SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Real-time PCR Test
Device Trade Name:	*therascreen*® BRAF V600E RGQ PCR Kit
Device Procode:	OWD
Applicants Name and Address:	QIAGEN GmbH QIAGEN Strasse 1 Hilden 40724 Germany
Date(s) of Panel Recommendation:	None
Premarket Approval Application:	P190026
Date of FDA Notice of Approval:	April 15, 2020

II. INDICATIONS FOR USE

The *therascreen* BRAF V600E RGQ PCR Kit is a real-time PCR test for the qualitative detection of V600E mutations in the BRAF gene using genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) human colorectal cancer (CRC) tumor tissue.

The *therascreen* BRAF V600E RGQ PCR Kit is an *in vitro* diagnostic device intended to be used as an aid in selecting patients with metastatic colorectal cancer (mCRC) whose tumors carry the BRAF V600E mutation for treatment with BRAFTOVI (encorafenib) in combination with cetuximab.

The *therascreen* BRAF V600E RGQ PCR Kit is for use on the Rotor-Gene Q MDx (US) instrument.

The *therascreen* BRAF V600E RGQ PCR Kit is intended for *in vitro* diagnostic use.

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the *therascreen* BRAF V600E RGQ PCR Kit labeling.

V. DEVICE DESCRIPTION

The *therascreen* BRAF V600E RGQ PCR Kit is a real-time qualitative polymerase chain reaction (PCR) kit designed to detect the V600E mutation (T1799A) in exon 15 codon 600 of the BRAF gene. The *therascreen* BRAF V600E RGQ PCR System uses:

- QIAGEN QIAamp DSP DNA FFPE Tissue Kit.
- QIAGEN *therascreen* BRAF V600E RGQ PCR Kit.
- QIAGEN Rotor-Gene Q MDx Instrument (RGQ) MDx Instrument with Automated data analysis and results interpretation using Rotor-Gene AssayManager® (RGAM) software version 2.1, Gamma MDx plug-in and *therascreen* BRAF V600E FFPE Assay Profile.

The *therascreen* BRAF V600E RGQ PCR Kit is supplied in a 24-reaction package size (with scope of analyzing 24 clinical samples in 6 x 4 PCR runs (i.e., 6 samples + 2 run controls per PCR run x 4 PCR runs)) and each kit contains the following components are shown in Table 1.

Table 1. Kit Components

Component	Purpose
Control Reaction Mix	To detect BRAF gene exon 3. It is used to determine the amount of amplifiable DNA present in the sample and is a factor used in the mutation analysis calculations.
V600E Mutation Reaction Mix:	Tor the identification of V600E on the BRAF gene exon 15*
BRAF Positive Control (PC):	PCR amplification control template
Nuclease and DNA free water	for No Template Control (NTC)
Nuclease and DNA free water for sample dilution	
Taq polymerase	

* The BRAF Mutation Reaction mix will not distinguish between V600E and V600E Complex (V600Ec) mutations. V600Ec is a very rare somatic mutations in CRC. The assay is not analytically validated for V600Ec.

A. Specimen Preparation

The assay is validated for use with FFPE CRC specimens with a minimum of 5% tumor content. Initial preparation of specimens for use with the *therascreen* BRAF V600E RGQ PCR Kit follows standard pathology procedures. Tumor tissue is typically fixed in 10% neutral buffered formalin and then embedded in paraffin to form FFPE blocks. FFPE tissue blocks 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. Two non-stained tissue sections are scraped from the slide for Deoxyribonucleic Acid (DNA) extraction.

DNA is manually extracted and purified using the QIAGEN QIAamp DSP DNA FFPE Tissue Kit following the standard extraction method with thefollowing modifications:

- Two FFPE sections are used per extraction.
- Ethanol evaporation temperature is set at 35.0°C to 39.0°C for a minimum of 10 minutes.
- Proteinase K digestion is performed for 1 hour.
- The extracted DNA sample is eluted with two elution steps consisting of elution buffer (ATE) of 60 µl each, resulting in a final elution volume of 120 µl from each sample extraction run.

B. Qualification of DNA Samples

Determination of DNA input for the assay is based on DNA qualification and is performed using the Control Reaction Mix to assess the amount of amplifiable DNA. Samples will be qualified for further Mutation Assessment if the amount of the amplifiable DNA (represented by the Ct value of the Control Reaction Mix) is determined to be within the pre-defined Control Working Range (CWR). The acceptable measurable range of the assay (Control CT 20.95–33.00).

C. PCR Amplification and Detection

The *therascreen* BRAF V600E RGQ PCR Kit uses Amplification Refractory Mutation System (ARMS®) and Scorpion technology to selectively amplify and detect the BRAF V600E mutation. The ARMS technique is based on the use of mutation sequence-specific PCR primers that allow amplification of test DNA only when the 3'- end of the primer is hybridized to the target mutation sequence. In the presence of a wild-type sequence or other non-targeted BRAF mutations, the ARMS primer will not completely hybridize, and amplification will not occur.

The Scorpion contains both primer and probe elements. The probe element contains fluorophore and quencher. When target sequence is not available, the fluorophore and quencher are in a closed configuration, i.e., the fluorophore and quencher are in such close proximity that fluorescence is reduced through collisional quenching. During PCR, the Scorpion primer element and ARMS primer will amplify the target sequences, for which the scorpion probe will bind to during the annealing step. The binding leads to the fluorophore and quencher being physically separated (open configuration) and fluorescence increases. The open configuration is more thermodynamically stable than the closed configuration.

The Scorpion probes used in the *therascreen* BRAF V600E RGQ PCR Kit are labeled with carboxyfluorescein (FAM) or hexachloro-fluorescein (HEX) fluorescent reporter dyes each with a distinct absorption and emission profile. The FAM probe is used for target detection (both Wild Type and BRAF V600E

mutant) and the HEX probe is used in the Internal Control Reaction (IC).

The *therascreen* BRAF V600E RGQ PCR Kit contains reagents that allow PCR amplification and qualitative detection of the mutation listed in Table 2.

Table 1: Mutation Targets of the *therascreen* BRAF V600E RGQ PCR Kit

Mutation	Exon	Base change	COSMIC ID*
V600E	15	1799T>A	476

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

D. Test Controls

The *therascreen* BRAF V600E 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. The Ct values obtained in the IC channel are checked to ensure the sample validity. If the RGAM fails to detect an in-range signal in the IC, the sample is reported as invalid and no BRAF V600E mutation status results for that sample are reported.

In addition to the IC, 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 BRAF V600E RGQ PCR Kit has been verified to work within a specific working range (i.e. upper and lower Control Reaction Ct values, this is also known as Control Working Range, CWR) and any samples that do not give Ct values within this range are invalidated by the RGAM software, plug-in and associated assay profile.

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

- **Internal Control (IC):** The BRAF V600E reaction mix contains reagents (ARMS primer, Scorpion probe and oligonucleotide template) for an IC reaction designed to detect failure of the reaction, e.g., due to PCR inhibition.
- **No Template Control (NTC):** An NTC tube 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 contains long oligo sequence representing BRAF mutations. Detection of the BRAF V600E target within acceptable ranges confirms the proper functioning of each of the reaction mixes in the kit.

For *therascreen* BRAF V600E RGQ PCR Kit test runs to be accepted as valid, the Ct values for PC, IC and NTC have to meet the following pre- defined criteria in Table 3.

Table 2: Run Validity Criteria for Controls

Reaction Mix	Control	Channel	Acceptable Ct range (inclusive)
Control	PC	Green (FAM)	27.82-33.85
V600E	PC	Green (FAM)	27.49-33.51
All	NTC	Green (FAM)	No Amp (that is, not ≤ 40.00)
All	NTC	Yellow (HEX) - IC	32.53-38.16

E. Instruments and Software

The *therascreen* BRAF V600E RGQ PCR Kit is designed to be used with the Rotor-Gene Q (RGQ) MDx 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 pass the excitation / emission optics every 150 milliseconds. The fluorescence signals indicate the progress of the PCR reactions. The Rotor-Gene Q MDx has six channels (six excitation sources and six detection filters). Two of these channels; green and yellow, are used with the *therascreen* BRAF V600E RGQ PCR Kit. Cycling parameters, data analysis and results interpretation for the *therascreen* BRAF V600E RGQ PCR Kit are performed by:

- RGAM version 2.1 Software.
- Gamma MDx plug-in version 1.0.0.
- Mutation Assessment Assay Profile:
  therascreen BRAF V600E FFPE MA MDx Assay Profile v1.0.0.
- Sample Assessment Assay Profile:
  therascreen BRAF V600E FFPE SA MDx.

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* BRAF V600E FFPE MA MDx 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.

F. Interpretation of Results

The cycle threshold (Ct) at which the instrument distinguishes the amplification generated fluorescence as being above the background signal is within the range of 0 to 40. Ct values generated by the Control and Mutation Reaction Mixes 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. Ct values generated by the Control and Mutation Reaction Mixes 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.

If the Sample Validity Criteria are met, the sample is analyzed for the presence of the BRAF V600E mutation. The difference in Ct values (ΔCt) between the Control Reaction and the Mutation Reaction is a qualitative measure of BRAF V600E mutation status and is calculated as:

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

Samples are classed as mutation positive (reported as "Mutation Detected") if they give a ΔCt less than or equal to the assay cut-off ΔCt value of 7.0 (Table 4). Above this value, the sample will be reported as "No Mutation Detected". Additional BRAF V600E assay software flags will report on invalid samples.

Table 4 Cycle Threshold (Ct)

Result	Interpretation
$\Delta Ct \leq 7.0$	No Mutation Detected
$\Delta Ct > 7.0$	Mutation Detected

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA cleared or approved alternatives for the testing of FFPE CRC tissue for BRAF V600E mutation status in the selection of patients who are eligible for a combined treatment with BRAFTOVI® (encorafenib) and ERBITUX®(cetuximab).

VII. MARKETING HISTORY

The *therascreen* BRAF V600E 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 BRAF V600E mutation results, which could impact patient treatment decisions. A false positive test result may lead to inappropriate treatment and potentially any adverse effects associated with treatment with a targeted BRAF inhibitor rather than standard of care treatments. A false negative test may prevent a patient benefitting from a targeted therapy.

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

The mutation status of samples was confirmed by an independent method. The percentage mutation or MAF was determined by a validated digital droplet PCR method.

