoD	117 / 120	97.50%	92.87%
H1047R	3x LoD	120 / 120	100.00%	96.97%
	LoD	120 / 120	100.00%	96.97%
H1047Y	3x LoD	120 / 120	100.00%	96.97%
	LoD	117 / 120	97.50%	92.87%
Q546E	3x LoD	120 / 120	100.00%	96.97%
	LoD	120 / 120	100.00%	96.97%
Q546R	3x LoD	119 / 119	100.00%	96.95%
	LoD*	139 / 140	99.29%	96.08%
WT	Ct 30	108 / 120	90.00%	83.18%

*Samples at LoD with E545A and Q546R PIK3CA mutations were evaluated for 6 days at one site by 3 operators, with 2 runs and 4 replicates for a total of 144 measurements.

For each sample, the proportion of correct mutation calls along with the corresponding two-sided exact 95% confidence limit are reported in the Table 11 below for reproducibility.

Table 11: Assay Reproducibility – Proportion of Correct Calls for PIK3CA Mutation Tested

Mutation	Template	Fractional Proportion of Valid Results	Percentage	Two-Sided Lower 95% Confidence Limit
C420R	3x LoD	240 / 240	100.00%	98.47%
	LoD	240 / 240	100.00%	98.47%
E542K	3x LoD	240 / 240	100.00%	98.47%

	LoD	237 / 239	99.16%	97.01%
E545A	3x LoD	240 / 240	100.00%	98.47%
	LoD*	431 / 432	99.77%	98.72%
E545D	3x LoD	240 / 240	100.00%	98.47%
	LoD	238 / 240	99.17%	97.02%
E545G	3x LoD	240 / 240	100.00%	98.47%
	LoD	240 / 240	100.00%	98.47%
E545K	3x LoD	240 / 240	100.00%	98.47%
	LoD	238 / 240	99.17%	97.02%
H1047L	3x LoD	240 / 240	100.00%	98.47%
	LoD	230 / 240	95.83%	92.47%
H1047R	3x LoD	240 / 240	100.00%	98.47%
	LoD	240 / 240	100.00%	98.47%
H1047Y	3x LoD	240 / 240	100.00%	98.47%
	LoD	234 / 240	97.50%	94.64%
Q546E	3x LoD	240 / 240	100.00%	98.47%
	LoD	240 / 240	100.00%	98.47%
Q546R	3x LoD	239 / 239	100.00%	98.47%
	LoD*	421 / 424	99.29%	97.95%
WT	Ct 30	222 / 240	92.50%	88.41%

*Samples at LoD with E545A and Q546R PIK3CA mutations were evaluated for 6 days across 3 sites, by 3 operators, with 2 runs and 4 replicates for a total of 144 measurements per site, 432 total.

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 XIII.

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 repeatability study. These estimates were reported along with the number of observations for the mean of ΔCt, Control Ct, and Mutant Ct values. Results by variance components and total variance are presented below for ΔCt. The column "N" included the number of data points that generated a ΔCt value. Across all variance components, the total standard deviation (SD) was ≤1.14 in all PIK3CA mutations tested. Across all mutant panel members, the SD was in general ≤0.52 for between-operators, between lots, between days, and between runs.

Table 12: Repeatability - Overall Mean, Standard Deviation (SD) for ΔCt – Between Lot, Between-run, Between-Operator, Between-Day, Between-Instrument and Total Variance

Mutation	LoD Level Tested	N	Mean	Between Kit Lot (SD)	Between Run (SD)	Between Operator (SD)	Between Day (SD)	Between Instrument (SD)	Within Run (SD)	Total (SD)
E542K	**3x LoD**	**120**	**1.95**	0.00	0.16	0.00	0.10	0.14	0.24	0.33
	LoD	**119**	**3.59**	0.00	0.21	0.00	0.09	0.11	0.35	0.43
E545K	**3x LoD**	**120**	**2.68**	0.02	0.14	0.08	0.10	0.08	0.30	0.36
	LoD	**120**	**4.41**	0.09	0.22	0.24	0.00	0.16	0.51	0.60
E545A	**3x LoD**	**120**	**1.32**	0.00	0.00	0.00	0.00	0.12	0.53	0.54
	LoD*	**144**	**2.79**	0.00	0.00	0.14	0.09	0.13	0.92	0.93
Q546E	**3x LoD**	**120**	**2.38**	0.08	0.00	0.00	0.14	0.00	0.39	0.41
	LoD	**120**	**4.71**	0.00	0.00	0.07	0.00	0.21	0.70	0.72
E545D	**3x LoD**	**120**	**2.83**	0.00	0.05	0.10	0.00	0.00	0.34	0.35
	LoD	**120**	**4.45**	0.02	0.00	0.05	0.15	0.07	0.58	0.60
H1047Y	**3x LoD**	**120**	**3.47**	0.05	0.14	0.06	0.00	0.00	0.33	0.37
	LoD	**120**	**4.94**	0.11	0.20	0.00	0.00	0.17	0.51	0.58
C420R	**3x LoD**	**120**	**0.96**	0.00	0.29	0.00	0.08	0.06	0.30	0.43
	LoD	**120**	**2.57**	0.00	0.11	0.22	0.14	0.15	0.61	0.67
H1047R	**3x LoD**	**120**	**2.34**	0.00	0.21	0.07	0.03	0.19	0.28	0.39
	LoD	**120**	**4.4**	0.00	0.00	0.08	0.00	0.19	0.56	0.58
E545G	**3x LoD**	**120**	**1.92**	0.08	0.15	0.00	0.00	0.00	0.26	0.31
	LoD	**120**	**5.1**	0.00	0.29	0.00	0.00	0.00	0.83	0.88
Q546R	**3x LoD**	**119**	**1.67**	0.14	0.18	0.00	0.00	0.19	0.41	0.48
	LoD*	**140**	**3.99**	0.13	0.00	0.00	0.11	0.30	0.78	0.83
H1047L	**3x LoD**	**120**	**4.87**	0.00	0.26	0.14	0.00	0.11	0.43	0.52
	LoD	**119**	**6.96**	0.12	0.00	0.00	0.52	0.13	1.04	1.14

*Samples at LoD with E545A and Q546R PIK3CA mutations were evaluated for 6 days at one site by 3 operators, with 2 runs and 4 replicates for a total of 144 measurements.

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. These estimates were reported along with the number of observations for the mean of ΔCt, Control Ct, and Mutant Ct values. Results by variance components and total variance are presented below for ΔCt. The column "N" included the number of data points that generated a ΔCt value. Across all mutant panel members, the SD was in general ≤0.36 for within site, between-lots, between instrument, between days, and between operators.

Table 13: Reproducibility - Overall Mean, Standard Deviation (SD) for ΔCt –Between-run, Within – Site, Between Site, Between Lot, Between-Operator, Between-Day, Between-Instrument and Total Variance

Mutation	LoD Level Tested	N	Mean	Between Site (SD)	Between Run, Within Site (SD)	Between Operator (SD)	Between Instrument (SD)	Between Day (SD)	Between Lot (SD)	Within Run (SD)	Total (SD)
E542K	**3x LoD**	**240**	**1.85**	0.03	0.00	0.02	0.23	0.06	0.07	0.34	0.40
E542K	**LoD**	**239**	**3.49**	0.00	0.20	0.00	0.30	0.00	0.00	0.43	0.54
E545K	**3x LoD**	**240**	**2.73**	0.00	0.14	0.05	0.08	0.09	0.03	0.29	0.34
E545K	**LoD**	**240**	**4.43**	0.00	0.23	0.13	0.18	0.00	0.05	0.47	0.56
E545A	**3x LoD**	**240**	**1.27**	0.00	0.10	0.06	0.08	0.00	0.00	0.53	0.55
E545A	**LoD**	**432**	**2.85**	0.00	0.11	0.00	0.17	0.08	0.08	1.06	1.09
Q546E	**3x LoD**	**240**	**2.41**	0.00	0.12	0.00	0.00	0.06	0.11	0.41	0.43
Q546E	**LoD**	**240**	**4.78**	0.00	0.00	0.04	0.15	0.10	0.00	0.74	0.77
E545D	**3x LoD**	**240**	**2.86**	0.05	0.00	0.09	0.09	0.09	0.00	0.35	0.37
E545D	**LoD**	**239**	**4.55**	0.10	0.27	0.00	0.15	0.17	0.06	0.61	0.71
H1047Y	**3x LoD**	**240**	**3.45**	0.00	0.14	0.00	0.09	0.00	0.02	0.39	0.42
H1047Y	**LoD**	**240**	**4.93**	0.00	0.12	0.00	0.18	0.00	0.05	0.54	0.58
H1047R	**3x LoD**	**240**	**2.38**	0.00	0.14	0.04	0.11	0.04	0.00	0.32	0.37
H1047R	**LoD**	**240**	**4.53**	0.11	0.09	0.00	0.15	0.00	0.00	0.60	0.63
C420R	**3x LoD**	**240**	**1.01**	0.00	0.23	0.00	0.11	0.00	0.16	0.32	0.42
C420R	**LoD**	**240**	**2.71**	0.07	0.22	0.07	0.17	0.00	0.12	0.56	0.64
E545G	**3x LoD**	**240**	**1.89**	0.00	0.16	0.02	0.05	0.00	0.07	0.28	0.34
E545G	**LoD**	**240**	**5.04**	0.00	0.26	0.00	0.00	0.00	0.00	0.80	0.84
Q546R	**3x LoD**	**239**	**1.57**	0.14	0.12	0.04	0.12	0.00	0.11	0.44	0.49
Q546R	**LoD**	**423**	**3.97**	0.00	0.17	0.00	0.34	0.09	0.08	0.77	0.86
H1047L	**3x LoD**	**240**	**5**	0.22	0.36	0.17	0.07	0.02	0.09	0.46	0.63
H1047L	**LoD**	**239**	**7.15**	0.17	0.22	0.26	0.22	0.15	0.17	1.24	1.32

*Samples at LoD with E545A and Q546R PIK3CA mutations were evaluated for 6 days across 3 sites, by 3 operators, with 2 runs and 4 replicates for a total of 144 measurements per site, 432 total.

