SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Real Time PCR
Device Trade Name:	THxID™ BRAF Kit for use on the ABI 7500 Fast Dx Real-Time PCR Instrument
Device Procode:	OWD
Applicant's Name and Address:	bioMérieux, Inc. 595 Anglum Rd. Hazelwood, MO 63042
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P120014

Date of FDA Notice of Approval:	May 29, 2013
Expedited:	Granted priority review status on August 31, 2012 because the availability of the device is in the best interest of the patients.

II. INDICATIONS FOR USE

The THxID™ BRAF kit is an In Vitro Diagnostic device intended for the qualitative detection of the BRAF V600E and V600K mutations in DNA samples extracted from formalin-fixed paraffin-embedded (FFPE) human melanoma tissue. The THxID™ BRAF kit is a real-time PCR test on the ABI 7500 Fast Dx system and is intended to be used as an aid in selecting melanoma patients whose tumors carry the BRAF V600E mutation for treatment with dabrafenib [Tafinlar®] and as an aid in selecting melanoma patients whose tumors carry the BRAF V600E or V600K mutation for treatment with trametinib [Mekinist™].

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the THxID™ BRAF Kit labeling.

V. DEVICE DESCRIPTION

The BRAF assay is intended for the detection of V600E (T1799A) and V600K (GT1798-

1799AA) mutations in exon 15 of the BRAF oncogene in DNA samples extracted from formalin-fixed, paraffin-embedded (FFPE) melanoma skin tissue and invaded lymph nodes. The following components comprise the overall device:

- THxID™ BRAF PUR Kit – provides reagents for nucleic acid isolation from FFPE tissue sections.
- THxID™ BRAF AMP Kit – provides reagents for automated real-time PCR amplification and detection of target DNA present in the extracted nucleic acids using primer pairs and oligonucleotide probes labeled with different fluorescent dyes.
- Applied BioSystems® 7500 Fast Dx Real-time instrument with the Sequence Detection System (SDS) Software version 1.4 with THxID™ BRAF templates.
- THxID™ -BRAF Software – BRAF assay specific software converts the SDS file into a B-RAF mutation test report on a separate computer for this purpose.

Specimen Requirements and DNA extraction: The claimed specimens for this assay are formalin-fixed paraffin embedded melanoma skin and lymph node specimens. At least 20 mm^2 of tissue when using a 10 µM section, or 40 mm^2 of tissue when using a 5 µM section, is required to perform the assay. FFPE tissue sections are macro-dissected with a scalpel according to the pathologists indication if less than 80% of the tumor cells are present in the section, or if the section contains necrotic tissue, fatty tissue, hemorrhagic tissue, or non-tumor, melanin rich area. The tissue is deparaffinized and lysed by incubation with proteinase K to digest tissue and enhance removal of formaldehyde. The solution is centrifuged through a column to remove contaminants from the sample. The DNA is washed and eluted following a short incubation with sodium azide solution. The DNA is eluted in a total volume of 55 µL to 60 µL.

Amplification and Detection: The THxID™ BRAF assay uses Amplification Refractory Mutation System (ARMS) technology which selectively amplifies the targeted mutant allele when the terminal 3'-end of the primer is hybridized to the target DNA sequence. The Taq DNA polymerase distinguishes between the match and mismatch at the 3'-end of each unique forward primer. In the presence of a wild-type sequence, the forward primers will not completely hybridize and the amplification will not occur. One primer pair is specific to the BRAF gene and allows the amplification of a non-polymorphic gene area of exon 13, which is used as an internal control (IC). Two primer pairs are specific to the mutations V600E and V600K respectively and allow the amplification of mutated fragments leading to the identification of these BRAF mutations in exon 15. The assay is not designed to detect the wild-type sequence for codon 600. Two different target probes, each labeled with a different dye, allow the simultaneous detection of the BRAF internal control and a BRAF mutation if present. The amount of DNA present in the sample is determined by the amount of fluorescence detected by the instrument during allele amplification and is expressed as a function of Ct values. Crossing threshold (Ct) values for BRAF mutant and internal control (IC) are calculated using the ABI Instrument automatic baseline and automatic threshold features of the SDS Software version 1.4 (auto Ct).