1. Correlation with the Reference Method

The analytical accuracy of the *therascreen* BRAF V600E RGQ PCR Kit was assessed by comparison of results an orthogonal method. A total of 606 CRC DNA samples collected and extracted from the BEACON clinical trial were tested by a validated Sanger Sequencing reference method. Samples were consecutively enrolled into the study based on the date of testing to minimize bias. Samples were also selected based on results obtained with the BRAF V600E clinical trial assay (CTA): 275 CTA positive, 275 CTA negative and 56 CTA indeterminate samples were selected. Of these, 600 samples returned Sanger testing results (including both valid and invalid/indeterminate). Of these samples, 79 samples (13%) were indeterminate with *therascreen* BRAF V600E RGQ PCR Kit and 136 were indeterminate by Sanger. A total of 417 were valid for both BRAF V600E

and Sanger Sequencing. Agreement. was demonstrated by assessing the positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) from both of sets of results. Table 5 demonstrates an overall agreement of 95%. Breakdown of the results including indeterminate and the results (both valid and indeterminate by both methods) are summarized in Table 6. The measures of agreement, with adjusting for the enrichment of CTA positives are shown in Table 7.

Table 5: Distribution of Results (Sanger sequencing versus BRAF V600E Kit)

		Bi-Directional Sanger BRAF Result			
		Indeterminate	**Mutation Detected**	**No Mutation Detected**	**Total**
***therascreen* BRAF V600E Result**	**Indeterminate**	32	24	23	79
	Mutation	44	192	20	256
	No Mutation	60	0	205	265
	Total	136	216	248	600

Table 6. Agreement in Overall Mutation Status between *therascreen* BRAF V600E RGQ PCR Kit and Sanger Sequencing (as reference method), unadjusted analysis

Measure of Agreement	N	Percent Agreement	Lower Two-sided 95% Confidence Limit*	Upper Two-sided 95% Confidence Limit*
Overall Percent	397/417	95.20	92.69	97.05
Positive Percent	192/192	100.00	98.10	100.00
Negative Percent	205/225	91.11	86.61	94.49

*Confidence intervals were calculated using Clopper-Pearson (Exact) Binomial.

Table 7: Agreement between *therascreen* BRAF V600E RGQ PCR Kit and Sanger Sequencing (as reference method), adjusted analysis

Agreement Measure	Estimate	Lower Two-sided 95% Confidence Limit*	Upper Two-sided 95% Confidence Limit*
NPA	94.24%	91.83%	96.50%
OPA	96.23%	94.57%	97.76%
PPA	100.00%	N/A**	N/A**

* calculated using a non-parametric bootstrap method

** not calculated in cases where estimated value is 100.00%

2. Analytical Sensitivity

a) Analytical Sensitivity-Limit of Blank (LoB)

The LoB is defined as the highest measurement result that is likely to be observed for a blank sample. A total of 96 replicates were tested (water was used as test sample), no amplification was observed in either reaction (the Control and Mutation reactions). To assess performance of the *therascreen* BRAF V600E RGQ PCR Kit in the absence of mutant-positive template and to ensure that a blank sample does not generate an analytical signal that may indicate a low concentration of mutation, samples with no template were evaluated. The results demonstrated no detectable control or mutant CT value in any of the control or mutation reaction tubes. Therefore, the LoB for the *therascreen* BRAF V600E RGQ PCR Kit was set to Ct value of 40, the maximum PCR cycle number used for the Kit.

b) Analytical Sensitivity -ΔCt Analytical Cut-off Determination

During assay development, the assay Cut-off was determined using 82 CRC FFPE samples including both positive and negative samples (characterized by highly sensitive orthogonal methods) for the BRAF V600E mutation. The analytical ΔCt cut-off value was determined based on false-positive rates and false-negative rates as shown in Table 8.

Table 8: False Positive rate and False Negative Rate for Chosen Assay Cut-off

	False Negative		False Positive		Overall False Rate	
Cut-Off	**Fraction**	**Percentage**	**Fraction**	**Percentage**	**Fraction**	**Percentage**
ΔCt 7	0 / 37	0.000	1 / 45	2.2%	1 / 82	1.2%

c) Analytical Sensitivity-Limit of Detection (LoD)

The measurable range (CWR) for the therascreen BRAF V600E RGQ PCR Kit is based on the amount of amplifiable DNA in the specimen as determined by the Control reaction CT value. The stated measurable range for the assay is defined by the control CT pre-specified range of 20.95 to 33.00. The LoD is the minimum percentage of mutant DNA that can be detected in a background of wild-type DNA, when the total amplifiable DNA is within the stated input range and still below the threshold cutoff ΔCT value. The LoD is defined as the lowest amount of analyte in a sample that can be detected with at least a 95% probability as determined by a probit analysis. The LoD was determined using 10 clinical CRC samples representing high, medium and low DNA input levels. Samples were positive for the BRAF V600E mutation and the mutation allele frequency (MAF) was established using an independently validated

ddPCR method.

A five or six-point MAF dilution series was made for each of the clinical samples by serially diluting mutant samples in a clinical wild type (WT) DNA background. These dilution series were then tested in multiple replicates using 2 lots of the *therascreen* BRAF V600E RGQ PCR Kit over a period of ≥ 3 days with multiple instruments and operators. LoD values were determined using the results following a probit analysis. The LoD values are reported in Table 9.

Table 9: LoD Values for the *therascreen* BRAF V600E RGQ PCR Kit

DNA Input Levels	Control Ct Range	LoD (MAF)
High	≥20.95– ≤ 25.00	2%
Medium	>25.00 – ≤ 29.38	3.5%
Low	>29.38 – ≤ 33.00	7.8%

The LoD values were further verified by an independent clinical sample set including seven Resection (RES) and Core Needle Biopsy (CNB) FFPE CRC samples.

d) Analytical Sensitivity-Effect of DNA Input on ΔCt

When samples at different total DNA levels contain the same proportion of mutant DNA, it is expected that the measured ΔCT values will remain consistent. DNA extracted from 4 FFPE CRC samples (two wild-type and two mutant) were used to prepare pools of DNA with the lowest achievable control reaction CT and diluted across a minimum of six dilution levels that span the entirety of the CWR (measurable range of the assay).

For each sample tested, the linear, quadratic and cubic regression models were fitted to the data with ΔCT (for positive samples) or Control CT values (for negative samples) as the response variable, and dilution number as the continuous explanatory variable. The regression model results were reported along with the corresponding standard errors, 95% confidence limits and p-values. Linearity was demonstrated for all samples throughout the measurable range tested, there were no statistically significant differences in the regression models. Some quadratic and cubic effects were observed but the maximum difference predicted was within the precision limit (i.e., <2x standard deviation). Therefore, ΔCt values were consistent across the full DNA input range.

3. Analytical Specificity

a) Analytical Specificity-Primer and Probe Specificity

The purpose of this study was to demonstrate the theoretical specificity for the primers and probes used within the *therascreen* BRAF V600E RGQ PCR Kit. Multiple analyses were conducted to ensure that each oligonucleotide (Oligo) used in the therascreen BRAF V600E RGQ PCR Kit binds only to target sequence and not to sequences from other species or to non-target human genome sequences. Each primer and probe design were checked against the nucleotide (nr/nt) database using a blastn search and against the Human Genomic and Transcript (Human G+T) database using a blastn search. BLAST was also performed between each of the therascreen BRAF V600E RGQ PCR Kit primer and probe sequences to the IC template. Additional analysis was also performed to confirm that each oligo included in the therascreen BRAF V600E RGQ PCR Kit does not self-react or cross-react with each other to generate unspecific amplicons. This study concluded that the design of therascreen BRAF V600E RGQ PCR Kit does not produce non-specific amplifications against other species or human genome sequences.

b) Analytical Specificity-Cross Reactivity

Cross-reactivity was evaluated by testing the *therascreen* BRAF V600E RGQ PCR Kit with non-targeted mutations on the BRAF V600 codon (V600Ec, V600D, V600K, V600R, V600M, and V600G) at high DNA input level and various MAF. Cell line gDNA samples were used in this study in order to obtain samples at challenging levels (i.e., high DNA input). Six replicates were assessed for each sample at each MAF level.

The assay showed no cross reactivity to V600K, V600R, V600M, V600G at all concentrations (up to 100% MAF) tested. Cross reactivity was observed for V600Ec and V600D using cell line gDNA. The design of *therascreen* BRAF V600E RGQ PCR Kit does not distinguish between V600E and V600Ec mutations. However, V600Ec was not able to be validated as a target because no clinical specimens were identified in the BEACON study.

The cross reactivity of BRAF V600D was confirmed using cell line DNA. No BRAF V600D positive clinical specimens were identified in the clinical study due to the low prevalence of BRAF V600D in the CRC population (0% reported in the COSMIC database at the time this summary report is written).

c) Analytical Specificity-Cross Contamination

To evaluate the potential occurrence of cross contamination during the DNA extraction and subsequent *therascreen* BRAF V600E RGQ PCR Kit testing procedures (entire system), this study was performed using high DNA input level Positive BRAF V600E clinical samples (MT) and Wild-type clinical samples (WT to BRAF V600E). The assessment of cross contamination was carried out by extracting high positive MT samples and WT within the same DNA extraction runs and followed by testing these samples in a "checker board" pattern (contained both WT and MT samples) PCR runs and "WT only" runs. Any WT samples that produced false positive results would be used as evidence of "cross contamination".

In total, 131 WT replicates were tested, and the observed percentage of correct mutation calls was 100%, demonstrating no cross contamination of the WT samples by high positive mutant samples sharing the same DNA extraction, RGQ and run set up procedure.

d) Analytical Specificity-Interfering Substances

The effect of potential interfering substances from both endogenous and exogenous substances was measured by comparison of correct call and ΔCt values between interferent spiked and control spiked extracts. Nine potential interfering substances were tested:

- Hemoglobin, a potential endogenous interfering substance that may be present in FFPE tissue samples.
- Potential exogenous interferents from the extraction procedure using the QIAamp DSP DNA FFPE Tissue Kit that may be introduced into the sample during the DNA extraction process:
    - Paraffin Wax
    - Ethanol
    - Xylene
    - Extraction Buffers (AW1, ATL, AW2 and AL)
    - Proteinase K

Four (4) clinical FFPE CRC tissue samples (2 MT and 2 WT) were used in this study. The evaluation was performed by testing 9 replicates per interferent using the *therascreen* BRAF V600E RGQ PCR Kit. All results were as expected showing no statistically significant difference in Control Ct (WT samples) or Delta Ct (Mutant samples) (P values were > 0.05 for all interferent versus Interferent-free control). No false calls were observed for any test samples. In conclusion, none of the interferents tested had any impact on the *therascreen* BRAF V600E RGQ PCR Kit performance.

e) Analytical Specificity-Interfering Substances

The presence of necrotic tissue in the range of 30–100% within CRC FFPE samples was evaluated in seven "high necrotic tissue" samples identified in the procured clinical CRC population (approximately 250 in total, only 7 contained necrotic tissue equal to or greater than 30%), no false BRAF V600E results were generated by the *therascreen* BRAF V600E RGQ PCR Kit (confirmed by Pyro-sequencing) and no association between % necrosis and Control Ct values was observed (following serial dilution of samples with necrotic tissue of ≥ 80%).