6. **<u>Lot-to-Lot Interchangeability</u>**

The objective of this study was to demonstrate lot-to-lot interchangeability and to demonstrate consistency of the mutation status across the QIAamp DSP DNA FFPE Tissue Kit and the *therascreen* PIK3CA RGQ PCR Kit. The study utilized three lots of the FFPE Extraction Kit and three lots of the *therascreen* PIK3CA RGQ PCR Kit

to test mutation positive breast cancer FFPE clinical specimens and cell line FFPE samples representing the 11 mutations assays along with WT FFPE clinical samples. A total of 22 samples were used: 13 mutation positive clinical breast FFPE samples, three WT clinical breast FFPE specimens, and six FFPE cell line samples. Samples were used without any modification or dilution. Each sample was extracted with three lots of QIAamp FFPE Extraction Kits. Two replicate extractions were carried out per QIAamp FFPE Extraction Kit, to give 6 extractions per sample. All extracts were tested with three different lots of the *therascreen* PIK3CA RGQ PCR Kit for a total of 396 data points, including 342 mutation positive and 54 WT data points, reported across all 9 PIK3CA Kit and extraction kit combinations. Further a total of 44 data points, including 38 mutation positive and 6 WT data points, would be reported per kit lot and extraction lot combination. Mutant samples were tested with the Control assay and the mutation assay of interest. WT samples were tested with the full *therascreen* PIK3CA RGQ PCR Kit.

The percentage of correct overall mutation call and specific mutation calls were reported across all samples and kit lots, along with the corresponding two-sided exact 95% confidence limits. The percentage of correct overall mutation call was also reported for each PCR and extraction kit combination separately, along with the corresponding two-sided exact 95% confidence limits.

Of the nine kit combinations, eight were found to give mutation positive results (E542K and E545K) for WT sample 1. Based on these findings, investigations were performed to determine the sample status of WT Sample 1. It was found that WT Sample 1 had been misclassified as a WT sample. An assessment of the data determined that removal of this sample from the dataset did not affect the capability of the study to determine Lot Interchangeability.

In addition to the removal of the WT sample 1, C420R extract 2 was removed due to sample invalidity. The number of data points was therefore reduced. A total of 375 data points, including 339 mutation positive and 36 WT data points, were reported across all 9 PCR kits and extraction kit combinations. A total of 41 data points per PCR kit lot and extraction kit lot 1 combination was reported including 37 mutation positive samples and 4 WT data points. A total of 42 data points per PCR kit lot and both extraction kit lot 2 and extraction kit lot 3 combinations was reported including 38 mutation positive samples and 4 WT data points.

The purpose of the lot interchangeability study was to determine that extraction and PCR kits are interchangeable. With the removal of WT Sample 1, the data from 2 WT samples was used during the statistical analysis.

The proportion of correct overall mutation calls was 96.80% for each *therascreen* PIK3CA RGQ PCR Kit lot and Extraction Kit lot combination. For extraction kit lot 3 and all PCR kit combinations, the proportion of correct overall mutation call was 90.48% and for extraction kit lot 1 and 2 and all PCR kit combinations, the proportion of correct overall mutation calls was 100.00%.

In conclusion, this study showed that lot-to-lot interchangeability has no impact on assay performance. It also showed that combining different lots of the PIK3CA RGQ PCR Kit and FFPE Extraction Kit does not have an effect on the ability to determine a correct mutation call.

7. **Specimen Handling – Reproducibility**

The objective of this study was to assess sample handling variability, specifically within the DNA extraction as part of the PIK3CA Kit test system process at three different sites. Four mutation positive clinical FFPE breast cancer specimens and six mutation positive cell line FFPE samples representing all 11 mutation assays along with one WT clinical FFPE breast cancer specimen were used in this study. Twelve (12) sections of 11 samples (10 mutant and one WT) were distributed evenly across three different sites. Six extractions per sample (two sections per extraction per sample) were performed at each of the three different sites one located in the United Kingdom, and two in the USA. Testing of the extracts using the PIK3CA Kit was performed at the site in the United Kingdom. Extractions were performed at all three sites using one kit lot of QIAamp DSP DNA FFPE Tissue Kit. There were 48 FFPE sections required for each sample; these FFPE sections were randomized and split into 24 extract sets. These extract sets were then distributed evenly across the three testing sites, six extracts per study site. The remaining six extracts were retained as a contingency set.

Each mutation assessment run tested all six extractions of one sample from each site with the full PIK3CA Kit, that is, with testing with all 6 reaction mixes included in the kit. In total 198 replicates across three sites (that is, 6 replicates at each of the 3 sites for each of the 10 mutation positive samples and 1 WT sample) were planned for evaluation. Sixty-six (66) replicates within sites (that is, 6 replicates at each site for each of the 10 mutation positive samples and 1 WT sample) were planned for testing.

In the final dataset, one run (contained 6 data points) was removed as it was invalid, due to a PC out of specification. This run was not repeated as sufficient data points to conduct the primary analysis were collected from all other associated runs. Due to this, the study has 192 data points instead of 198. There is no impact on the study design by this reduction in sample size. The original power calculations are not impacted, and the power remains >99% over all extractions. When comparing the results of the samples across all three sites, the percentage of correct mutation calls for mutation positive and WT samples was 100.00%. Across specific PIK3CA mutation calls, the proportion of correct calls was 97.92%.

8. **Guardbanding**

The objective of the guard banding studies was to establish the robustness of the PIK3CA Kit. The following studies were conducted to: (1) assess the tolerance of the PIK3CA Kit to temperature variations in the annealing step during PCR that could be introduced by the RGQ instrument, (2) determine the effect of varying

reagent volume on the mutation status of samples called by the PIK3CA Kit, (3) determine the effect of varying reagent mixing (4) determine the effect of varying the thaw time of the PIK3CA Kit reagents and samples and (5) determine the effect of varying set-up time on the mutation status called by the therascreen PIK3CA RGQ PCR Kit. For all guardband studies, a WT sample and a representative mutation in each of the reaction mixes within the kit was assessed at 3xLoD and low DNA input.

a. Cycling Guardband

The study was designed to determine the tolerance of the PIK3CA Kit to temperature variations of the annealing step during PCR that could be introduced by the RGQ instrument.

The standard cycling conditions for the *therascreen* PIK3CA RGQ PCR Kit are denaturation at 95°C for 30 seconds and annealing at 60°C for 60 seconds. Annealing temperature was tested across a seven-point range 60°C ±2°C. The 59°C to 61°C temperature range was selected to represent temperatures within the RGQ dynamic temperature specification, two conditions, 58°C and 62°C were selected to represent temperatures outside the RGQ specification range. Mutant samples were tested with 4 replicates per run over three runs generating a total of 12 replicates per temperature condition. WT samples were tested with 2 replicates per run. A total of 10 replicates were generated per temperature condition.

When the PCR annealing temperature was varied by ±2°C, correct calls across all tested mutation assays, for each temperature condition, within the dynamic temperature range was 100.00%.

b. Volumetric Guardbanding

The objective of this study was to determine the effect of varying reagent volume on the mutation status of samples called by the PIK3CA Kit. Six samples, a representation of each of the PIK3CA Kit tubes (five mutants and one WT) were tested.

The standard volumes as stated in the instructions for use are 19.83µL RM, 0.17µL Taq and 5.00µL of sample. The volumetric tolerance was tested by varying the volume of each individual component while keeping the volume of the other components constant. Each component volume was varied by ±6% as shown below (Table 14).

Table 14: Volumetric conditions to be tested

Condition	Reaction Mix Volume per reaction (µL)	*Taq* Volume per reaction (µL)	Total Mastermix Volume per reaction (µL)	Sample Volume per reaction (µL)	Total Volume per reaction (µL)
1 (Standard)	19.83	0.17	20.00	5	25

Condition	Reaction Mix Volume per reaction (µL)	*Taq* Volume per reaction (µL)	Total Mastermix Volume per reaction (µL)	Sample Volume per reaction (µL)	Total Volume per reaction (µL)
2	18.64	0.17	19.81	5	23.81
3	21.02	0.17	21.19	5	26.19
4	19.83	0.16	19.99	5	24.99
5	19.83	0.18	20.01	5	25.01
6	19.83	0.17	20.00	4.7	24.70
7	19.83	0.17	20.00	5.3	25.3

This represents the total error that can be introduced by pipetting calculated by relative accuracy and precision stated in the pipette specifications.

Mutant samples were tested with a total of 12 replicates were generated per volume condition. WT samples were tested with one replicate per run. A total of 10 replicates were generated per volume condition. The PC and NTC were also be tested as a sample with the seven volume conditions. A total of 4 replicates were generated per volume condition.