Instrument and Software: The THxID™ BRAF assay is run on the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with the Sequence Detection System (SDS) Software version 1.4. This system is a real-time nucleic acid amplification and detection platform that measures fluorescence signals and converts them into comparative quantitative readouts using fluorescent detection of dual-labeled hydrolysis probes. The THxID™ BRAF assay uses the automatic baseline and threshold features of the SDS Software version 1.4 [Auto Crossing threshold (Ct)] to analyze the data obtained after the amplification run. The use of these features is mandatory for the editing of the results report, i.e., any manual change of these settings by the user will be detected and lead to an error preventing the issuance of a diagnostic result.
Four THxID™ BRAF templates configure the instrument for a BRAF run, reducing the risk of misuse of the instrument and wrong positioning of samples. The proper template is chosen by the user according to the number of clinical samples to be tested in the run. The THxID™ BRAF templates file is loaded on the computer that runs the instrument and on which the SDS software is installed. The user creates an .sds run file for the instrument according to the instructions given in the Package Insert.

The THxID™ BRAF Software gives an interpretation of data obtained from the ABI 7500 Fast Dx Real-Time PCR instrument and SDS software version 1.4. The THxID™ BRAF Software imports raw data from the specified SDS file, checks the validity of the run and automatically interprets the results of the SDS file created by the SDS Software by indicating whether the mutation has been detected or not for each clinical sample. It uses an algorithm to interpret the patient samples results and generates a PDF formatted report with qualitative assessment of the BRAF mutation status for patient samples. The interpretation of the sds file requires 2 steps: (1) Transfer a copy of the sds result file to the THxID™ BRAF computer; and (2) Generate the THxID™ BRAF Mutation Test Report (PDF formatted report).

Test Controls: Controls are used throughout the entire procedure from sample preparation to PCR to ensure the performance of the THxID™ BRAF test.

Internal Control (IC): The internal control is a non-polymorphic region of exon 13 in the BRAF gene. This target should be detected (within a pre-established acceptance range) in every sample reaction. It controls the cell lysis efficiency, DNA extraction and purification efficiency, and absence of PCR inhibitors.

Positive Control: The positive control is composed of a mixture of V600E and V600K and WT plasmids. The positive control is carried through the entire PCR process in each run to detect THxID™ BRAF assay failures (reagents and instruments). In the event of the positive control failing, the whole run is deemed invalid (no result is provided for clinical samples) All samples and controls should be re-tested.

Negative Control: The negative control (i.e., a no template control) consists of an empty tube that follows the whole process beginning with the xylene treatment step. The negative control is performed in each run to control the absence of

contamination in the whole process. The expected result is a negative outcome. Failure of the negative control invalidates the whole run.

Acceptable Ct Values for Positive and Negative Run Controls

	Positive Control	**Negative Control**
Internal Control (IC) Ct values	26.5 < Ct value <31.5	Ct value > 37
	AND	AND
Mutant target Ct values (V600E or V600K)	28.9 < Ct value < 35.7	Ct value > 39
	AND	AND
ΔCt values	1.0 < Ct value < 5.6	Not applicable

Interpretation of Results: The THxID™ BRAF software interprets the results automatically and highlights the presence of valid or invalid results in the generated report. The 2 possible outcomes for Positive and Negative Controls are "Valid" or "Invalid". The result validity of a clinical specimen is determined first by the Internal Control (IC) Ct value that should fall within pre-specified limits to guarantee that the samples contained adequate DNA. If a specific amplification is detected for the mutant target, a ΔCt value is calculated by subtracting the IC Ct from the Ct value of the mutant reaction (V600E or V600K).