4. Precision of the Assay

a) Repeatability, Intermediate Precision and Lot Interchangeability

The objective of this study was to demonstrate the repeatability, intermediate precision and lot to lot variation of the *therascreen* BRAF V600E RGQ PCR Kit. The study used 9 FFPE clinical CRC samples. Each sample was normalized to the target DNA input level and percentage mutation as shown in Table 10. The positive samples % mutant allele were quantified by digital droplet PCR (ddPCR). Each test panel members were tested in a three-day period with 3 operators, 3 instrument and 3 kit lots included. In total 27 PCR runs were performed with 81 replicates per test panel member. The precision based on the proportion of correct calls is provided in Table 11 below. The variance component analysis output is provided in Table 12. The results demonstrated that the assays are reproducible across the range of allele frequencies

Table 3: Test Panel Members for the Precision Studies

Panel Member	Mutant Status	Acquisition Method	DNA input level (Targeted Ct)	% Mutation Level*
1	WT	RES	Low (Ct 31)	N/A
2	WT	RES	Medium (Ct 27.5)	N/A
3	Low Positive V600E (below LoD)	CNB	Low (Ct 31)	N/A
4	V600E	RES	High (Ct 23.5)	LoD (2.0%)
5	V600E	RES	Medium (Ct 27.5)	LoD (3.5%)
6	V600E	CNB	Low (Ct 31)	LoD (7.8%)
7	V600E	RES	Low (Ct 31)	LoD (7.8%)
8	V600E	RES	Low (Ct 31)	2xLoD (15.6%)
9	V600E	RES	Medium (Ct 27.5)	2xLoD (7.0%)

Table 11: Proportion of Correct Calls for Each Test Panel

Grouping Variable(s)			Proportion		Two-Sided 95% Confidence Limit	
Sample	Acquisition Method	Mutation Level	Fraction	Percentage	Lower	Upper
Test Panel 1	RES	WT	81 / 81	100.00%	95.55%	100.00%
Test Panel 2	RES	WT	81 / 81	100.00%	95.55%	100.00%
Test Panel 3	CNB	Low Positive (below LoD)	78 / 81	96.30%	89.56%	99.23%
Test Panel 4	RES	LOD	81 / 81	100.00%	95.55%	100.00%
Test Panel 5	RES	LOD	81 / 81	100.00%	95.55%	100.00%
Test Panel 6	CNB	LOD	80 / 81	98.77%	93.31%	99.97%
Test Panel 7	RES	LOD	81 / 81	100.00%	95.55%	100.00%
Test Panel 8	RES	2 X LOD	81 / 81	100.00%	95.55%	100.00%
Test Panel 9	RES	2 X LOD	79 / 79	100.00%	95.44%	100.00%

Table 12: Variance Components Analysis for Repeatability and Intermediate Precision Evaluation

Alignment	Mutation Level	Number of Amplified	Number of	Mean	Between Run	Between Kit Lot (SD, % CV)	Between Operator (SD, % CV)	Between Instrument	Between Day (SD, % CV)	Between Template	Residual (SD, % CV)	#Total (SD, % CV)
Delta Ct	2 X LOD	160	0	3.57	(0.0844, 2.36%)	(0.0647, 1.81%)	(0.0559, 1.56%)	(0.0000, 0.00%)	(0.0445, 1.25%)	(1.5281, 42.75%)	(0.4967, 13.90%)	(1.1981, 33.52%)
	LOD	323	1	3.90	(0.0653, 1.68%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(1.3368, 34.32%)	(0.4267, 10.95%)	(1.2365, 31.74%)
Green Ct (Control Assay)	2 X LOD	160	0	28.75	(0.1349, 0.47%)	(0.0000, 0.00%)	(0.0926, 0.32%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(2.9368, 10.22%)	(0.2591, 0.90%)	(2.1049, 7.32%)
	LOD	324	0	27.73	(0.0913, 0.33%)	(0.0476, 0.17%)	(0.0361, 0.13%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(3.8626, 13.93%)	(0.1695, 0.61%)	(3.3562, 12.10%)
	WT	243	0	29.91	(0.0517, 0.17%)	(0.0314, 0.11%)	(0.0000, 0.00%)	(0.0063, 0.02%)	(0.0000, 0.00%)	(2.1832, 7.30%)	(0.2490, 0.83%)	(1.8045, 6.03%)
Green Ct (V600E Assay)	2 X LOD	160	0	32.32	(0.0000, 0.00%)	(0.0934, 0.29%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(1.4071, 4.35%)	(0.4463, 1.38%)	(1.0955, 3.39%)
	LOD	324	0	31.63	(0.1527, 0.48%)	(0.0419, 0.13%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(2.9075, 9.19%)	(0.4400, 1.39%)	(2.5646, 8.11%)

The table above shows the standard deviation estimated for each of the investigational factors. The precision study met the prespecified acceptance criteria.

b) Reproducibility Study

The purpose of this study was to assess the between-site precision (reproducibility) of the *therascreen* BRAF V600E RGQ PCR Kit at 3 clinical testing sites. The same "Test Panel Members" were used for both the Reproducibility (this study) and the Repeatability and Intermediate Precision Study (Section 6a). At each test site, three runs were performed (split between two operators, two RGQ instruments) over a three-day testing period. Each test panel was tested with 3 replicates in each PCR run, for a total of 27 replicates per test panel per site (81 reps across all three sites). The proportion of correct mutation calls for all test panels across three sites is provided in Table 13.

The proportion of correct mutation calls per site was provided for Test Panel 6 only in Table 14 (as Test Panel 6 was the only sample that produced one single incorrect call).

Table 13: Percentage Correct Mutation Call across 3 Sites for Reproducibility Study

Grouping Variable(s)			Proportion		Two-Sided 95% Confidence Limit	
Sample	**Acquisition Method**	**Mutation Level**	**Fraction**	**Percentage**	**Lower**	**Upper**
Test Panel 1	**RES**	**WT**	81 / 81	100.00%	95.55%	100.00%
Test Panel 2	**RES**	**WT**	80 / 80	100.00%	95.49%	100.00%
Test Panel 3	**CNB**	**WT**	81 / 81	100.00%	95.55%	100.00%
Test Panel 4	**RES**	**LOD**	81 / 81	100.00%	95.55%	100.00%
Test Panel 5	**RES**	**LOD**	81 / 81	100.00%	95.55%	100.00%
Test Panel 6	**CNB**	**LOD**	80 / 81	98.77%	93.31%	99.97%
Test Panel 7	**RES**	**LOD**	81 / 81	100.00%	95.55%	100.00%
Test Panel 8	**RES**	**2 X LOD**	81 / 81	100.00%	95.55%	100.00%
Test Panel 9	**RES**	**2 X LOD**	81 / 81	100.00%	95.55%	100.00%

Table 14: Proportion of Correct Mutation Calls Per Site (for Test Panel 6)

Grouping Variable(s)				Proportion		Two-Sided 95% Confidence Limit	
Sample	**Acquisition Method**	**Mutation Level**	**Site**	**Fraction**	**Percentage**	**Lower**	**Upper**
Test Panel 6	**CNB**	**LOD**	**1**	26 / 27	96.30%	81.03%	99.91%
			2	27 / 27	100.00%	87.23%	100.00%

Grouping Variable(s)				Proportion		Two-Sided 95% Confidence Limit	
Sample	Acquisition Method	Mutation Level	Site	Fraction	Percentage	Lower	Upper
			3	27 / 27	100.00%	87.23%	100.00%

The summary statistics output for the Reproducibility study is provided in Table 15. The table shows the standard deviation estimated for each of the investigational factors. For example, the site-to-site observed variation (corresponding to the reproducibility of the assay) in ΔCt for 2xLOD samples is equal to 0.08 ΔCt.

Table 45: Summary Statistics Outputs for Reproducibility

Analysis Variable	Mutation Level	Number of Amplified	Number of Non-Amplified	Mean	Between Site (SD, % CV)	Between Run Key Order (SD, % CV)	Between Template (SD, % CV)	Between Operator Within Site (SD, % CV)	Between Instrument (SD, % CV)	Between Day Within Site (SD, % CV)	Residual (SD, % CV)	#Total (SD, % CV)
Delta Ct	2 X LOD	162	0	3.64	(0.0800, 2.20%)	(0.0587, 1.61%)	(1.4756, 40.50%)	(0.1004, 2.75%)	(0.0439, 1.21%)	(0.1006, 2.76%)	(0.4654, 12.77%)	(1.1570, 31.75%)
	LOD	323	1	3.89	(0.0460, 1.19%)	(0.0747, 1.92%)	(1.3340, 34.33%)	(0.0507, 1.31%)	(0.0000, 0.00%)	(0.0704, 1.81%)	(0.4486, 11.54%)	(1.2459, 32.07%)
Green Ct (Control Assay)	2 X LOD	162	0	28.61	(0.1208, 0.42%)	(0.0803, 0.28%)	(2.9372, 10.27%)	(0.0830, 0.29%)	(0.0511, 0.18%)	(0.0000, 0.00%)	(0.1878, 0.66%)	(2.0976, 7.33%)
	LOD	324	0	27.62	(0.1260, 0.46%)	(0.0770, 0.28%)	(3.8494, 13.93%)	(0.0240, 0.09%)	(0.0624, 0.23%)	(0.0000, 0.00%)	(0.1518, 0.55%)	(3.3452, 12.11%)
	WT	242	0	29.82	(0.0821, 0.28%)	(0.0893, 0.30%)	(2.1804, 7.31%)	(0.0478, 0.16%)	(0.0279, 0.09%)	(0.0000, 0.00%)	(0.2150, 0.72%)	(1.7971, 6.03%)
Green Ct (V600E Assay)	2 X LOD	162	0	32.25	(0.2626, 0.81%)	(0.1537, 0.48%)	(1.4599, 4.53%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.4617, 1.43%)	(1.1639, 3.61%)
	LOD	323	1	31.50	(0.1957, 0.62%)	(0.0849, 0.27%)	(2.9052, 9.22%)	(0.0780, 0.25%)	(0.0000, 0.00%)	(0.0810, 0.26%)	(0.4253, 1.35%)	(2.5652, 8.14%)

c) Lot Interchangeability

To demonstrate lot-to-lot interchangeability for the *therascreen* BRAF V600E RGQ PCR Kit, 3 kit lots were used to test 8 clinical CRC specimens including both confirmed MT and WT to BRAF V600E (The same test panel members as the Repeatability Study were used, except that Test panel 3 CNB WT was removed due to it being a low positive sample). The lowest proportion of correct calls was 96.30% for each *therascreen* BRAF V600E RGQ PCR Kit lot (Table 16) showing the reproducibility of the kit lots.