Four assays (E542K, Q546E, E545A and H1047L) a 100.00% correct mutation call was observed for these assays, across all conditions tested. For the E545K assay, 11 out of 12 mutation calls were correct. One mutation call for condition 6 gave a double positive mutation status. E545K was detected with a ΔCt value of 2.60 and E545D was reported with a ΔCt 7.05. The impact of volumetric variation on assay performance was low as the change in ΔCt was small (-0.480ΔCt) and no false negative mutation calls were made within the conditions tested.

c. Guardband Mixing

To determine the effect of varying reagent mixing, conditions that could be potentially introduced by the user, e.g. no mixing and vortexing, were evaluated against the standard, inversion 10 times. Six samples, a representation of each of the PIK3CA Kit tubes (five mutants and one WT) were tested.

The standard mixing conditions as stated in the instructions for use is to mix all reagents by inverting each tube 10 times. The following mixing conditions (as could be introduced by the user) were tested for, once a master mix containing both the reaction mix (Tubes 1 to 6) and the Taq DNA polymerase was made:

- Inversion 10 times (standard condition per the instructions for use (IFU))
- Vortex

- No mixing

Intermittent drop outs of mutation positive samples for the no mixing condition were observed for the E542K and E545K assays. The difference in mean ΔCt values for no mixing-inversion for the Q546R assay was 1.578. No difference was observed for all other assays tested.

In conclusion, this study indicated that there is an impact on assay performance if mixing is not performed correctly. The Instructions For Use states that the required mixing method is inversion 10 times. However, this study showed that mixing by vortex is also a suitable method.

d. Thawing guardband

The objective of this study was to determine the effect of varying the thaw time of the *therascreen* PIK3CA RGQ PCR Kit reagents and samples. Six samples, a representation of each of the PIK3CA Kit tubes (five mutants and one WT) were tested.

The tolerance of reaction mix, Taq and sample was tested against two thawing conditions, thaw time between ≥1 and ≤4.5 hours.

Both thawing conditions were tested on each run. Mutant samples were tested with six replicates per run over two runs and a total of 12 replicates were generated per condition. WT samples were tested with three replicates per run over four runs. A total of 12 replicates were generated per condition. Both PC and NTC were tested with one replicate per run for each condition on mutant runs and one replicate per run for condition 1 on WT runs.

The thawing of kits and templates was staggered, with condition 1 reaction mix removed for thawing 3.5 hours after condition 2 and 1 hour prior to set-up. The time of removal from the freezer and set-up start time was recorded in the worksheets for each condition. During set-up, master mix was prepared in duplicate and in bulk for some runs.

For each mutation sample, the differences in mean ΔCt, between the instructions for use stated thaw time of 1 hour, and the guardband tested thaw time of 4.5 hours were within acceptable limits. The objective of the study was met; the impact of thaw time between 1 - 4.5 hours on assay performance was determined to be low as the change in ΔCt was small (i.e. within the pre-specified limits), and no incorrect mutation calls were made for either mutant or WT samples.

e. Set-up guardband

The objective of this study was to determine the effect of varying set-up time on the mutation status called by the PIK3CA Kit. Six samples, a representation of each of the PIK3CA Kit tubes (five mutants and one WT) were tested.

Due to complicated run layouts for some studies it can take up to 1.5 hours to set-up. The Instructions For Use state a run should be set-up and ran on the RGQ immediately, the standard set-up condition was Condition 1. The following conditions, which could be introduced by the user, were tested in this study:

1. Set-up and immediate RGQ run (Standard Condition)
2. Set-up (up to 1.5 hours at room temperature then RGQ run)
3. Set-up (up to 4.5 hours at room temperature then RGQ run)
4. Set-up (up to 7.5 hours at room temperature then RGQ run)
5. Set-up (up to 1.5 hours at room temperature), store for 16h at 2-8°C prior to performing RGQ run

This study involved the setting up of 20 runs with 4 runs per condition assessed, to determine the robustness of the assay to environmental stresses during set-up. Twelve replicates were tested per condition. The five conditions that were assessed were a standard run preparation and run immediately on an RGQ, followed by four non- standard conditions where the runs were set-up at room temperature for a prolonged time frame with one condition, involving an overnight incubation step at 2 - 8°C. The set-up of each run consisted of noting down the time the set-up began and the end time being when the run file was created on the RGQ.

To establish the tolerance of the *therascreen* PIK3CA RGQ PCR Kit to set-up variations, the impact of set-up times on ΔCt and mutation call was determined. The data obtained shows that varying the set-up time has no effect on the mutation call of the kit except for H1047L for Condition 5. For H1047L the data shows that for Condition 5 has an impact on the ΔCt for H1047L. To mitigate the risk of a false mutation call, the instructions for use state that runs must not be left overnight. The data obtained shows that varying the set-up and storage time up to 7.5 hours at room temperature followed by an RGQ run, has no effect on the performance of the *therascreen* PIK3CA RGQ PCR Kit. The recommended maximum set-up time is 8.5 hours at room temperature; this includes 1-hour thaw time and 7.5 hours for set-up and storage. The recommended maximum set-up time is 8.5 hours at room temperature; this includes 1-hour thaw time and 7.5 hours for set-up and storage

9. **Cross-contamination**

The objective of this study was to demonstrate the absence of PCR cross contamination of the WT samples by mutant samples within the DNA extraction and

run set-up procedure. The study focused on the DNA extraction of FFPE samples utilizing one kit lot of the FFPE Extraction Kit to identify any cross contamination associated with routine use of the PIK3CA Kit. Contamination could potentially occur at any stage of the testing procedure. This study was designed to investigate the probability of cross contamination during the whole testing procedure (DNA extraction and subsequent PIK3CA Kit).

This study was performed with H1047R (the mutation with highest prevalence) and WT FFPE cell line samples. Two independent sets of samples referred to as "Set A" and "Set B" were extracted following a pre-defined extraction matrix.

Two operators performed the extractions. Eighteen extractions (9 per set) were carried out for the mutation positive (H1047R) samples. 42 extractions (21 per set) were carried out for the WT samples. The extracts were mutation assessed across ten PCR runs; five per sample set were set-up consecutively by the same operator using the same equipment and RGQ instrument, with no other runs set-up using this instrument between these runs. Extracts were tested with the Control assay reaction mix (PIK3CA Kit Tube 1) and mutation of interest (PIK3CA Kit Tube 6).

Per the study design, ten mutation assessment runs were planned and initially carried out. Sufficient data points were collected from the 9 valid runs and they were submitted for statistical analysis. One sample was invalid due to no IC Ct in tube 6 reaction mix, but the sample was not repeat tested as sufficient data points from the associated extraction run had been collected for statistical analysis.

After removal of invalid data points, 189 WT replicates were available for statistical analysis. The observed percentage of correct mutation calls for WT samples was 100%, demonstrating no cross contamination of the WT samples by mutant samples sharing the same DNA extraction and run set up procedure.

10. **Stability Studies**

a. Stability of FFPE Clinical Specimens and Extracted DNA from FFPE Specimens

The objective of this study was to demonstrate the long-term stability of samples stored in different conditions. The stability of specimens was assessed for: (1) FFPE section stability (paraffin dipped and undipped) stored at room temperature or 4ºC and (2) Extracted FFPE gDNA sample stability stored at -80ºC.

FFPE sections from 40 FFPE breast cancer tissue specimens, consisting of WT and mutant samples, were used for the cut FFPE tissue sections long term stability study. The FFPE sections were subjected to different storage conditions and time points, 10 specimens for each of the storage condition. Ten (10) breast cancer FFPE tissue specimens consisting of WT and mutant blocks were used for DNA extraction for the FFPE DNA samples long-term stability study.

The long-term stability for the cut FFPE tissue sections and long-term DNA storage stability study evaluated 5 time-points for sampling, including the baseline (T0), 6th month, 12th month, 24th month, and 33th month.

The FFPE sections were subjected to 4 testing conditions consisting of the dipped sections and undipped at room temperature (RT) and 4 °C. Forty (40) FFPE tissue specimens, consisting of WT and mutant blocks, were used for the study, 10 blocks for each testing condition.

The sections were grouped in 4 sets, 2 sets of the sections (dipped and undipped) assigned to the room temperature and 2 sets of the sections (dipped and undipped) assigned to 4 °C for long-term storage. A set of freshly cut sections from each block were used for DNA extraction immediately post-sectioning and testing results served as the baseline (T0) results.

The 4 sets of sections were subjected to 6 time-points for the cut FFPE tissue sections long term stability study. At each time point, the sections were sampled for DNA extraction and tested, and testing results were compared to the baseline (T0) results for determination of the section's stability at that time point and condition.

The long-term DNA storage stability study evaluated the same 5 time points for sampling as the cut FFPE tissue section long term stability. Ten (10) of breast cancer FFPE tissue specimens, including WT and mutant blocks, were used for this study. DNA samples from the same blocks were pooled to ensure enough testing material and prevent tumor heterogeneity affecting testing results. Aliquots of DNA samples from each specimen were subjected to different time points and stored at -80 °C. At each time point, the DNA aliquots were used for DNA stability testing using the PIK3CA PCR Mutation Assays.

Testing time points at baseline, 6, 12, 24 and 33 months for the long-term stability study for FFPE cut sections and FFPE gDNA was performed to assess PIK3CA mutation status.