Ct Values Used to Interpret Results

IC Ct values		**Mutant target Ct values (V600E or V600K)**		**ΔCt values (Ct mutant - Ct IC)**	**Result for the considered mutation**
Ct ≤ 20	Values not considered when Ct ≤ 20				Invalid
20 < Ct < 31.5	AND	Ct < 38.5	AND	ΔCt ≤ -10	Invalid
				-10 < ΔCt < 7	Mutant detected
				ΔCt ≥ 7	Mutant not detected
		Ct ≥ 38.5 or no Ct*	AND	ΔCt not considered	Mutant not detected
31.5 ≤ Ct < 32.5	AND	Ct < 38.5	AND	ΔCt ≤ -10	Invalid
				-10 < ΔCt < 7	Mutant detected
				ΔCt ≥ 7	Invalid
		Ct ≥ 38.5 or no Ct*	AND	ΔCt not considered	Invalid
Ct ≥ 32.5or no Ct*	Values not considered when Ct IC≥ 32.5				Invalid

The final result is based on the ΔCt value: (Ct mutant – Ct IC) = ΔCt

- If the ΔCt value is below the threshold value then a V600E or V600K BRAF mutation is present,
- If the ΔCt value is above the threshold value then no V600E or V600K BRAF mutation is present or it is below the limit of detection.
- If no specific amplification is detected for the mutant targets (V600E and V600K not detected), the sample will be characterized as BRAF mutation-negative.

Result Reports

Reported Result	Description
BRAF mutation-negative	V600E and V600K mutations not detected
V600E mutation	V600E mutation detected
V600K mutation	V600K mutation detected
V600E and V600K mutations	V600E and V600K mutations detected
Invalid	Run was invalid

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA-cleared or approved alternatives for the testing of formalin-fixed, paraffin-embedded melanoma tissue for BRAF V600E *and* BRAF V600K mutation status in the selection of patients who are eligible for dabrafenib (Tafinlar®) and tramatenib (Mekinist™) treatment.

VII. MARKETING HISTORY

The THxID™ BRAF 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 as expected or failure to correctly interpret test results may lead to incorrect BRAF test results and subsequently improper patient management decisions on melanoma treatment. For the specific adverse events that occurred in the clinical studies, please see Section X below.

IX. SUMMARY OF PRECLINICAL STUDIES

Formalin-fixed, paraffin-embedded (FFPE) specimens used in the analytical performance studies were assessed for tumor content (% tumor cells), melanin content, and presence of necrotic tissue by a pathological review. The genetic status of the samples on the V600 locus was determined by bi-directional Sanger sequencing. All samples were macrodissected when the tumor content in the sample was less than 80%, or diluted consistent with instructions to users. Additionally, because melanin inhibition may lead to an invalid result, for samples with invalid results, a 1:4 dilution of the sample was made and retested, as indicated in the instructions for use.

A. Laboratory Studies

1. Correlation to Reference Method

The primary study objective was to evaluate the performance of the THxID™ BRAF test in the detection of BRAF V600E and V600K mutations in clinical samples in malignant melanoma specimens collected from clinical trials with dabrafenib and tramatenib by assessing the positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) with bi-directional sequencing Sanger as a reference method. Consecutive samples were

collected and compared to bi-directional Sanger sequencing results. The number of samples was selected on the basis of supporting a pre-specified point estimates for PPA and NPA. Pre-specified acceptance criteria for the sequencing Phred score were > 40. There were 898 samples available for testing. Excluding all invalids and QNS samples (total 43) there were 35 discordant cases [35/(898-43) = 4.1%]. Two samples determined to be V600D were detected by the THxID™ BRAF assay as V600E. The overall results are shown in the table below:

Agreement Between THxID – BRAF assay and Bi-directional Sequencing for All Samples