Table 16: Proportion of Correct Calls per Kit Lot

Grouping Variable(s)			Proportion		Two-Sided 95% Confidence Limit	
Mutation Level	**Method**	**Kit Lot**	**Fraction**	**Percentage**	**Lower**	**Upper**
2 X LOD	**RES**	**1**	53 / 53	100.00%	93.28%	100.00%
		2	53 / 53	100.00%	93.28%	100.00%
		3	54 / 54	100.00%	93.40%	100.00%
LOD	**RES**	**1**	81 / 81	100.00%	95.55%	100.00%
		2	81 / 81	100.00%	95.55%	100.00%
		3	81 / 81	100.00%	95.55%	100.00%
	CNB	**1**	27 / 27	100.00%	87.23%	100.00%
		2	27 / 27	100.00%	87.23%	100.00%
		3	26 / 27	96.30%	81.03%	99.91%
WT	**RES**	**1**	54 / 54	100.00%	93.40%	100.00%
		2	54 / 54	100.00%	93.40%	100.00%
		3	54 / 54	100.00%	93.40%	100.00%

d) Sample Handling and Tumor Heterogenicity

A study was conducted to assess sample handling variability, including site variation, within the DNA extraction procedures.

This study focused on the DNA extraction of clinical FFPE samples to demonstrate that different laboratories produce the same results starting from the same clinical samples (different sections taken from the same FFPE block).

Eight FFPE CRC clinical specimens representing different DNA input levels and mutation levels were extracted at three different laboratories using three different lots of the QIAamp DSP DNA FFPE Tissue Kit. For each of the 8 FFPE specimens (4 WT and 4 MT), 36 sections were distributed evenly across three different sites.

A total of 6 extractions per sample (two sections per extraction) were performed at each of the three different test sites using 3 extraction kit lots (18 extraction in total per RES sample). A total of 12 extractions per CNB sample (2 sections per extraction) were performed at a single test site using 3 extraction kit lots. The extracted DNA samples were tested at a single site using a single lot of the *therascreen* BRAF V600E RGQ PCR Kit. The percentage of correct mutation calls for MT and WT samples across 3 sites is provided in Table 17.

The results observed for extractions across multiple FFPE sections were highly concordant suggesting tumor heterogenicity would not impact on the *therascreen* BRAF V600E RGQ PCR Kit test results when neoplastic cells are confirmed to be present by a pathologist.

Table 5: Proportion of Correct Calls by Extraction Site

Grouping Variable(s)			Proportion		Two-Sided 95% Confidence Limit	
Sample Level	**Acquisition Method**	**Extraction Site**	**N Correct / N Total**	**Proportion**	**95% Lower Confidence Limit**	**95% Upper Confidence Limit**
MT	RES	Site 1	24 / 24	100.00%	85.75%	100.00%
	RES	Site 2	24 / 24	100.00%	85.75%	100.00%
	RES	Site 3	24 / 24	100.00%	85.75%	100.00%
WT	CNB	Site 1	12 / 12	100.00%	73.54%	100.00%
	RES	Site 1	23 / 24	95.83%	78.88%	99.89%
	RES	Site 2	23 / 24	95.83%	78.88%	99.89%
	RES	Site 3	24 / 24	100.00%	85.75%	100.00%

5. Tumor Cell Content

Different proportions of Tumor Content (TC%) were investigated to demonstrate that the *therascreen* BRAF V600E RGQ PCR Kit can detect BRAF V600E mutations across a range of sections with different amounts of tumor. Samples with no neoplastic cells were not qualified for inclusion in this test.

The TC% against BRAF V600E results were plotted using data obtained from the BEACON clinical trial (the sub-population selected for the Accuracy study). A total of 599 samples were included in the analysis. Within this population, 18 samples had TC% $\leq$ 5% (13 samples were negative, and 5 samples were positive with the *therascreen* BRAF V600E RGQ PCR Kit). Otherwise the results demonstrated that tumor content does not correlate with the CWR Ct values and that the assay works across the range of tumor content.

6. Specimen Handling

The first objective of this study was to confirm that the *therascreen* BRAF V600E RGQ PCR Kit mutation status would remain unchanged when testing DNA samples extracted with and without macrodissection from specimens with low TC%. Sixty clinical CRC FFPE samples with TC% ranging from 5% to 50% were extracted with and without macrodissection and then tested with the *therascreen* BRAF V600E RGQ PCR kit. 58 samples produced valid results. The PPA, NPA and OPA between the macrodissected and non-macrodissected extraction procedures are reported in Table 18.

Table 16: Agreement Summary Table between Extraction Method with and without Macrodissection

Measure of Agreement	Frequencies	Percent Agreement	Clopper-Pearson (Exact) Binomial Lower Two-sided 95% Confidence Limit	Clopper- Pearson (Exact) Binomial Upper Two- sided 95% Confidence Limit
Overall Percent Agreement	58/58	100.00	93.84	100.00
Positive Percent Agreement	18/18	100.00	81.47	100.00
Negative Percent Agreement	40/40	100.00	91.19	100.00

The second objective of this study was to verify that BRAF V600E mutation detection at close to LoD level would not have been impacted by samples extracted with and without macrodissection.

Positive clinical samples were extracted with and without macrodissection and tested at LoD level in multiple replicates. Both with and without macrodissection groups produced a positive rate ≥ 95% meeting the study acceptance criterion for LOD samples (Table 19). A Fisher Exact test also demonstrated no statistically significant difference observed between the 2-extraction methods Table 20).

Table 17. Proportion of correct calls for Macro and Non-Macro Extraction Methods

Grouping Variable(s)	Proportion		Two-Sided 95% Confidence Limit	
Sample Process	Fraction	Percentage	Lower	Upper
Macro	28 / 28	100.00%	87.66%	100.00%
Non-Macro	27 / 28	96.43%	81.65%	99.91%

Table 20: Fishers Exact Test – Difference between Macro and Non-Macro Extraction Methods

Obs	diff	Risk	Percentage Difference (%)	p-value
1	Macro - Non-macro	0.04	3.57	1.000

7. Guard Band Studies

For all Guard Band studies, two clinical CRC WT and two FFPE CRC MT at approximately 2x LoD were used.

a) Volumetric Guard Band

The objective of this study was to verify the tolerance of the *therascreen* BRAF V600E RGQ PCR Kit to volumetric variations that can be introduced by the end user.

The volumetric tolerance was tested by varying the volume of each individual component while keeping the volume of the other components constant. Each component volume was varied by ± 6%. This represents the total error that can be introduced by pipetting, calculated using accuracy and precision data for a standard pipette.

When the individual component volume was varied by ±6% the difference in means of Delta Ct (For MT sample) or Control Ct (For WT samples) between each pair (test vs nominal conditions) were assessed to be within +/- 2×SD (calculated in the Repeatability Study for the *therascreen* BRAF V600E RGQ PCR Kit). The variation observed due to the test conditions are therefore within the normal variation expected with the *therascreen* BRAF V600E RGQ PCR Kit. In addition, 100% correct mutation calls were observed, for all tested samples, for all conditions.

b) Thermal Cycling Guard Band

The objective of this study was to verify the tolerance of the BRAF Kit to temperature variations of the annealing step during routine PCR cycling. When the PCR annealing temperature was varied by ±1°C, the difference in means of ΔCt (For MT sample) or Control Ct (For WT samples) between each pair (test vs nominal conditions) were assessed to be within +/- 2×SD (calculated in the Repeatability Study for the *therascreen* BRAF V600E RGQ PCR Kit), except for one WT sample at 59°C condition. However, the proportion of correct calls across all samples, all test conditions was 100%.

c) Master Mix Guard Band

This study was designed to verify the robustness of the *therascreen* BRAF V600E RGQ PCR system during PCR setup, more specifically the master mix to a prolonged time on the bench prior to the addition of samples.

The effect of keeping Master mix at ambient temperature for up to 4 hours or in the fridge (2°C to 8°C) for up to 24 hours on the *therascreen* BRAF V600E RGQ PCR Kit mutation status was evaluated. The difference in means of Delta Ct (For MT sample) or Control Ct (For WT samples) between each pair (test vs nominal conditions) were assessed to be within +/- 2×SD (calculated in the Repeatability Study for the *therascreen* BRAF V600E RGQ PCR Kit). The proportion of correct mutation calls for all test samples was 100%.

d) Extraction Guard Band Study

The objective of this study was to confirm the extraction tolerance of the *therascreen* BRAF V600E RGQ PCR Kit to potential variation introduced during routine execution in the extraction protocol.

Eight clinical FFPE CRC specimens (4 WT and 4 MT) were used to assess different temperature and length (minutes) of proteinase K incubation and formalin de-crosslinking as shown in Table 21.

Table 81: Variations Introduced in to the Extraction Protocol

Extraction Step	Low	Nominal	High
Proteinase K temp.	53°C	56°C	59°C
Proteinase K time	30 min	60 min	90 min
De-crosslinking temp.	85°C	90°C	95°C
De-crosslinking time	30 min	60 min	90 min

Although some conditions did not meet the ±2xSD of the intermediate precision (calculated in the Repeatability Study for the *therascreen* BRAF V600E RGQ PCR Kit) acceptance criteria for WT samples, the frequency of correct calls

observed for all test conditions was 100.00% (note: there were 2 false positive calls observed in the nominal condition for proteinase K).

8. Specimen Stability

a) Stability of FFPE Sections and Extracted DNA

The stability of specimens was assessed for:

- FFPE section stability stored at room temperature (in dark).
- Extracted FFPE gDNA sample stability stored at -20ºC (-35ºC to -15ºC).

The study tested 6 CRC samples (3 WT samples and 3 BRAF V600E MT samples) over a period of 25 months (N+1 month, to claim FFPE section and DNA stability for up to 24 months) at 9 different time points (including initial testing) with three replicates per sample tested per test point. FFPE Stability was assessed from T=0 through T=8 only. DNA stability was assessed at T=9, T=10, T=11, T=12 and T=13. The T=12 timepoint was performed to provide an n+1 timepoint, as 33 weeks will be claimed as maximum stability.

The frequency of correct call for all test samples was 100% for both FFPE sections and gDNA. FFPE section stability supported a stability claim of 24 months (N-1timepoint). Extracted DNA data supported a stability claim of 33 months when stored under appropriate conditions.

b) Kit Stability

The *therascreen* BRAF V600E RGQ PCR Kit stability testing included

- Real time storage (shelf-life)
- In-Use stability (including up to 6 freeze-thaw cycles and open vial, post-transport simulation).
- Transport simulation study (integrated in Real-Time and In-Use stability study).