Using the results obtained at T0 as reference, the point estimate of the percent agreement on mutation status along with its associated two-sided 95% Wilson Score confidence interval was calculated at each time point tested under each of the four testing conditions (RT Dipped, RT Undipped, 4 °C Dipped and 4 °C Undipped). For each of these four testing conditions at all time-points achieved 100% agreement (10/10) with an associated two-sided 95% Wilson Score confidence interval of (72.2%, 100.0%). Similarly using the results obtained at T0 as reference, the point estimate of the percent agreement on mutation status along with its associated two-sided 95% Wilson Score confidence interval was calculated at the 6th, 12th, 24th and 33rd month time-points. At each time-point evaluating FFPE gDNA stability 100% agreement (10/10) was achieved with T0 with an associated two-sided 95% Wilson Score confidence interval of (72.2%, 100.0%).

The study demonstrated that cut FFPE tissue sections are stable after 33 months at room temperature and 4 °C in dipped and undipped storage conditions; and that gDNA derived from cut FFPE tissue sections are stable after 33 months stored at -80 °C.

Stability of the DNA extracted from FFPE clinical specimens was demonstrated in the P110027 approval of the *therascreen*® KRAS RGQ PCR Kit.

b. Kit Stability

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 study (integrated in Real-Time and In-Use stability study)

For all Real-Time and In-Use stability study test time points the same batch of extracted tissue DNA clinical samples (H1047R mutant positive samples at 2xLoD and WT) as well as Cell Line mutant samples at 3xLoD level were used. 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 XIII.

B. <u>Animal Studies</u>

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, under Investigational Device Exemption (IDE) G160122. The Phase III study for alpelisib (also known as CBYL719C2301 or SOLAR-1), and the bridging study between the Clinical Trial Assay (CTA) and the *therascreen* PIK3CA RGQ PCR Kit were the clinical basis of the PMA approval decision.

SOLAR-1 (NCT02437318) was a randomized, double-blind, placebo-controlled Phase III trial of PIQRAY plus fulvestrant versus placebo plus fulvestrant in patients with HR-positive, HER2-negative, advanced or metastatic breast cancer whose disease had progressed or recurred on or after an aromatase inhibitor (AI)-based treatment (with or without CDK4/6 combination). The trial primary objective was to determine whether treatment with alpelisib in combination with fulvestrant prolongs progression free survival (PFS) compared to treatment with placebo in combination with fulvestrant based on local radiological assessment using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 for patients with PIK3CA mutant status. Efficacy in the PIK3CA non-mutant cohort was assessed as a proof of concept (PoC) in a secondary objective.

SOLAR-1 initiated in July 2015 with PIK3CA mutation testing conducted with the PIK3CA PCR Mutation Clinical Trial Assay (CTA) using FFPE tumor biopsy specimens prior to patient enrollment. SOLAR-1 PIK3CA mutation screening transitioned to the QIAGEN *therascreen* PIK3CA RGQ PCR Kit on September 28, 2016. Prior to implementation of the RGAM and Tissue Assay Profile software, the *therascreen* PIK3CA RGQ PCR Kit with manual analysis and results interpretation was used to identify patients for enrollment in the SOLAR-1 study. Enrollment for the SOLAR-1 non-mutant cohort ended on 21 December 2016. Screening continued to identify PIK3CA mutation positive patients for the SOLAR-1 mutant cohort until completion of randomization (21 July 2017).

The automated *therascreen* PIK3CA RGQ PCR Kit with RGAM and Tissue Assay Profile with automatic data scan (AUDAS) disabled was used to retrospectively retest all tissue samples from patients enrolled in SOLAR-1 based on the original CTA to support a clinical bridging study. A subsequent *in silico* software bridging study demonstrated high agreement of the *therascreen* PIK3CA RGQ PCR Kit with manual analysis to the fully automated final market ready *therascreen* PIK3CA RGQ PCR Kit with RGAM, Gamma MDx Plug-in and associated Tissue Assay Profile software with AUDAS enabled and to the automated *therascreen* PIK3CA RGQ PCR Kit with RGAM and Tissue Assay Profile with AUDAS disabled used in the clinical bridging study. Hereafter the *therascreen* PIK3CA RGQ PCR Kit with manual analysis, the automated *therascreen* PIK3CA RGQ PCR Kit

with RGAM and Tissue Assay Profile with AUDAS disabled and the final market ready *therascreen* PIK3CA RGQ PCR Kit with RGAM, Gamma MDx Plug-in and associated Tissue Assay Profile software with AUDAS enabled are referred to as *therascreen* PIK3CA RGQ PCR Kit or PIK3CA Kit.

The *therascreen* PIK3CA RGQ PCR Kit clinical study included the entire SOLAR-1 randomized population to demonstrate safety and efficacy of the *therascreen* PIK3CA RGQ PCR Kit for identification of HR+, HER2- advanced breast cancer patients with PIK3CA mutations eligible for treatment with alpelisib. A summary of the clinical study is presented below.

A. Study Design

Patients were treated starting July 23, 2015. The data cut-off date for efficacy analysis in PIK3CA mutant cohort and safety for both cohorts is June 12, 2018 while the data cut-off for efficacy analysis in PIK3CA non-mutant cohort is December 23, 2016. 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.

SOLAR-1 was a pivotal Phase III, randomized, double-blind, placebo controlled, international, multicenter study of alpelisib in combination with fulvestrant in postmenopausal women, and men 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 500 mg 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).

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 on 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.

Patients were treated until disease progression, unacceptable toxicity, death, or discontinuation from the study treatment for any other reason. Dose adjustments for alpelisib/placebo were permitted for safety concerns but not for fulvestrant.

The primary endpoint for the 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. The key secondary endpoint was overall survival (OS) for patients with a PIK3CA mutation.

1. **Patient Information and Consent**

   In the SOLAR-1 study, informed consent was obtained from each patient in writing prior to any screening procedures. Patient informed consent for use of samples for the *therascreen* PIK3CA RGQ PCR Kit development (including the retrospective retesting) was included in the SOLAR-1 clinical study Model Patient Information and Informed Consent. Completed informed consent forms were not collected, nor made available to the Device Investigator; informed consent was collected by the clinical investigator sites and only samples for which informed consent was obtained were sent for testing. The study was explained to the patient by the Investigator or designee, who answered any questions, and written information was also provided. Ensuring patient informed consent was obtained before sample processing was the responsibility of the clinical investigator site.

2. **Tumor Specimens and Testing**

   The Clinical Performance Study utilized samples that were collected and tested (archived tissue or new biopsy) at screening or banked as part of the SOLAR-1 clinical study. Patient samples (RES/CNB) were collected at the SOLAR-1 clinical investigational sites. Patient samples consisted of FFPE blocks, 4-5µm FFPE sections mounted on glass slides, or fresh tissue samples fixed and shipped in 70% ethanol. Samples were shipped from the collection sites at ambient conditions to the clinical testing site.

   Tissue samples that were fixed and received in ethanol were processed into FFPE blocks at the clinical testing site. If samples were received as fresh tissue or an FFPE block, sectioning and mounting on glass slides occurred at the clinical testing site. FFPE slides allocated for the retrospective retesting could be either undipped or paraffin dipped for long term stability and stored at either room temperature or 4˚C until retrospective retesting. Sectioning and staining of samples were performed per the clinical testing site procedures. The pathologist assessment of the H&E included marking the tumor ROI, evaluation of the tumor content, tumor area and percent (%) necrosis in the ROI marked. Samples were stored at ambient temperature at the clinical testing site until gDNA extraction. Genomic DNA could be stored at 2–8°C for 1-week post extraction, or at -15 to -35°C for up to 8 weeks before use. Extracted DNA, if not used immediately, was stored at -15 to -35°C until results were reported. Residual DNA was stored at -80°C +/- 10°C.

3. **Key Clinical Inclusion and Exclusion Criteria**

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

- Patient has advanced (loco regionally recurrent not amenable to curative therapy or metastatic) breast cancer.
- Patients may be relapsed with documented evidence of progression while on (neo) adjuvant endocrine therapy or within 12 months from completion of (neo)adjuvant endocrine therapy with no treatment for metastatic disease;
- relapsed with documented evidence of progression more than 12 months from completion of (neo)adjuvant endocrine therapy and then subsequently progressed with documented evidence of progression while on or after only one line of endocrine therapy for metastatic disease;
- newly diagnosed advanced breast cancer, then relapsed with documented evidence of progression while on or after only one line of endocrine therapy
- 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 negative breast cancer defined as a negative in situ hybridization test or an IHC status of 0, 1+ or 2+. If IHC is 2+, a negative in situ hybridization (FISH, CISH, or SISH) test is required by local laboratory testing.
- Identified PIK3CA status (mutant or non-mutant; determined by a Novartis designated laboratory)
- Adults (female postmenopausal or men) ≥ 18 years old
- 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 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

4. **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 weeks 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 overall survival events was reached or if the study had stopped for other reasons. During the survival follow-up, data related 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.

5. **Clinical Endpoints**

The primary endpoint was to determine whether treatment with alpelisib in combination with fulvestrant prolongs PFS compared to treatment with placebo in combination with fulvestrant in postmenopausal women and men with patients with HR positive, HER2-negative, breast cancer. The primary endpoint was investigator-assessed PFS in the PIK3CA mutant cohort per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1.