		Bi-directional Sequencing							
THxID-BRAF Result	BRAF V600 mutations			**E and K mutations not detected**					
		V600E	**V600K**	**V600D**	**V600R**	**WT**	**Invalid[1]**	**QNS[2]**	**Total**
	V600E	341	2	2		21	7		373
	V600K	1	57			2			60
	V600E&K		2			2			4
	E and K mutations negative	6	2		11	406	5		430
	Invalid[1]	6	1			20	2		29
	QNS[2]					2		1	3
	Total	354	64	2	11	453	14		899

[1]No result was obtained; [2] QNS = Quantity Not Sufficient for testing

For the purposes of analyzing agreement between the THxID™ BRAF kit and Sanger sequencing, any specimen that was deemed E or K was considered mutation positive and any sequencing result not E or K was deemed E and K mutation negative. Analyses were conducted with and without the THxID™ BRAF assay invalids and QNS samples. Agreement was not impacted by specimen type or melanin content (data not shown). The results met the prespecified criteria.

Agreement between the THxID™ BRAF assay and Sanger sequencing for all samples

	Including THxID™ BRAF invalids	**Without THxID Invalids**
	No. of concordance / No of tests (%) [95% CI]	**No. of concordance / No of tests (%) [95% CI]**
Positive Percent Agreement (PPA) for V600E and V600K	403/418 96.4% [94.2%; 97.8%]	403/411 98.1% [96.2%; 99.0%]
Negative Percent Agreement (NPA)	417/464 89.9% *[86.8%; 92.3%]	417/444 93.9% [91.3%; 95.8%]

	Including THxID™ BRAF invalids	Without THxID Invalids
	No. of concordance / No of tests (%) [95% CI]	**No. of concordance / No of tests (%) [95% CI]**
Overall Agreement	820/882 92.3% [91.1%; 94.5%]	820/855 95.9% [94.4%; 97.0%]

The accuracy of the V600E and V600K allele were individually assessed. THxID™ BRAF invalids were included in this analysis (QNS and Sanger invalids excluded). 1Negative agreement for V600E was based on the total non-V600E alleles. Negative agreement for V600K was based on the total non-V600K alleles. Agreement was not impacted by specimen type (data not shown). Accuracy for the V600E allele was 96.3% and accuracy for the V600K alleles was 92.2%.

	V600E –with invalids	V600K –with invalids
	No. of concordance / No of tests (%) [95% CI]	**No. of concordance / No of tests (%) [95% CI]**
Positive Percent Agreement (PPA)	341/354 96.3% [(93.8, 97.8]	59/64* 92.2 (79.7; 94.7)
Negative Percent Agreement (NPA)	503/528 99.2% (93.1, 96.8)	813/817 99.5 (98.8 to 99.8)

*Two samples with V600E&K and Sanger shows V600K, and Sanger can only report one mutation

2. <u>Analytical Sensitivity</u>

a) <u>Limit of Blank (LoB) – No Template/Exclusivity</u>

To assess performance of the THxID™ BRAF Kit in the absence of the template and to ensure that sample with wild-type (WT) DNA or a sample with a V600E or V600K mutation does not generate an analytical signal that results in a false result, cell lines and clinical FFPE melanoma samples covering all testing conditions (i.e., WT, V600E and V600K for skin & lymph node) were tested at high DNA concentrations to determine the level of background amplification in the absence of the target. A total of 3 cell lines and 6 clinical samples were tested at the highest DNA concentration per reaction (350 ng /µL; total 750ng) claimed. A total of 60 replicates per sample were evaluated over 3 runs and one assay reagent lot. Skin samples were limited to 150 ng/ µL and 20 replicates due to lack of tissue. No background amplification of the unexpected target DNA occurred. Ct values are reported as undetermined for the appropriate reactions. Four results were reported as invalid and the remaining results reported as "mutant not detected" and no unexpected amplification or wrong results occurred

b) Limit of Detection (LoD)

The claimed range for DNA input for the assay is 20-700 ng DNA per reaction. For the THxID™ BRAF Kit, the limit of detecting mutant DNA in a background of wild-type DNA is defined as the lowest mutation level in a specimen for which the assay yields a positive result in 95% of the of the test replicates for each mutation positive sample. Determination of the LoD was performed by testing (1) melanoma cell line FFPE blocks; and (2) FFPE melanoma skin and lymph node clinical specimens. Two different test lots were used for these evaluations.