For all Real-Time and In-Use stability Test Timepoints (TTP), the same batch of pooled extracted DNA clinical samples (BRAF V600E WT and MT samples at approximately 2xLoD) were used. Transport Simulation and In-Use stability were assessed using three *therascreen* BRAF V600E RGQ PCR Kits. Real-Time stability was assessed using three *therascreen* BRAF V600E RGQ PCR Kit lots. A minimum of 12 WT and MT replicates were tested at each TTP for the Real-Time stability study. A minimum of 6 WT and MT replicates were tested at each TTP for In-Use stability.

Real Time Stability Testing

Real-Time stability testing was performed at 4 testing time points up to 10 months (TTP0, TTP4, TTP7 and TTP10) generated from WT and BRAF V600E MT (2xLoD) samples. The Real-Time timepoints for WT and MT samples showed 100% correct calls. The Real-time stability study supported stability of the BRAF V600E Kit at 10 months of storage at the defined temperature conditions. The Real-time stability claim is for 10 months at --30 to –15°C. Real-Time stability (closed bottle, post-transport simulation) planned for up to 25 months.

In-Use Stability Testing

In-use stability testing was performed at 3 testing time points up to 7 months (TTP0, TTP4 and TTP7). For all In-use time points tested, the percentage of correct mutation status was 100%. The In-use stability claim is for 6-freeze/thaw cycles (N-1) for the first 7 months of storage at -20°C.

Transport Simulation Study

Study kits were exposed to conditions designed to simulate the extremes of environmental factors that may be experienced during the distribution from the manufacturing site to the customer. To ensure the worst-case scenario was simulated, 3 cycles of transport conditions were applied. The first and second cycle were proposed to simulate the transportation to the warehouses and subsequent interim storage; the third cycle represented transport to the customer. Kits subjected to transport conditions were also used for Real-time and In-use studies. For the Real-time stability study 1 kit lot was subjected to simulated transport cycles in the final packaging after time point 0. For the In-use study, all kit lots were subjected to transport conditions before time point zero to reflect the customer use. The time points and conditions are shown in Table 22 below. The transport simulation study was integrated into the Real-time study. The transport simulation claim is 9 months at -20°C.

Table 22:Transport Simulation Conditions

Cycle	Duration	Place	Temperature
1	5 days ± 2 hours	On dry ice	n/a
	2 days ± 2 hours	Freezer	-30°C to -15°C
2	5 days ± 2 hours	On dry ice	n/a
	2 days ± 2 hours	Freezer	-30°C to -15°C
3	5 days ± 2 hours	On dry ice	n/a
	2 days ± 2 hours	Freezer	-30°C to -15°C
	Storage until testing is required	Freezer	-30°C to -15°C

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Array BioPharma Inc (Array) is the developer of encorafenib for the treatment of metastatic colorectal (mCRC) patients with a BRAF V600E mutation. BRAF V600 mutations lead to constitutive activation of BRAF kinase and sustained RAS/RAF/MEK/ERK pathway signaling, resulting in increased cell proliferation and survival. Encorafenib is a BRAF kinase inhibitor and cetuximab is an EGFR inhibitor.

Array was the sponsor of the BEACON study (Clinical trial number ARRAY-818-302; NCT01909453). Eligible patients were required to have BRAF V600E mutation-positive metastatic colorectal cancer (CRC), as detected using an investigational QIAGEN *therascreen* BRAF V600E RGQ polymerase chain reaction (PCR) Kit clinical trial assay (CTA). Clinical studies of the device were initiated on September 28, 2016 and were conducted under an approved investigational device exemption. Patients were enrolled into the BEACON study between October 9, 2016 and January 31, 2019 with data cutoff date of February 11, 2019. The database for this PMA included test results from 1688 patients (11 patients were removed due to no patient information available in the database).

The BEACON study used results from local BRAF V600E local laboratory testing (LDT) to identify patients for enrollment. The QIAGEN CTA Assay was used as central laboratory confirmation of each enrolled subject's BRAF mutation status or to determine BRAF V600E mutation status for patients without a local LDT result. The validated CDx assay contains software design changes to the CTA assay used in the BEACON study and therefore upon completion of the BEACON study, a Bridging Study was conducted; both to demonstrate concordance between the LDTs and CDx assay and between the CTA and CDx assays and to assess the impact of the use of LDT's on mutation prevalence and efficacy reporting.

The efficacy and safety of the combination of encorafenib and cetuximab was evaluated in Array's BEACON study. The BEACON study and the bridging study between the Clinical Trial Assay (CTA) and the *therascreen* BRAF V600E RGQ PCR Kit were the clinical basis for providing evidence of clinical performance. The *therascreen* BRAF V600E RGQ PCR Kit has been validated by QIAGEN as a companion diagnostic device (CDx) for a combination of BRAFTOVI® (encorafenib) and cetuximab, for the treatment of BRAF V600E mutant mCRC.

This device SSED comprises a summary of the device clinical performance studies using the BEACON clinical study results to support the assurance of safety and effectiveness of the *therascreen* BRAF V600E RGQ PCR Kit when used in accordance with its intended use.

The study analyses conducted and summarized in this SSED are as follows:

- Efficacy of the drug combination based on the CTA results.
- Efficacy of the drug combination using the CDx results, including adjustment for the use of LDTs during patient pre-screening (i.e., prior to BRAF CTA test).

The major efficacy outcome measure was overall survival (OS). The results demonstrated that BRAFTOVI in combination with cetuximab demonstrated a statistically significant improvement in OS for mCRC patients whose tumors have BRAF V600E mutations detected by the QIAGEN *therascreen* BRAF V600E RGQ PCR Kit

A. Study Design – BEACON Clinical Trial

The BEACON CRC Study (ARRAY-818-302; NCT01909453), was a Phase 3 multicenter, randomized, open-label, 3-arm study designed to evaluate the efficacy and safety of encorafenib + cetuximab + binimetinib (referred to as the Triplet arm) and encorafenib + cetuximab (referred to as the Doublet arm) versus Investigator's choice of either irinotecan/cetuximab or FOLFIRI/cetuximab (Control arm) in patients with BRAF V600E mCRC whose disease had progressed after 1 or 2 prior regimens in the metastatic setting. Randomization was stratified by Eastern Cooperative Oncology Group (ECOG) performance status (0 versus 1), prior use of irinotecan (yes versus no), and cetuximab product used (US-licensed versus EU-approved). The primary endpoints of the BEACON study were OS (Triplet arm vs. Control arm) and confirmed ORR by Blinded independent central review (BICR) per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 (Triplet arm vs. Control arm). The secondary endpoints of the BEACON study were OS and ORR by BICR per RECIST v1.1 of the Doublet Arm compared against the Control Arm.

This SSED presents analysis from the Doublet Arm against the Control Arm, in order to align with NDA labelling. Two committees were involved in the conduct of the BEACON study: a Steering Committee (SC) and a Data Monitoring Committee (DMC). The SC was appointed to oversee the study conduct. A detailed description of the SC responsibilities, membership and procedures were outlined in the SC Charter.

A detailed description of the DMC responsibilities, membership and operations were outlined in the DMC Charter. The control group was treated with a legally marketed alternative with similar indications for use.

1. Inclusion and Exclusion Criteria for Molecular Screening

Enrollment in the BEACON study occurred two ways.

- Population 1: Patients who satisfied the Screening eligibility criteria, were enrolled based on local BRAF test result (LDT), and were retrospectively confirmed to have the BRAF V600E mutation status with the central CTA, or
- Population 2: Patients were tested prospectively to determine BRAF V600E mutation status with the CTA..

a) Molecular Pre-screening Inclusion Criteria

All of the following inclusion criteria had to be met for a patient to be eligible to undergo molecular tumor pre-screening:

- Provide a signed and dated Prescreening informed consent document
- Age ≥ 18 years at time of informed consent
- Histologically- or cytologically-confirmed CRC that is metastatic
- Eligible to receive cetuximab per locally approved label with regard to tumor RAS status
- Able to provide a sufficient amount of representative tumor specimen (primary or metastatic, archival or newly obtained) for central laboratory testing of BRAF and KRAS mutation status (minimum of 6 slides; optimally up to 15 slides).

b) Molecular Pre-screening Exclusion Criteria

Patients meeting any of the following criteria at Prescreening were not eligible to undergo molecular tumor prescreen:

- Leptomeningeal disease
- History or current evidence of RVO or current risk factors for RVO (e.g., uncontrolled glaucoma or ocular hypertension, history of hyperviscosity or hypercoagulability syndromes)
- Known history of acute or chronic pancreatitis
- History of chronic inflammatory bowel disease or Crohn's disease requiring medical intervention (immunomodulatory or immunosuppressive medications or surgery) ≤ 12 months prior to randomization
- Concurrent neuromuscular disorder that is associated with the potential of elevated CK (e.g., inflammatory myopathies, muscular dystrophy, amyotrophic lateral sclerosis, spinal muscular atrophy)
- Known history of human immunodeficiency virus (HIV) infection
- Known history of Gilbert's syndrome or is known to have any of the following genotypes: UGT1A1*6/*6, UGT1A1*28/*28, or UGT1A1*6/*28
- Known contraindication to receive cetuximab or irinotecan at the planned doses; refer to the most recent cetuximab and irinotecan summary of product characteristics (SPC) or local label as applicable
- Prior anti-EGFR treatment
- More than 2 prior regimens in the metastatic setting
  - Disease relapse during treatment or within 6 months following adjuvant therapy will be considered metastatic disease.
  - Maintenance therapy given in the metastatic setting will not be considered a separate regimen.

2. <u>Clinical Inclusion and Exclusion Criteria for Patient Enrollment</u>

In the event of a positive CTA mutation result, a patient was confirmed eligible and enrolled if they also satisfied the remaining eligibility criteria. Otherwise, the patient was excluded.

a) Enrollment Inclusion Criteria (abridged list)

All of the following inclusion criteria had to be met for a patient to be eligible to be included in this study:

- Provide a signed and dated Screening informed consent document
- Age ≥ 18 years at time of informed consent
- Histologically- or cytologically-confirmed CRC that is metastatic
- Presence of BRAF V600E in tumor tissue as previously determined by a local assay at any time prior to Screening or by the central laboratory. Notes:
    - Only PCR and NGS-based local assays results will be acceptable
    - If at any time in the Phase 3 portion of the study there is lack of BRAF V600E confirmation by the central laboratory (for any reason including discordance and inadequate available tissue) in 37 total patients or discordance (a valid result of "no BRAF V600E mutation" as determined by the central laboratory) between the local assay and the central laboratory in 18 patients, all subsequent patients will be required to have BRAF V600E determined by the central laboratory prior to enrollment.
    - Central testing cannot be repeated to resolve discordances with a local result once the central laboratory delivers a definitive result (positive or negative).
    - If the result from the central laboratory is indeterminate or the sample is deemed as inadequate for testing, additional samples may be submitted.
    - Results from local laboratories with more than 1 discordant result leading to patient enrollment will not be accepted for further patient enrollment.
    - Sites with more than 2 randomized patients having indeterminate results after initiation of protocol version 6 will be required to enroll all subsequent patients based only on central laboratory assay results.