6. **Clinical Bridging study**

The clinical bridging study between CTA used to enroll patients in SOLAR-1 prior to the use of the *therascreen* PIK3CA RGQ PCR Kit for enrollment in the SOLAR-1 trial was conducted to establish the concordance between the CTA and the QIAGEN *therascreen* PIK3CA RGQ PCR Kit (PIK3CA Kit). The clinical bridging study was aimed at establishing the clinical validity of the *therascreen* PIK3CA RGQ PCR Kit in identifying PIK3CA mutation positive patients for treatment with alpelisib in combination with fulvestrant against placebo in combination with fulvestrant by assessing efficacy of the QIAGEN's PIK3CA Kit.

Samples from all patients enrolled by the CTA were eligible for retrospective retesting with the PIK3CA Kit based on the following criteria:

Sample inclusion criteria

- Samples from all randomized patients enrolled by the CTA in the SOLAR-1 clinical trial
- Availability of adequate sample to generate a PIK3CA Kit test result, including pathology review to confirm the presence of tumor and determine tumor content and area

Sample exclusion criteria

- Lack of clear patient identification or label on stored patient sample
- Obvious physical damage of stored patient sample
- Insufficient sample
- No tumor present

The H&E stained section of each specimen previously reviewed by a pathologist during SOLAR-1 screening was re-reviewed to determine the number of sections required for DNA extraction of CNB samples and to determine if macrodissection was required for RES samples prior to DNA extraction. Paraffin-dipped slides for each patient were deparaffinized by incubating at 60ºC for 1 hour and cooling at ambient temperature for 5 minutes. Operators retesting the samples were blinded to previous CTA results. One of the following results was assigned to an individual sample:

- PIK3CA Mutation Detected and the specific mutation
- No Mutation Detected
- Invalid – based on flags assigned during analysis by RGAM

The diagnostic device study included the entire SOLAR-1 randomized population to demonstrate safety and efficacy of the *therascreen* PIK3CA RGQ PCR Kit for identification of HR+, HER2- advanced breast cancer patients with PIK3CA mutations eligible for treatment with alpelisib.

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

B. Accountability of PMA Cohort

A total of 1442 patients were screened for SOLAR-1. As part of the screening process, PIK3CA mutation testing was performed on 1244 of the 1442 patients using either the CTA (542 patients) or the PIK3CA Kit (702 patients). PIK3CA test records for 198 patients were not available due to patient withdrawal of consent or physician decision (28 patients), screen failure for other reasons prior to obtaining a PIK3CA result (103 patients), or incorrect/insufficient sample (67 patients).

Of the 542 patients tested by the CTA, 395 were randomized to the SOLAR-1 study based on PIK3CA status (169 patients PIK3CA mutation positive, 226 patients PIK3CA mutation negative) and 147 patients were not randomized to SOLAR-1 (28 patients with invalid PIK3CA mutation results and 119 screen failures for other reasons or withdrawal from the study).

Enrollment for the SOLAR-1 non-mutant cohort was substantially complete when the PIK3CA Kit was implemented for SOLAR-1, therefore the PIK3CA Kit predominantly selected PIK3CA mutation positive patients for the SOLAR-1 mutant cohort. Of the 702 patients tested by the PIK3CA Kit during screening for SOLAR-1, 177 were randomized (172 PIK3CA mutation positive, 5 PIK3CA mutation negative) and 525 patients were not randomized to SOLAR-1 (43 patients with invalid PIK3CA mutation results, 482 patients screen failed (including due to mutation negative result or withdrew from the study).

Samples from the 395 CTA-enrolled patients were retrospectively retested with the PIK3CA Kit and yielded 389 PIK3CA Kit-evaluable patients (98.5%) with 6 PIK3CA Kit-unevaluable patients (all PIK3CA mutation negative by CTA). The CTA-enrolled population was used for the PIK3CA Kit/CTA concordance analysis. The PIK3CA Kit efficacy analyses included the 177 PIK3CA Kit-enrolled patients and the 395 CTA-enrolled patients (all retrospectively retested with the PIK3CA Kit). The Figure 1 and Table 15 below outline the specimen accountability and sample disposition to the bridging study.

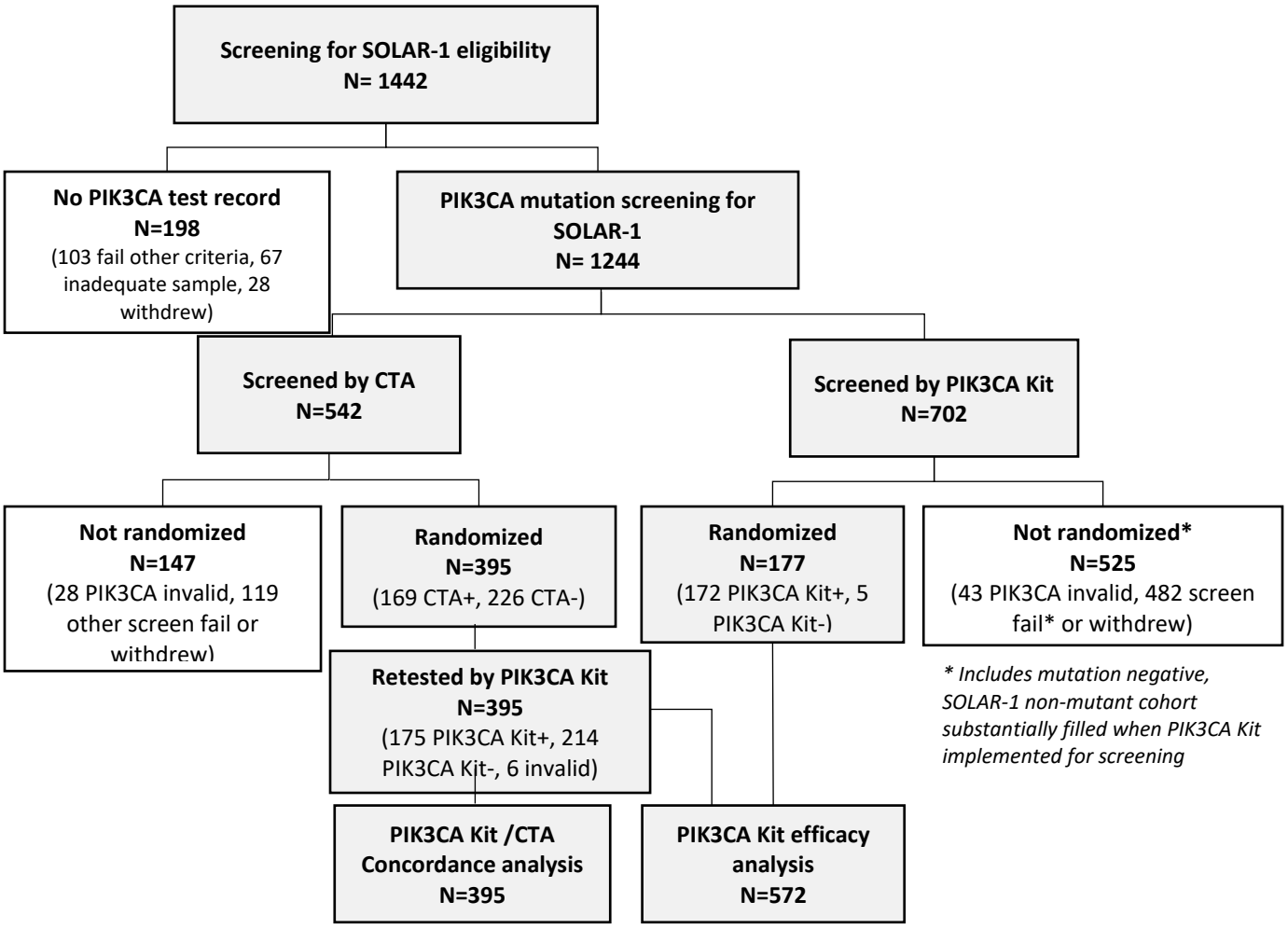


Figure 1. Specimen accountability

Table 15. Disposition of All Screened Subjects (All Screened Subjects)

Characteristics	Total patients
All screened	1442
Randomized by CTA	395
Tested as positive	169
Treated in Alpelisib arm	85
Treated in Placebo arm	84
Tested as negative	226
Treated in Alpelisib arm	112
Treated in Placebo arm	114
Randomized by PIK3CA Kit	177
Tested as positive	172
Treated in Alpelisib arm	84
Treated in Placebo arm	88
Tested as negative	5
Treated in Alpelisib arm	3
Treated in Placebo arm	2
Not randomized	870
Tested by CTA	147
Positive	46
Negative	73
Invalid	28
Tested by PIK3CA Kit	525
Positive	105
Negative	377
Invalid	43
No test record	198
Treated is defined as Planned	

Mutation Prevalence in the PIK3CA Kit-positive Population in SOLAR-1

There were 341 patients enrolled in the PIK3CA mutant cohort and 231 enrolled in the PIK3CA non-mutant cohort. Of the 341 patients enrolled in PIK3CA mutant cohort in SOLAR-1, 169 patients were enrolled based on central testing with the CTA, and 172 were enrolled based on testing with the therascreen PIK3CA RGQ PCR Kit. Of the 169 patients enrolled based on CTA results in the PIK3CA mutant cohort, 164 had PIK3CA mutant positive status confirmed by the *therascreen* PIK3CA RGQ PCR Kit. Of the 226 patients enrolled based on CTA results in the PIK3CA non-mutant cohort, 11 had PIK3CA mutant positive status determined by the *therascreen* PIK3CA RGQ PCR Kit.