Clinical samples: LoD values were calculated independently for both lymph and skin specimens. LoD values were determined using serial dilutions of DNA extracted from macrodissected mutant specimens blended with DNA extracted from wild-type specimens. Serial dilutions from three DNA concentrations spanning the claimed input range were evaluated (20ng, 350 ng, and 700ng) for V600E and two concentrations for V600K (because a high concentration specimen could not be obtained.) Mutant samples were presumed to be 100% mutant based on the ΔCt value. Mutant content was diluted from this value.

The LoD was estimated by calculating the 95% predicted hit rate using Probit analysis. Results for skin and lymph node were similar The LoD was determined as the highest value obtained. The data support a claimed LoD of 5% mutant DNA in a background of wild-type DNA for V600E and V600K positive FFPE skin and lymph node specimens across the DNA input range.

FFPE cell line DNA: Dilution series using DNA extracted from FFPE cell lines with either the V600E or V600K mutation were diluted to a final concentration of 5% mutant in a background of wild-type DNA extracted from FFPE cell line. A total of 24 replicates (12 replicates per lot) at very low DNA input concentration (30ng/reaction) was evaluated for each mutation on two reagent lots. The V600E FFPE cell line was homozygous and the V600K FFPE cell line contained 66.7% mutant DNA. LoD was confirmed to be < 5%.

Confirmatory Testing: DNA was extracted from WT and mutant clinical FFPE specimens, or melanoma cell line FFPE blocks, and combined to create the required samples, each containing 5% mutation. The FFPE specimens were from skin or lymph node tissue. Samples represented a range of total DNA inputs within the claimed range (20-700 ng/reaction) and outside of this range (10 and 1000 ng/reaction). Two FFPE clinical specimens with high melanin contents were included. A total of 20 replicates each were evaluated. For each sample, the 20 replicates were tested in one PCR run. The study included one lot of the THxID™ BRAF assay, which was a different lot than the two used for LoD Determination (above).

For each sample in the study, including clinical FFPE specimens and cell line FFPE blocks, the observed hit rate (mutation positive rate) was 100% (20/20

replicates) [95% confidence interval 83.9% – 100%], meeting the acceptance criteria of ≥ 95% hit rate (≥19/20 replicates) for each sample. A summary of confirmation of LoD results for the clinical FFPE specimens and the melanoma cell line FFPE blocks is shown in the following table.

Condition	**Tissue Type**	**Mutant allele at 5%**	**Mutation positive / tested**
10 ng/reaction (below DNA range)	Lymph node	V600E	20/20
	Skin	V600K	20/20
20 ng/reaction (lower limit of DNA range)	Lymph node	V600E	20/20
	Skin	V600E	20/20
	Lymph node	V600K	20/20
	Skin	V600K	20/20
High melanin content*	Skin	V600E	20/20
	Lymph node	V600K	20/20**
700 ng/reaction (high end of range)	Lymph node	V600E	20/20
	Skin	V600E	20/20
	Lymph node	V600K	20/20
	Skin	V600K	20/20
1000 ng/reaction (above DNA range)	Lymph node	V600E	20/20
	Lymph node	V600K	20/20
Melanoma FFPE cell line blocks	Melanoma cell line	V600E	20/20
	Melanoma cell line	V600K	20/20

* DNA input for high melanin samples were at 60 ng/reaction for the V600E sample and 526 ng/reaction for the V600K sample and melanin content were respectively of 80.7% and 73.6%.