- Able to provide a sufficient amount of representative tumor specimen (primary or metastatic, archival or newly obtained) for confirmatory central laboratory testing of BRAF and KRAS mutation status (minimum of 6 slides; optimally up to 15 slides).
- Eligible to receive cetuximab per locally approved label with regard to tumor RAS status
- Progression of disease after 1 or 2 prior regimens in the metastatic setting. Notes:
    - Disease relapse during treatment or within 6 months following adjuvant therapy will be considered metastatic disease.
    - Patients who have received 2 prior regimens (i.e., those entering the study in the 3rd line setting), must have received or have been offered and refused prior oxaliplatin unless it was contraindicated due to underlying conditions.
    - Maintenance therapy given in the metastatic setting will not be considered a separate regimen.

- In the Phase 3 portion of study, the number of patients having received 2 prior regimens will be limited to 215 (35% of the total randomized). Patients with 2 prior regimens who have entered Screening at the time that the limit has been reached will be permitted to continue into the study if they are otherwise determined to be eligible.
- Evidence of measurable or evaluable non-measurable disease per RECIST, v1.1
- ECOG PS of 0 or 1

b) Enrollment Exclusion Criteria (abridged list)

Patients meeting any of the following criteria at screening were not included in the study:

- Prior treatment with any RAF inhibitor, MEK inhibitor, cetuximab, panitumumab or other EGFR inhibitors
- Prior irinotecan hypersensitivity or toxicity that would suggest an inability to tolerate irinotecan 180 mg/m2 every 2 weeks
- Symptomatic brain metastasis Notes: Patients previously treated or untreated for this condition who are asymptomatic in the absence of corticosteroid and anti-epileptic therapy are allowed. Brain metastases must be stable for ≥ 4 weeks, with imaging (e.g., magnetic resonance imaging [MRI] or computed tomography [CT]) demonstrating no current evidence of progressive brain metastases at screening.
- Leptomeningeal disease
- History or current evidence of RVO or current risk factors for RVO (e.g., uncontrolled glaucoma or ocular hypertension, history of hyperviscosity or hypercoagulability syndromes)
- Use of any herbal medications/supplements or any medications or foods that are strong inhibitors or inducers of cytochrome P450 (CYP) 3A4/5 ≤ 1 week prior to the start of study treatment
- Known history of acute or chronic pancreatitis
- History of chronic inflammatory bowel disease or Crohn's disease requiring medical intervention (immunomodulatory or immunosuppressive medications or surgery) ≤ 12 months prior to randomization
- Impaired cardiovascular function or clinically significant cardiovascular diseases, including any of the following:
  - History of acute myocardial infarction, acute coronary syndromes (including unstable angina, coronary artery bypass graft [CABG], coronary angioplasty or stenting) ≤ 6 months prior to start of study treatment;
  - Symptomatic congestive heart failure (i.e., Grade 2 or higher), history or current evidence of clinically significant cardiac arrhythmia and/or conduction abnormality ≤ 6 months prior to start of study treatment, except atrial fibrillation and paroxysmal supraventricular tachycardia.
- Uncontrolled hypertension defined as persistent elevation of systolic blood pressure ≥ 150 mmHg or diastolic blood pressure ≥ 100 mmHg despite current therapy
- Impaired hepatic function, defined as Child-Pugh class B or C

- Impaired GI function or disease that may significantly alter the absorption of encorafenib or binimetinib (e.g., ulcerative diseases, uncontrolled vomiting, malabsorption syndrome, small bowel resection with decreased intestinal absorption)
- Concurrent or previous other malignancy within 5 years of study entry, except cured basal or squamous cell skin cancer, superficial bladder cancer, prostate intraepithelial neoplasm, carcinoma in-situ of the cervix, or other noninvasive or indolent malignancy without Sponsor approval

- History of thromboembolic or cerebrovascular events ≤ 6 months prior to starting study treatment, including transient ischemic attacks, cerebrovascular accidents, deep vein thrombosis or pulmonary emboli
- Concurrent neuromuscular disorder that is associated with the potential of elevated CK (e.g., inflammatory myopathies, muscular dystrophy, amyotrophic lateral sclerosis, spinal muscular atrophy)
- Treatment with any of the following:
  - Cyclical chemotherapy within a period of time that was shorter than the cycle length used for that treatment (e.g., 6 weeks for nitrosourea, mitomycin-C) prior to starting study treatment
  - Biologic therapy (e.g., antibodies) except bevacizumab or aflibercept, continuous or intermittent small molecule therapeutics, or any other investigational agents within a period of time that is ≤ 5 half-lives (t1/2) or ≤ 4 weeks (whichever is shorter) prior to starting study treatment
  - Bevacizumab or aflibercept therapy ≤ 3 weeks prior to starting study treatment
  - Radiation therapy that included > 30% of the bone marrow
- Residual CTCAE ≥ Grade 2 toxicity from any prior anticancer therapy, with the exception of Grade 2 alopecia or Grade 2 neuropathy
- Known history of HIV infection
- Active hepatitis B or hepatitis C infection
- Known history of Gilbert's syndrome or is known to have any of the following genotypes: UGT1A1*6/*6, UGT1A1*28/*28, or UGT1A1*6/*28
- Known contraindication to receive cetuximab or irinotecan at the planned doses; refer to the most recent cetuximab and irinotecan SPC or local label as applicable

3. Follow-up Schedule

All patients were scheduled to return for follow-up examinations every 6 weeks (± 7 days) from the date of for the first 24 weeks of treatment, then every 12 weeks (± 7 days) thereafter until disease progression, withdrawal of consent, initiation of subsequent anticancer therapy, patient was lost to follow-up, death or defined end of study. Following discontinuation of the treatment period, patients were followed for survival every 3 months until withdrawal of consent, lost to follow-up, death or defined end of study.

4. Clinical Endpoints

This SSED presents analysis from the Doublet Arm against the Control Arm (which was the secondary endpoint), in conjunction with the corresponding NDA (210496) (See Section D. Safety and Effectiveness Results).

The Endpoints were the following:

- OS, defined as the time from randomization to death due to any cause, of Doublet Arm vs. Control Arm
- Confirmed ORR (by BICR) per RECIST, v1.1 of Doublet Arm vs. Control Arm

B. Accountability of PMA Cohort

A total of 1677 patients were screened for participation in the BEACON study, including both the SLI/JSLI cohorts and the randomized Phase 3. Of these, 975 patients (58.1%) discontinued either the pre-screening or screening phase and were not enrolled. A total of 665 patients were randomized. The majority of patients who were not enrolled or randomized due to inclusion/exclusion criteria not met did not have the required BRAF V600E mutation (56.9%), followed by patients who did not meet other inclusion/exclusion criteria (30.7%) and patients for whom no further informed consent was obtained (12.3%).

Of the 665 enrolled patients, 220 were included in the Doublet arm and 221 enrolled in the Control arm.

To conduct the Bridging study analysis, in total, 1934 test results, representing 1688 patients (patient ID) were included in the "QIAGEN Merged database". 11 patient IDs had no associated patient information available. This accounts for the lower total patient number screened for the BEACON study (1677), compared to patient IDs in the Bridging Study (1688). Of the 1677 patients screened, 1488 patient samples provided CTA results.

189 patients were excluded from analyses in the Bridging study because either they provided no CTA result (161) or they presented with no neoplastic cells (28). A further cohort of 39 patients from a safety lead in a Japanese subset were removed, which left 1449 patients included in bridging analyses. In the population screened with LDT a prevalence of close to 100% is expected. The non-screened population presents the most representative estimate of prevalence.

Table 93: Bridging Study Population Prevalence

BEACON Population	CTA Prevalence	CDx Prevalence
Population 1 with LDT screening	97.8% (544 / 556)	97.2% (520 / 535)
Population 2 without LDT screening	38.3% (285 / 745)	37.0% (280 / 756)
Overall	63.7% (829 / 1301)	62.0% (800 / 1291)

C. Study Population Demographics and Baseline Parameters

The CTA evaluable and CTA non-evaluable populations were consistent in terms of key demographic, specimen and sample handling characteristics (Table 24) and therefore the CTA evaluable population is representative of the wider study population.

Region, tumor content (%), MSI status, presence of liver metastases at baseline and number of organs involved based on target and non-target lesion assessment were statistically significantly different (at the 5% significance level) between the CTA evaluable and CTA non-evaluable patients.

Table 4: Demographic, Disease and Specimen Characteristics Data Comparing CTA Evaluable and CTA Non-Evaluable Patients (Population: Population 1 and 2)

	CTA Evaluable	CTA Unevaluable	pValue
Total	1301	182	
Age (yrs)			
Mean	59.4	57.9	0.144
Std	12	12.5	
Min	18	24	
Median	61	59	
Max	91	86	
N	1301	182	
Gender [N (%)]			
Female	633 (48.7)	83 (46.1)	0.51
Male	666 (51.3)	97 (53.9)	
MISSING	2	2	
Region [N (%)]			
Europe	685 (52.7)	64 (35.2)	<.001 #
North America	115 (8.8)	4 (2.2)	
Rest of World	501 (38.5)	114 (62.6)	
ECOG Status [N (%)]			
0	314 (50.3)	17 (63.0)	0.198
1	310 (49.7)	10 (37.0)	
MISSING	677	155	
Prior use of Irinotecan [N (%)]			
N	309 (49.5)	11 (40.7)	0.372
Y	315 (50.5)	16 (59.3)	
MISSING	677	155	