Overall, the PIK3CA Kit positive population consisted of 347 patients, 175 from retrospective retesting of the CTA-enrolled patients and 172 randomized to SOLAR-1 based on the PIK3CA Kit result as summarized in the Table 16 below.

Table 16. Comparison of PIK3CA Kit Mutation Results Between the CTA-enrolled Patients and the PIK3CA Kit-enrolled Patients (FAS)

PIK3CA Kit results	CTA-enrolled		PIK3CA Kit-enrolled		All	
	Positive	Negative	Positive	Negative	Positive	Negative
	N=169 (%)	N=226 (%)	N=172 (%)	N=5 (%)	N=341 (%)	N=231 (%)
Positive	164 (97.0)	11 (4.9)	172 (100)	0	336 (98.5)	11 (4.8)
Negative	5 (3.0)	209 (92.5)	0	5 (100)	5 (1.5)	214 (92.6)
Missing	0	6 (2.7)	0	0	0	6 (2.6)

Within the PIK3CA Kit-positive population, all 11 of the targeted mutation variants were present. The most prevalent PIK3CA mutation variants in the PIK3CA Kit-positive patients were H1047R, E545K and E542K as shown below.

Table 17. Comparison of Mutation Prevalence Between the CTA-enrolled Patients and the PIK3CA Kit-enrolled Patients in the PIK3CA Kit-positive Patients (FAS)

PIK3CA Kit mutations	CTA-enrolled N=175 (%)	PIK3CA Kit-enrolled N=172 (%)	All N=347 (%)
Exon 7			
C420R	3 (1.7)	3 (1.7)	6 (1.7)
Exon 9			
E542K	31 (17.7)	35 (20.3)	66 (19.0)
E545A	4 (2.3)	0	4 (1.2)
E545D	1 (0.6)	5 (2.9)	6 (1.7)
E545G	5 (2.9)	4 (2.3)	9 (2.6)
E545K	41 (23.4)	50 (29.1)	91 (26.2)
Q546E	0	1 (0.6)	1 (0.3)
Q546R	0	2 (1.2)	2 (0.6)
Exon 20			
H1047L	17 (9.7)	7 (4.1)	24 (6.9)
H1047R	83 (47.4)	77 (44.8)	160 (46.1)
H1047Y	2 (1.1)	3 (1.7)	5 (1.4)

A mutation positive patient may have more than 1 mutation

Concordance of the PIK3CA Kit to the CTA is described below. Of the 169 CTA mutation positive tissue samples, 164 were PIK3CA Kit-positives. Of the 226 CTA mutation negative tissue samples, 209 were PIK3CA Kit-negative and 6 were invalid. There were 16 discordant results, 11 false positive results (PIK3CA Kit+, CTA-) and 5 false negative results (PIK3CA Kit-, CTA+). Of the discordant samples, 13 were low mutant fraction (near the PIK3CA Kit cut-off) and/or had low tumor content.

Table 18. Contingency table between the PIK3CA Kit and CTA results (FAS, CTA-enrolled)

PIK3CA Kit Results	CTA Results		
	Positive	Negative	Total
Positive	164	11	175

PIK3CA Kit Results	CTA Results		
	Positive	**Negative**	**Total**
Negative	5	209	214
Invalid	0	6	6
Total	169	226	395

Agreement (PPA, NPA and OPA) between the PIK3CA Kit and CTA, with and without invalid PIK3CA Kit results, was calculated using the CTA results as reference and shown below. The point estimates of PPA, NPA and OPA were 97.0%, 95.0% and 95.9% respectively, when excluding PIK3CA Kit invalid results. The lower bounds of the 95% Clopper-Pearson confidence intervals for PPA (93.2%) and NPA (91.2%).

Table 19. Bridging Study Agreement Analysis between the PIK3CA Kit and CTA based on the CTA results (Full analysis set, CTA-enrolled)

Measure of agreement	Without invalid PIK3CA Kit results		With invalid PIK3CA Kit results	
	Percent agreement (N)	95% CI [(1)]	Percent agreement (N)	95% CI [(1)]
PPA	97.0% (164/169)	(93.2%, 99.0%)	97.0% (164/169)	(93.2%, 99.0%)
NPA	95.0% (209/220)	(91.2%, 97.5%)	92.5% (209/226)	(88.2%, 95.6%)
OPA	95.9% (373/389)	(93.4%, 97.6%)	94.4% (373/395)	(91.7%, 96.5%)

[(1)] The 95% Confidence Interval (CI) calculated using the Clopper-Pearson Exact method

C. Study Population Demographics and Baseline Parameters

Demographics and baseline disease characteristics, tumor burden, and prior antineoplastic therapy were well-balanced between the study arms and cohorts with or without a PIK3CA mutation as shown in the tables below. Patients in the PIK3CA mutation cohort had a median age of 63 years (range 25 to 92). In this cohort, 54% were < 65 years, 45% patients were 65 years but < 85 years, and four patients were ≥ 85 years. The patients included were White (66%), Asian (22%), Black or African American (1%), and Other (5%).

Within the PIK3CA mutantion cohort, 169 patients were randomized to receive PIQRAY in combination with fulvestrant and 172 patients were randomized to placebo in combination with fulvestrant. Within this cohort, 170 (50%) patients had liver/lung metastases and 20 (6%) patients had prior CDK4/6 inhibitor treatment. In the PIQRAY plus fulvestrant arm of the cohort with a PIK3CA mutation, 98% of patients had received prior hormonal therapy, 48% as treatment of metastatic disease and 52% as adjuvant therapy as the last setting prior to entry into the trial. Primary endocrine resistance was observed in 13%, and secondary endocrine resistance was observed in 72% of patients in the alpelisib plus fulvestrant arm.

Table 20. Demographics and baseline characteristics (FAS, PIK3CA mutant cohort enrolled by either CTA or PIK3CA Kit)

Demographic variable	PIQRAY plus fulvestrant N=169 n (%)	Placebo plus fulvestrant N=172 n (%)	All Subjects N=341 n (%)
Age (years)			
Mean (SD)	62.7 (10.22)	64.0 (9.99)	63.3 (10.11)
Median	63.0	64.0	63.0
Sex – n (%)			
Female	168 (99.4)	172 (100)	340 (99.7)
Male	1 (0.6)	0	1 (0.3)
Race – n (%)			
White	117 (69.2)	109 (63.4)	226 (66.3)
Asian	34 (20.1)	40 (23.3)	74 (21.7)
Other	8 (4.7)	10 (5.8)	18 (5.3)
Unknown	8 (4.7)	8 (4.7)	16 (4.7)
Black or African American	1 (0.6)	3 (1.7)	4 (1.2)
American Indian or Alaska native	1 (0.6)	2 (1.2)	3 (0.9)
ECOG performance status – n (%)			
0	112 (66.3)	113 (65.7)	225 (66.0)
1	56 (33.1)	58 (33.7)	114 (33.4)
Missing	1 (0.6)	1 (0.6)	2 (0.6)

Table 21. Demographics and baseline characteristics (FAS, PIK3CA non-mutant cohort enrolled either by CTA or PIK3CA Kit)

Demographic variable	PIQRAY plus fulvestrant N=115 n (%)	Placebo plus fulvestrant N=116 n (%)	All subjects N=231 n (%)
Age (years)			
Mean (SD)	62.5 (9.01)	62.3 (10.61)	62.4 (9.83)
Median	62.0	63.0	62.0
Sex – n (%)			
Female	115 (100)	116 (100)	231 (100)
Race – n (%)			
White	82 (71.3)	69 (59.5)	151 (65.4)
Asian	25 (21.7)	26 (22.4)	51 (22.1)
Unknown	6 (5.2)	9 (7.8)	15 (6.5)
Other	1 (0.9)	7 (6.0)	8 (3.5)
Black or African American	1 (0.9)	3 (2.6)	4 (1.7)
American Indian or Alaska native	0	2 (1.7)	2 (0.9)
ECOG performance status – n (%)			
0	84 (73.0)	79 (68.1)	163 (70.6)
1	30 (26.1)	37 (31.9)	67 (29.0)
Missing	1 (0.9)	0	1 (0.4)

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%).

a. Safety Results - Adverse device effects that occurred in the PMA clinical study

No adverse events were reported in connection with the studies used to support this PMA with the final market ready *therascreen* PIK3CA RGQ PCR Kit.

2. Effectiveness Results

a. Overall Efficacy

Efficacy results from the SOLAR-1 trial for the PIK3CA mutant cohort Full Analysis Set (FAS) are presented in Table 22 and Figure 2 below.

PFS results for the PIK3CA mutant cohort by investigator assessment were supported by consistent results from a blinded independent review committee (BIRC) assessment.