** After 1:4 dilution of the eluate. Before dilution each of the 20 replicates was invalid. In accordance with the troubleshooting section, the eluate was diluted 1:4 in Buffer ATE and re-tested with results as described in the table.

c) <u>Genomic DNA Input Range</u>

The claimed genomic input range is 10ng/µL to 350ng/ µL, i.e., a total of 20ng of DNA to 700ng of DNA input when 2µL is used for each reaction. The THxID - BRAF Kit does not require the user to obtain a specific concentration of DNA as determined by spectrophotometry because an internal control Ct value is used to measure adequate DNA integrity. The claimed range was validated two ways: (1) Validation of the DNA input range was shown in the limit of detection, and

linearity studies demonstrated that replicates across the claimed DNA input range produce correct results; and (2) DNA from the 891 eluates from the patient specimens evaluated in the accuracy studies were measured by spectrophotometry prior to testing to ensure that users will typically obtain between 10ng/µL to 350ng/µL per extraction using the range of allowable tumor specimen. The results showed that 94.6% of DNA values were within 10-350ng/µL (total range was 2 to 1764ng/µL). Of these samples, 6.3% had DNA concentrations outside of the claimed range with 25 clinical specimens exceeding the claimed upper limit and 31 specimens had concentrations below the claimed lower limit. Of the 56 results, 8 were invalid. The package insert indicates that the optimal DNA concentration is less than 350ng/reaction.

3. **Validation of Control Ct Values**

The cut-off values for the positive control (PC), negative control (NC) and Internal Control (IC) using the BRAF interpretation algorithm for the THxID™ BRAF assay were validated in a study designed to ensure that errors due to failures occurring during set-up and amplification would be detected by the controls.

Positive control: The positive control is composed of a mixture of V600E, V600K and WT plasmids. The positive control is carried through the entire amplification process in each run to detect THxID™ BRAF assay failures (reagents and instruments). In the event of the positive control failure, the run is deemed invalid and no result is provided for clinical samples. Two independent studies were conducted to demonstrate the effectiveness of the positive control by simulating (1) 11 potential user-driven errors that can occur in assay set-up and amplification process using 3 lymph node specimens representing WT, V600E, and V600K, and belneded to represent the claimed LoD of 5% mutant in WT ratio; and (2) 6 potential user driven errors using 6 melanoma FFPE specimens blends with approximate LoD equivalent to 15% mutant to WT ratio. The study included the following six melanoma FFPE samples: 2 WT samples (1 lymph node, 1 skin); 2 V600E samples (1 lymph node, 1 skin); 2 V600K samples (1 lymph node, 1 skin). Both positive control and clinical procured specimens were tested with and without the simulated errors. The potential errors that were simulated were as follows:

- **Error 1**: no vortexing during preparation of the PCR amplification to mimic a breakdown of the vortex-type mixer (*steps 2, 6 and 9*).
- **Error 2**: no centrifugation before starting the amplification on the instrument, only tested on 96-well plate (*step 20*).
- **Error 3**: Primers & probes sphere re-suspended by master mix instead of dedicated diluent (*step1*).
- **Error 4**: substitution of master mix with primer diluent (no Master Mix in the reaction) (step 1).
- **Error 5**: Master mix frozen for at least 18 hours (recommended storage being +4°C) (step 5).
- **Error 6**: Use master mix re-suspension volume (110µl) instead of diluent volume (85µl) (step 1).

- **Error 7**: Primers & probes sphere re-suspended in 60µl instead of 85µl to mimic confusion with the elution volume (step 1).
- **Error 8**: 5µl of eluate or control added in the reaction instead of 2µl (step 12).
- **Error 9**: Re-suspend 2 Primers & probes spheres with 255µl to mimic the loss of one sphere in a mix (note in step 1).
- **Error 10**: Positive control sphere re-suspended in 85µl instead of 150µl to mimic confusion with the Primers & probes sphere re-suspension volume (step 1).
- **Error 11**: Primers & probes sphere re-suspended in 150µl instead of 85µl to mimic confusion with the control re-suspension volume (step 1).