	CTA Evaluable	CTA Unevaluable	pValue
Cetuximab Source [N (%)]			
EU-approved	573 (86.8)	27 (93.1)	0.568 #
US-licensed	87 (13.2)	2 (6.9)	
MISSING	641	153	
Sample type [N (%)]			
CNB	137 (10.6)	19 (10.4)	1.000 #
FNA	7 (0.5)	1 (0.5)	
RES	1148 (88.9)	162 (89.0)	
MISSING	9	0	
Tumor Content (%)			
Mean	46.2	42.2	0.038
Std	22.8	24.7	
Min	0	0	
Median	45	41	
Max	100	100	
N	1300	182	
MISSING	1	0	
Necrotic Tissue (%)			
Mean	2.9	2.2	0.291
Std	8.4	8.2	
Min	0	0	
Median	0	0	
Max	77	85	
N	1129	169	
<1	26	2	
MISSING	146	11	
MSI Status [N (%)]			
Abnormal High	53 (9.4)	0 (0.0)	<.001 #
Abnormal Low	2 (0.4)	0 (0.0)	
Normal	481 (85.6)	8 (33.3)	
Not Evaluable	26 (4.6)	16 (66.7)	
MISSING	739	158	
Presence of liver metastases at baseline [N (%)]			

	CTA Evaluable	CTA Unevaluable	pValue
N	907 (69.7)	156 (85.7)	<.001
Y	394 (30.3)	26 (14.3)	
Removal status of primary tumor [N (%)]			
Completely Resected	384 (76.3)	9 (100.0)	0.126 #
Partially Resected	119 (23.7)	0 (0.0)	
MISSING	798	173	
C-reactive protein (CRP) baseline level	0	0	
Mean	0	0	0.177
Std	0	0	
Min	0	0	
Median	0.5	0.1	
Max	647	29	
N	654	153	
MISSING			
Side of Tumor [N (%)]	35 (5.3)	3 (10.3)	
Both Sides	229 (34.7)	10 (34.5)	0.304 #
Left Colon	357 (54.1)	13 (44.8)	
Right Colon	39 (5.9)	3 (10.3)	
Unknown Colon	641	153	
MISSING			
Number of organs involved based on Target and Non-target lesion assessment [N (%)]	311 (23.9)	16 (8.8)	
3+	990 (76.1)	166 (91.2)	<.001
<=2			

D. Safety and Efficacy Results

1. Safety Results

The safety with respect to treatment with BRAFTOVI™ and cetuximab will not be addressed in detail in this SSED. Refer to United States product insert for safety information on these treatments. No Adverse Device Effects occurred in the PMA clinical study.

The safety of the *therascreen* BRAF V600E RGQ PCR device is related to its accuracy, as false results may lead to inappropriate treatment decisions. A false negative result would prevent a patient from receiving a potentially beneficial treatment. A false positive result

would potentially expose the patient to an ineffective treatment with possible adverse effects associated with the therapeutic.

The accuracy of the BRAF V600E kit relative to the validated reference method of Sanger Sequencing, was determined using specimens from the BEACON study. The estimated OPA, PPA, and NPA between the BRAF V600E Kit and Sanger Sequencing (with Sanger as the reference method) were 95.20%, 100.00% and 91.11% respectively, demonstrating that the BRAF V600E Kit has high accuracy when compared to the reference method and that the possibility of false results is very low. Additionally, the use of the BRAF V600E kit poses minimum safety hazard to patients, as biopsy specimens are routinely used in diagnosis and staging of mCRC.

2. Effectiveness Results-Based on Mutation Detection with CTA

The analysis of effectiveness was based on the Secondary Efficacy endpoints of OS and ORR for Doublet Arm vs Control Arm in 665 mCRC patients with BRAF V600E mutation positive assessed by a clinical trial assay (CTA). The Secondary endpoints were used (rather than Primary endpoints) in order to align with amended NDA labelling.

Overall Survival

The study demonstrated statistically and clinically significant superiority in OS for the Doublet arm vs. the Control arm as summarized in Table 25 and Figure 1. The analysis of OS found 40% reduction in risk of death was observed for the Doublet arm compared to the Control arm (HR 0.60, 95% CI: 0.45, 0.79). The median OS in the Doublet arm was 2.99 months longer than that in the Control arm, with median OS estimates using Kaplan-Meier methodology of 8.41 months (95% CI: 7.46, 11.04) in the Doublet arm and 5.42 months (95% CI: 4.76, 6.57) in the Control arm (p = 0.0002, stratified log-rank test).

Table 25: Efficacy Results from BEACON CRC

	BRAFTOVI with cetuximab N = 220	**Irinotecan with cetuximab or FOLFIRI with cetuximab N = 221**
Overall Survival		
Number of Events (%)	93 (42)	114 (52)
Median OS, months (95% CI)	8.4 (7.5, 11.0)	5.4 (4.8, 6.6)
HR (95% CI)[a,b]	0.60 (0.45, 0.79)	
P-value[a,c]	0.0003	
Overall Response Rate (per BICR)		
ORR (95% CI)[d]	20% (13%, 29%)	2% (0%, 7%)
CR	5%	0%
PR	15%	2%
P-value[a,e]	<0.0001	
Median DoR, months (95% CI)	6.1 (4.1, 8.3)	NR (2.6, NR)

CI = Confidence interval; CR = Complete response; DoR = Duration of response; HR = Hazard ratio; NR = Not reached; ORR = Overall response rate; OS = Overall survival; PFS = Progression-free survival; PR = Partial response.

[a] Stratified by ECOG PS, source of cetuximab (US-licensed versus EU-approved) and prior irinotecan use at randomization.

[b] Stratified Cox proportional hazard model.

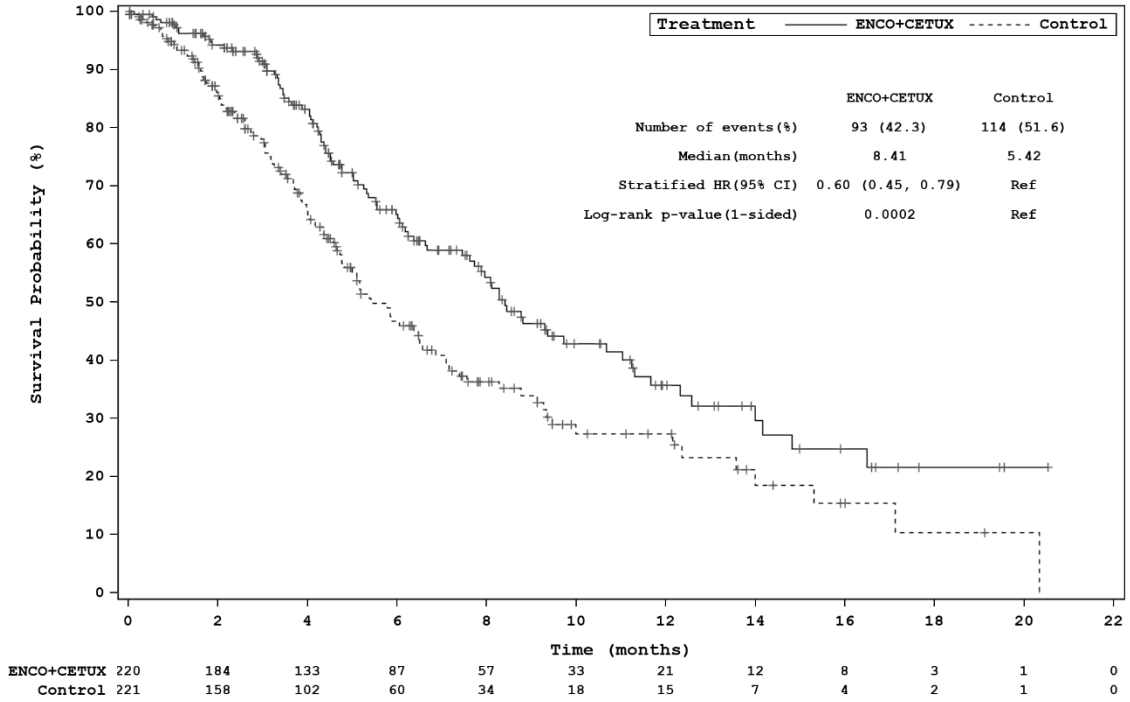
[c] Stratified log-rank test, tested at alpha level of 0.0084.

[d] BRAFTOVI/cetuximab arm (n=113) and control arm (n=107).

[e] Cochran-Mantel-Haenszel test; tested at alpha level of 0.05.

[f] Stratified log-rank test, tested at alpha level of 0.0234.

Figure 1: Kaplan-Meier Curves for Overall Survival in BEACON CRC (Randomized Phase 3, Full Analysis Set)



Abbreviations: CETUX = cetuximab; CI = confidence interval; ENCO = encorafenib; HR = hazard ratio; OS = overall survival; Ref = reference; vs. = versus
+ indicates censoring.

Overall Response Rate

The primary analysis of confirmed ORR by BICR found a significantly higher response rate in the Doublet arm compared to the Control arm in the Phase 3 Response Efficacy Set (20.4% vs. 1.9%) ($p < 0.0001$, Cochran Mantel Haenszel test). Confirmed Complete Response (CR) by BICR was observed in 5.3% and 0.0% of patients in the Doublet and Control arms, respectively (data not shown).

3. Effectiveness Results-based on mutation detection with CDx in BEACON study

In the BEACON study, the BRAF V600E mutation status for screening and enrollment of patients was determined by CTA. The concordance between the *therascreen* BRAF V600E RGQ PCR Kit (CDx) and the CTA was assessed. The measures of negative percent agreement (NPA), positive percent agreement (PPA) and overall agreement (OA) including the respective Clopper-Pearson exact two-sided 95% confidence intervals calculated from population 2 are provided in Table 26.

Table 106: Measures of Agreement Between CTA and CDx (CTA and CDx Evaluable patients from Population 2)

Reference Method	Measure of Agreement	Frequencies	Percent Agreement	Clopper-Pearson (Exact) Binomial Lower Two-sided 95% Confidence Limit	Clopper-Pearson (Exact) Binomial Upper Two-sided 95% Confidence Limit
N/A	OA	679/680	99.85	99.18	100.00
CDx	PPA	263/264	99.62	97.91	99.99
	NPA	416/416	100.00	99.12	100.00
CTA	PPA	263/263	100.00	98.61	100.00
	NPA	416/417	99.76	98.67	99.99

Efficacy analyses were conducted in terms of OS and ORR using data from randomized CDx positive patients (LDT+&CTA+&CDx+ in population 1 and CTA+&CDx+ in population 2) of the BEACON clinical trial population.

Note, these analyses do not include results from any CDx positive patients who were not randomized and so do not include results from any non-randomized CDx+ patients with negative LDT or CTA results. Therefore, these analyses do not include the entire CDx+ intended use population. Analyses to account for non-randomized CDx positive patients, thus including the entire CDx+ intended use population, are presented in Section 4.

Overall Survival

For the OS efficacy analysis, the median survival time in the Doublet arm was 8.4 months, compared to a median survival time of 5.2 months in the Control arm and the stratified hazard ratio was 0.550 (95% CI: (0.406, 0.744)), (Table 27). These results are similar to those reported from the BEACON study using CTA to enroll patients.