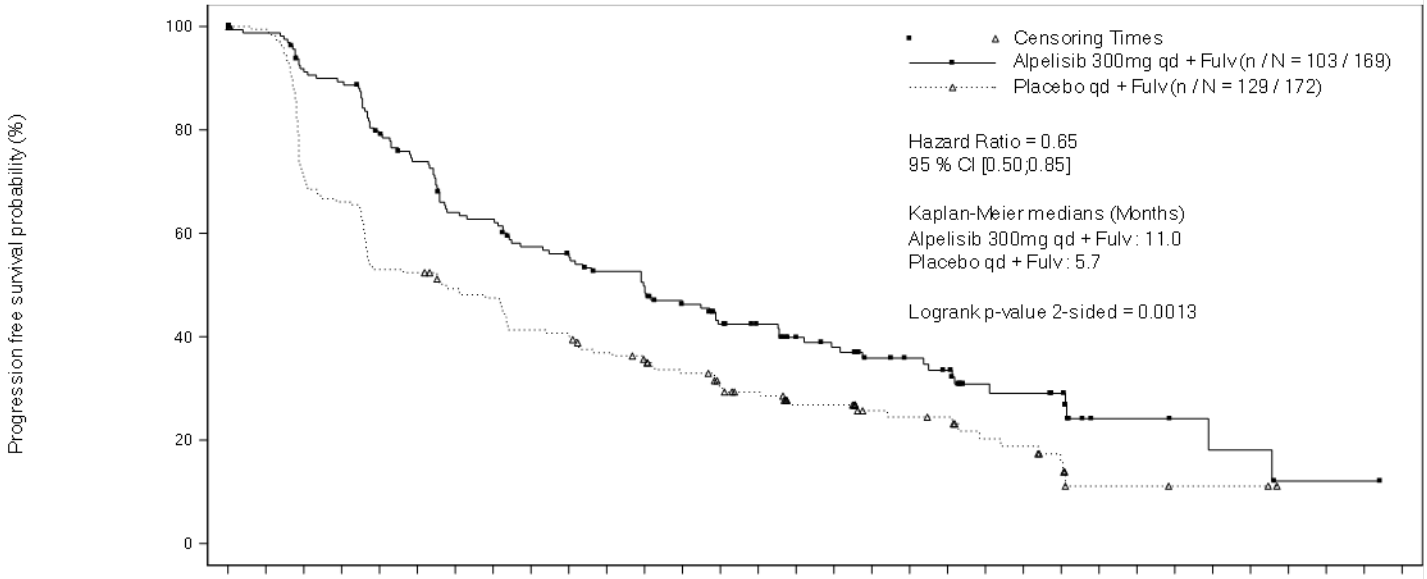
Table 22. Efficacy Results in SOLAR-1 (Patients with a PIK3CA Mutation per Investigator Assessment)

	PIQRAY plus fulvestrant	**Placebo plus fulvestrant**
Progression-free survival	**N = 169**	**N = 172**
Number of PFS events – n (%)	103 (61)	129 (75)
Median PFS months (95% CI)	11.0 (7.5, 14.5)	5.7 (3.7, 7.4)
Hazard ratio (95% CI)[1]	0.65 (0.50, 0.85)	
p-value[2]	0.0013	

[1] Cox proportional hazards model are stratified by prior CDK4/6 inhibitor usage and presence of lung/liver metastases. Confidence interval is obtained using Wald method.

[2] p-value obtained from 2-sided stratified logrank test

Figure 2. Kaplan-Meier Progression Free Survival Curves in the Cohort of Patients with a PIK3CA Mutation per Investigator Assessment in SOLAR-1



Number of subjects still at risk

Time (Months)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Alpelisib 300mg qd + Fulv	169	158	145	141	123	113	97	95	85	82	75	71	62	54	50	43	39	32	30	27	17	16	14	5	5	4	3	3	1	1	1	0
Placebo qd + Fulv	172	167	120	111	89	88	80	77	67	66	58	54	48	41	37	29	29	21	20	19	14	13	9	3	3	2	2	2	0	0	0	0

- Stratified Logrank test and stratified Cox model using strata define by (i) prior CDK 4/6 inhibitor use. (ii) presence of liver and/or lung metastases.

Clinical efficacy in the PIK3CA Kit-positive population was estimated by pooling the hazard ratios calculated for: 1) the CTA-enrolled patients that were PIK3CA Kit-positive after retest and 2) the PIK3CA Kit-enrolled

patients that were PIK3CA Kit-positive. Analysis was conducted with two pooling schemes:

- With pooling weighted by inverse variance of the log HR, the estimated hazard ratio was 0.64 (95% CI: 0.48, 0.85)
- Analysis weighted by sample size showed consistent results (estimated hazard ratio was 0.64 (95% CI: 0.48, 0.85)

Clinical efficacy of alpelisib in combination with fulvestrant for the PIK3CA Kit-positive population (HR = 0.64, 95% CI: 0.48, 0.85) was similar to the clinical efficacy in the SOLAR-1 PIK3CA mutant cohort (HR = 0.65; 95% CI: 0.50, 0.85).

In the PIK3CA Kit-positive subpopulations:

- The PFS HR for the 164 CTA-positive/PIK3CA Kit-positive patients was 0.67 (95% CI: 0.45, 1.01). Median PFS was 11.0 months for the alpelisib plus fulvestrant arm vs. 5.5 months for the placebo plus fulvestrant arm.
- The PFS HR for the 172 PIK3CA Kit-enrolled PIK3CA Kit-positive patients was 0.64 (95% CI: 0.43, 0.95). Median PFS was 9.4 months for the alpelisib plus fulvestrant arm vs. 5.7 months for the placebo plus fulvestrant arm.
- Median PFS for the 11 CTA-negative/PIK3CA Kit-positive patients was not estimable due to few events.

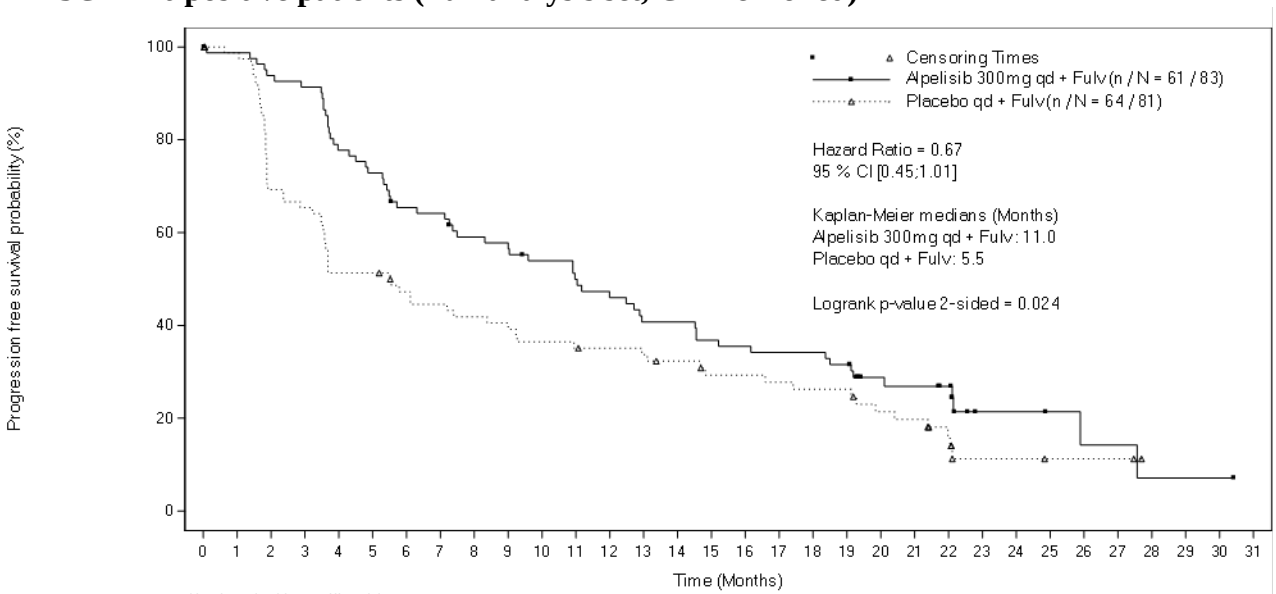
Table 23. PFS (months) in the CTA-positive, PIK3CA Kit-positive patients (CTA-enrolled) and in the PIK3CA Kit-positive patients randomized by the PIK3CA Kit (PIK3CA Kit-enrolled)

	CTA-positive/PIK3CA Kit-positive		**PIK3CA Kit Positive/PIK3CA Kit-enrolled**	
	PIQRAY plus fulvestrant	**Placebo plus fulvestrant**	**PIQRAY plus fulvestrant**	**Placebo plus fulvestrant**
PFS	**N = 83**	**N = 81**	**N = 84**	**N = 88**
Number of PFS events – n (%)	61 (73.5)	64 (79.0)	42 (50.0)	63 (71.6)
Median PFS months (95% CI)	11.0 (7.4, 14.6)	5.5 (3.5, 9.0)	9.4 (5.8, 16.8)	5.7 (3.6, 9.0)
Hazard ratio (95% CI) [1]	0.67 (0.45, 1.01)		0.64 (0.43, 0.95)	
p-value [2]	0.024		0.026	

[1] Cox proportional hazards model are stratified by prior CDK4/6 inhibitor usage and presence of lung/liver metastases. Confidence interval is obtained using Wald method.

[2] p-value obtained from 2-sided stratified logrank test.