Table 27: Doublet vs Control: Overall Survival Efficacy Analysis for LDT+&CTA+&CDx+ patients in Population 1 and CTA+&CDx+ patients in population 2

	DoubletArm (N=188)	ControlArm (N=189)
Subjects (%) with events	80 (42.6%)	102 (54%)
Subjects (%) without events (censored)	108 (57.4%)	87 (46.0%)
Time to events (Months)[a]		
Median[a]		
95% CI for median	8.4	5.2
1st quartile	(7.5, 11.2)	(4.6, 6.5)
3rd quartile	4.5	3.1
	16.5	12.2
OS probability (95% CI)[b]		
3 Months		
6 Months	0.93 (0.88, 0.96)	0.77 (0.70, 0.83)
9 Months	0.66 (0.57, 0.73)	0.44 (0.35, 0.52)

12 Months	0.47 (0.37, 0.56)	0.32 (0.23, 0.40)
18 Months	0.36 (0.27, 0.46)	0.26 (0.18, 0.35)
21 Months	0.22 (0.12, 0.34)	0.08 (0.01, 0.25)
Stratified log-rank test[c]		
p-value (one-sided)	<.0001	
Unstratified Log-rank test[d]		
p-value (one-sided)	<.0001	
Hazard Ratio (relative to Control Arm)[c] (Stratified)	0.550	
95% CI	(0.406, 0.744)	
p-value for HR=1 (one-sided)	<.0001	
Hazard Ratio (relative to Control Arm)[d] (Unstratified)	0.569	
95% CI	(0.424, 0.763)	
p-value for HR=1 (one-sided)	<.0001	

[a] Median and quartiles are calculated using Kaplan-Meier method and confidence interval for median is calculated using the Brookmeyer-Crowley method.
[b] Survival probability and confidence interval are calculated based on Kaplan-Meier product-limit method and Greenwood's formulae.
[c] Stratification factors include ECOG status (1 vs. 0), prior use of irinotecan (yes vs no), cetuximab source (US licensed vs EU-approved)
[d] Unstratified analysis includes only treatment as a covariate in the Cox PH model.

4. Effectiveness results for CDx+ subjects with bridging analysis

Weighted average calculations were conducted to determine the clinical drug efficacy δ in CDx+ subjects.

$$\delta=\delta_1\times \Pr(\text{Population 1}) + \delta_2 \times \Pr(\text{Population 2})$$

Where δ_1 indicates the clinical drug efficacy in CDx+ subjects estimated from population 1 and δ_2 indicates the clinical drug efficacy in CDx+ subjects estimated from population 2.
δ_1 was estimated through bridging efficacy analysis from CTA+, LDT+ to CDx+ in population 1 and δ_2 was estimated through bridging efficacy analysis from CTA+ to CDx+ in population 2.

Table 28 show the hazard ratios for each of the values of r_1 and r_2 (r_1 indicates the proportion of (CTA- or LDT-) and CDx+ subjects efficacy to CTA+&LDT+&CDx+ subjects efficacy and r_2 indicates the proportion of CTA-&CDx+ subjects efficacy to CTA+&CDx+ subjects efficacy), with efficacy estimates based on stratified efficacy analyses, along with the corresponding non-parametric two-sided 95% confidence intervals. Stratification factors in the efficacy analysis include ECOG status (1 vs. 0), prior use of irinotecan (yes vs no), cetuximab source (US-licensed vs EU-approved).

With no efficacy in the non-randomized (CTA- or LDT-) and CDx+ patients in population 1 and CTA-&CDx+ patients in population 2 ($r_1 = 0$, $r_2 = 0$) the hazard ratio is 0.493, with the

same efficacy in the non-randomized (CTA- or LDT-) and CDx+ patients in population 1 and CTA-&CDx+ patients in population 2 as in the observed patients in population 1 and 2 respectively ($r_1 = 1$, $r_2 = 1$) the hazard ratio is 0.490 (Table 29). The change in hazard ratio values across the r_1 and r_2 values demonstrates the impact of the non-randomized CDx+ patients and of pooling population 1 and 2 is minimal on the hazard ratio. [The ORR in the Doublet arm was also analyzed and determined to be 17.6% (95% CI: (12.4%, 23.8%)) compared to 1.1% (95% CI: (0.1%, 3.8%) in the Control arm (data not shown)].

Table 28: Overall Survival: Stratified Weighted Efficacy (Hazard Ratio and 95% confidence interval) Summary Table

	r_1*					
r_2*	**0.0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
0.0	0.493(0.344,0.659)	0.493 (0.344,0.659)	0.492 (0.343,0.659)	0.492 (0.343,0.658)	0.491 (0.342,0.658)	0.491 (0.342,0.658)
0.2	0.493 (0.344,0.659)	0.492 (0.344,0.659)	0.492 (0.343,0.658)	0.492 (0.343,0.658)	0.491 (0.342,0.658)	0.491 (0.342,0.658)
0.4	0.493 (0.344,0.659)	0.492 (0.343,0.659)	0.492 (0.343,0.658)	0.492 (0.343,0.658)	0.491 (0.342,0.658)	0.491 (0.342,0.658)
0.6	0.493 (0.344,0.659)	0.492 (0.343,0.659)	0.492 (0.343,0.658)	0.491 (0.342,0.658)	0.491 (0.342,0.658)	0.491 (0.342,0.657)
0.8	0.492 (0.344,0.659)	0.492 (0.343,0.658)	0.492 (0.343,0.658)	0.491 (0.342,0.658)	0.491 (0.342,0.658)	0.491 (0.341,0.657)
1.0	0.492 0.343,0.659)	0.492 (0.343,0.658)	0.492 (0.343,0.658)	0.491 (0.342,0.658)	0.491 (0.342,0.657)	0.490 (0.341,0.657)

*: r_1, the proportion of (CTA- or LDT-) and CDx+ subject's efficacy to CTA+&LDT+&CDx+ subject's efficacy

r_2, the proportion of CTA-&CDx+ subject's efficacy to CTA+&CDx+ subject's efficacy

5. Subgroup Analyses

Not performed in this study.

The following preoperative characteristics were evaluated for potential association with outcomes: (e.g., sex/gender, site, age, race and ethnicity).

6. Pediatric Extrapolation

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

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 221 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 Pathology Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Effectiveness of treatment with a combination of BRAFTOVI® (encorafenib) and cetuximab, when used with the *therascreen* BRAF V600E RGQ PCR Kit, is shown in the primary efficacy analyses based on OS and ORR. By both assessments, significantly improved outcomes were observed in the treatment group compared to the control group using data from randomized CDx positive patients (LDT+&CTA+&CDx+ in population 1 and CTA+&CDx+ in population 2) of the BEACON clinical trial population. For the OS efficacy analysis, the median survival time in the Doublet arm was 8.4 months, compared to a median survival time of 5.2 months in the Control arm and the stratified hazard ratio was 0.550 (95% CI: (0.406, 0.744)). Weighted average calculations were conducted to determine the clinical drug efficacy in CDx+ subjects. With no efficacy in the non-randomized (CTA- or LDT-) and CDx+ patients in population 1 and CTA-&CDx+ patients in population 2, the hazard ratio of the Doublet arm versus the control arm in CDx+ subjects is 0.493 (95% CI: (0.344, 0.659)).

B. Safety Conclusions

The *therascreen* BRAF V600E RGQ PCR Kit is not expected to directly cause actual or potential adverse effects, but test results directly impact patient treatment. The risks of the *therascreen* BRAF V600E RGQ PCR Kit are associated with the potential mismanagement of patient treatment resulting from false results of the test. Failure of the

device to perform as expected or failure to correctly interpret test results may lead to incorrect BRAF V600E test results, and consequently improper patient management decisions.

A patient with a false positive result may undergo treatment with inappropriate expectation of therapeutic benefit. A patient with a false negative result may be treated without effective drugs, and not experience the potential benefit. Analytical performance in this submission demonstrates that the assay is expected to perform with high accuracy mitigating the potential for false results.

C. Risk-Benefit Analysis

The probable benefits of the *therascreen* BRAF V600E RGQ PCR Kit are based on data collected in the pivotal registrational BEACON trial of BRAFTOVI® (encorafenib) and cetuximab. For the OS efficacy analysis using data from randomized CDx positive patients (LDT+&CTA+&CDx+ in population 1 and CTA+&CDx+ in population 2) of the BEACON clinical trial population, the median survival time in the Doublet arm was 8.4 months, compared to a median survival time of 5.4 months in the Control arm and the stratified hazard ratio was 0.550 (95% CI: (0.406, 0.744)). With no efficacy in the non-randomized (CTA- or LDT-) and CDx+ patients in population 1 and CTA-&CDx+ patients in population 2, the hazard ratio of the Doublet arm versus the control arm in CDx+ subjects is 0.493 (95% CI: (0.344, 0.659)).

The risks of the *therascreen* BRAF V600E RGQ PCR Kit are associated with the potential mismanagement of patients' treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug combination that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen.

The likelihood of false results was assessed and showed acceptable analytical performance with overall agreement (OPA) to Sanger Sequencing of 95.20 (95% CI:(92.69%, 97.05%)) when excluding test invalids. The *therascreen* BRAF RGQ PCR Kit produced 100.00% (95% CI: (98.10%, 100.00%)) PPA and 91.11% (95% CI: (86.61%, 94.49%) NPA with Sanger Sequencing as the reference method, when excluding test invalids, in the accuracy study. The NPA determined during the accuracy study was as expected due to the lower sensitivity of Sanger Sequencing which was used as a reference method.

Treatment with the drug combination of BRAFTOVI® (encorafenib), and cetuximab provides meaningful clinical benefit to BRAF V600E mutant mCRC patients, as measured by OS. Given the available information, the data supports the conclusion that, in selecting specific BRAF V600E mutation positive mCRC patients using the *therascreen* BRAF V600E RGQ PCR Kit for treatment with BRAFTOVI® (encorafenib) and cetuximab 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 BEACON Study met its primary objective, demonstrating a substantial improvement in OS and ORR by the investigational therapy in patients with mCRC who have the *BRAF* V600E mutation relative to the control therapy. Collectively, the efficacy and safety results from the BEACON study demonstrate that a combination of BRAFTOVI® (encorafenib) and cetuximab has a favorable benefit-risk profile and is a useful treatment in patients with mCRC who have BRAF V600E mutation *therascreen* BRAF V600E RGQ PCR Kit.

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from BEACON support the utility of *therascreen* BRAF V600E RGQ PCR Kit as an aid in selecting CRC patients with BRAF V600E mutation for whom BRAFTOVI® (encorafenib) and cetuximab is indicated.

XIII. CDRH DECISION

CDRH issued an approval order April 15, 2020.

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