Figure 3. Kaplan-Meier plot of progression free survival by treatment in the CTA-positive, PIK3CA Kit-positive patients (Full analysis set, CTA-enrolled)

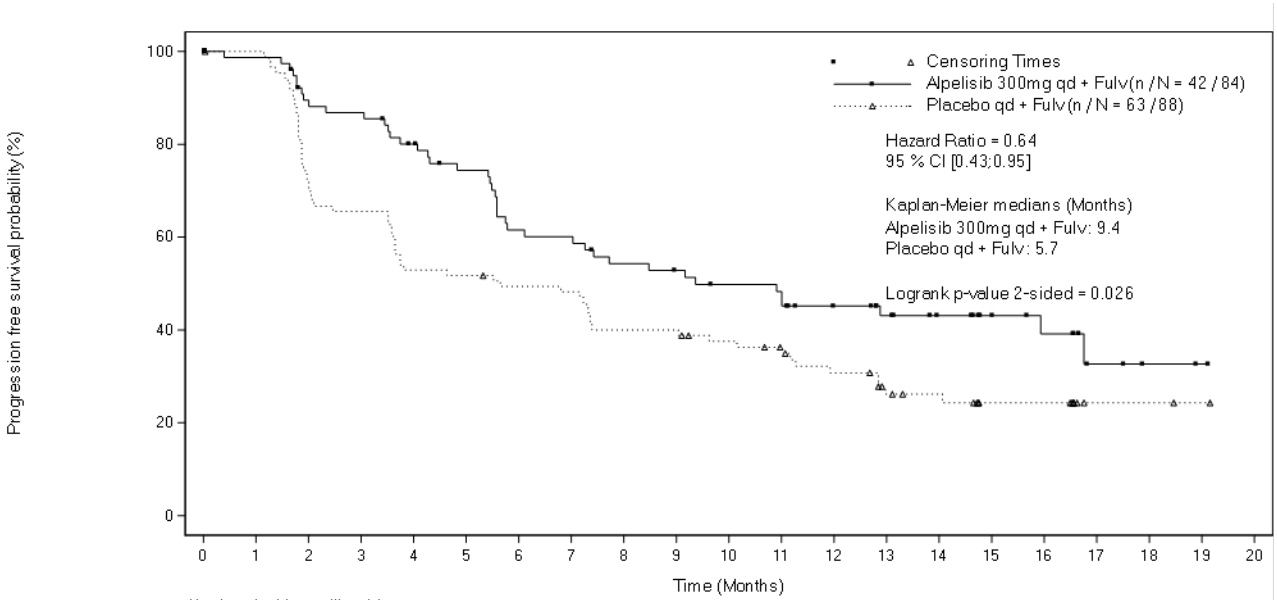


Number of subjects still at risk

Time (Months)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Alpelisib 300mg qd + Fulv	83	80	76	74	63	59	52	51	46	45	41	38	35	31	31	28	27	26	26	24	15	14	12	4	4	3	2	2	1	1	1	0
Placebo qd + Fulv	81	77	54	51	40	40	35	33	31	30	27	26	25	24	22	19	19	18	17	17	13	12	8	3	3	2	2	2	0	0	0	0

- Stratified Logrank test and stratified Cox model using strata define by (i) prior CDK 4/6 inhibitor use. (ii) presence of liver and/or lung metastases.

Figure 4. Kaplan-Meier plot of progression free survival by treatment in the PIK3CA Kit-positive patients randomized by the PIK3CA Kit (Full analysis set, PIK3CA-enrolled)



Number of subjects still at risk

Time (Months)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Alpelisib 300mg qd + Fulv	84	76	67	65	58	52	43	42	37	35	32	31	25	21	17	13	10	4	2	1	0
Placebo qd + Fulv	88	87	63	57	46	45	42	41	34	34	30	27	22	16	14	9	9	2	2	1	0

- Stratified Logrank test and stratified Cox model using strata define by (i) prior CDK 4/6 inhibitor use. (ii) presence of liver and/or lung metastases.

Sensitivity analysis: PIK3CA Kit-positive population

Sensitivity analysis against the missing PIK3CA Kit results demonstrated the robustness of the clinical efficacy estimate for PIK3CA Kit-positive patients. With pooling weighted by inverse variance of the log HR, the estimated hazard ratios were similar for:

- The case assuming all missing PIK3CA Kit results were concordant with the CTA results (HR = 0.64, 95% CI: 0.48, 0.85) and
- The case assuming all missing PIK3CA Kit results were discordant with the CTA results (HR = 0.63, 95% CI: 0.47, 0.84).

Analysis weighted by sample size showed consistent results with HR = 0.64 (95% CI: 0.48, 0.85) assuming missing PIK3CA Kit results were concordant with the CTA results, and HR = 0.63 (95% CI: 0.47, 0.84) assuming missing PIK3CA Kit results were discordant with the CTA results.

PFS was also estimated in the PIK3CA Kit -negative population. No PFS benefit was observed in patients with wildtype PIK3CA (HR = 0.85; 95% CI: 0.58, 1.25).

Efficacy Results for CNB vs. RES

The total SOLAR-1 PIK3CA mutant cohort based on the enrolling tissue result, regardless of consideration of tissue sample type, was well balanced across the treatment arms for the baseline demographic and disease characteristics. However, the patients with CNB samples vs RES samples showed imbalance by sample type and imbalance between treatment arms for the CNB patients, in particular for baseline disease characteristics. Many of the disease characteristics reflect more aggressive disease in the population represented by CNBs.

Overall, there were fewer patients with CNB samples compared to patients with RES samples (N= 103 for CNB vs N=244 with RES). The SOLAR-1 trial did not stratify for sample type; and a difference of ≥10% is observed in the allocation to treatment arm for the PIK3CA Kit positive patients with CNB samples with 58 (56%) patients in the alpelisib arm and 45 (44%) in the placebo arm compared to the more equally distributed PIK3CA Kit positive patients with RES samples with 115 (47%) patients in the alpelisib arm and 129 (53%) in the placebo arm.

The PFS HR was 0.91 (95% CI: 0.57, 1.46) for patients with CNB samples and the HR was 0.57 (95% CI: 0.41, 0.79) for patients with RES samples. The HR confidence interval for patients with CNB samples overlapped the confidence interval for patients with RES samples. The study was not powered for this subgroup analysis.

3. Subgroup Analyses
   Not performed for PIK3CA Kit analyses.

4. Pediatric Extrapolation
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population since it not applicable for the breast cancer indication.

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

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

In the SOLAR-1 PIK3CA mutant cohort , the median PFS for the alpelisib in combination with fulvestrant arm was 11.0 months, as compared to the placebo in combination with fulvestrant arm which was 5.7 months. This median PFS difference of 5.3 months is considered clinically meaningful in metastatic breast cancer. The clinical benefit of the *therascreen* PIK3CA RGQ PCR Kit was demonstrated by prospective and retrospective analysis of patients enrolled in the SOLAR-1 trial. Clinical efficacy of alpelisib in combination with fulvestrant for the companion diagnostic, the *therascreen* PIK3CA RGQ PCR Kit-positive population (HR = 0.64, 95% CI: 0.48, 0.85) was similar to the clinical efficacy in the SOLAR-1 PIK3CA mutant cohort (HR = 0.65; 95% CI: 0.50, 0.85). These hazard ratios were maintained when subjected to clinical sensitivity analysis.

B. Safety Conclusions

The risks of this device relate to false positive and false negative device results. A false negative result could potentially result in the patient forgoing potentially beneficial treatment with alpelisib with a demonstrated PFS benefit of about 5 months.

A false positive result could subject a patient to the toxicity of alpelisib while providing only limited expected benefit. The main toxicities of alpelisib are hyperglycemia, diarrhea, rash with a few cases of Stevens-Johnson syndrome in the dose finding studies, and nausea and vomiting which were mostly managed by oral medications as well as dose reductions. The most significant AE is the hyperglycemia. All of the AEs noted were anticipated for this class of drug. There were a few cases of pneumonitis so it was also listed in the warnings and precautions but those were very few cases.

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 PIQRAY (alpelisib), CBYL719C2301 (SOLAR-1), showing improved PFS in a defined population of patients with recurrent breast cancer. The clinical benefit of the *therascreen* PIK3CA RGQ PCR Kit was demonstrated by prospective and retrospective analysis of patients enrolled in the SOLAR-1 trial. Clinical efficacy of alpelisib in combination with fulvestrant for the companion diagnostic, the *therascreen* PIK3CA RGQ PCR Kit-positive population (HR = 0.64, 95% CI: 0.48, 0.85) was similar to the clinical efficacy in the SOLAR-1 PIK3CA mutant cohort (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 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. 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.

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. The use of this device to aid clinicians in identifying advanced breast cancer patients who may be eligible for treatment with PIQRAY (alpelisib) based on a PIK3CA

mutation detected result is expected to provide a benefit in progression-free survival of approximately five months. There is some uncertainty to this assessment based primarily on analytical performance factors of the device. Accordingly, post-market commitments with respect to the following analytical studies will be required: precision (repeatability and reproducibility), certain interference studies (adipose tissue), and study demonstrating PIK3CA Kit reagent stability.

In summary, considering all factors including conditions of approval (postmarket actions), the benefits of the use of QIAGEN *therascreen*® PIK3CA RGQ PCR Kit in patients with breast carcinoma are judged to outweigh the risks.

XIII. CDRH DECISION

CDRH issued an approval order with conditions for approval on May 24, 2019. The final conditions of approval cited in the approval order are described below.

1. Obtain additional repeatability and reproducibility data using 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 study data with representative mutation positive specimens from each reaction tube according to the agreed upon study proposal.

3. Evaluate potential interference from adipose tissue by evaluating PIK3CA mutation positive and negative SOLAR-1 clinical trial samples and update the labeling.

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

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.