The Positive Control was considered effective if it was reported as invalid in 4 / 4 replicates when tested under the same error condition that generated an invalid or incorrect result for an FFPE specimen. The results showed that for each of the PCR set-up errors evaluated in both studies, the Positive Control was invalid in 4 of 4 replicates whenever one or more replicates of the FFPE specimen tested under the same condition is incorrect or invalid. The results also showed that the user errors did not impact the results in many cases demonstrating the robustness of the assay overall.

Negative Control: The negative control follows the entire assay process, starting from the sample paraffin removal step. The negative control is performed in each run to control the absence of contamination in the whole process. In a valid negative control, no target amplification should be detected (Ct values are undetermined) or, if a late target specific amplification is detected, the corresponding Ct values should be greater than a value to be determined. Validation of the negative control was assessed by intentional contamination of key components with DNA extracted from mutant specimens: the lysis buffer (ATL), the elution buffer (ATE), and the primer diluent. The DNA samples used as contaminating materials were prepared from DNA extracted from procured clinical samples blended to reach 5% mutant to WT ratio and used at a very low concentration to represent a challenging sample. Each test with and without a contaminated component (lysis buffer, elution buffer or primer diluent) was carried out in quadruplicate. The results demonstrated that the negative control is more sensitive to contamination than a WT sample and that no false positive results occurred.

Internal Control: The internal control is a non-polymorphic region of exon 13 in the BRAF gene that should be detected (within the pre-established acceptance range) in every sample reaction. It controls the cell lysis efficiency, DNA extraction and purification efficiency, and absence of PCR inhibitors. Two independent studies were conducted using the 6 FFPE melanoma specimens 2 WT samples (1 lymph node, 1 skin); 2 V600E samples (1 lymph node, 1 skin); 2 V600K samples (1 lymph node, 1 skin) to demonstrate the effectiveness of the internal control by simulating 8 different error conditions as follows:

- **Error 1**: no deparaffinization with the xylene and ethanol before starting the lysis step -Start at the step 11.

- **Error 2**: no addition of the proteinase K -omit step 12.
- **Error 3**: no addition of 200µL of the Buffer AL to the sample and no addition of 200µL of ethanol -omit steps 17 to 19.
- **Error 4**: no addition of 500µL of buffer AW2 (wash buffer) -omit steps 30 to 34.
- **Error 5**: no centrifugation at full speed (approximately 20000 *g*) for 3 minutes at 18-25°C to dry the membrane completely and no incubation at 18-25°C for at least 1 minute -omit step 35 and step 40.
- **Error 6**: perform the extraction without adding ethanol in the buffer AW1 (25mL) and AW2 (30mL).
- **Error 7**: apply 120 µL instead of 60 µL of Buffer ATE to the center of the membrane -step 38 (it is tested if this step is done 2 times).
- **Error 8**: spiking of AL buffer (lysis buffer) in the eluate to mimic the presence of inhibitor- add 5µL of AL buffer in the eluate after step 41.

The results demonstrated the effectiveness of the IC control with the exception of error 8 (addition of ATL to the eluate) which failed in generating an invalid result and generated false negative results for the V600E PCR specimens and a V600K PCR specimen. This error condition did not systematically occur and does not reflect a realistic situation (5µL lysis buffer ending in the eluate). While it is very unlikely to occur (as confirmed by the performance studies shown here), a limitation in the package insert highlights the possible error.

4. Minimum Specimen Requirements

a) Tissue Area

A total of 199 clinical specimens with tissue areas ranging from 8-300 mm^2 were tested with the THxID BRAF assay. The specimens were both skin and lymph node, exhibited melanin content ranging 0 to 100%, were V600E, V600K or V600 wild-type, had varying tumor proportion, and were processed as either 1 x 10µm or 2x 5µm sections. The Ct values for these specimens were compared to the assay cut-offs and used to confirm the minimum quantity of tissue that can be used to give a valid result when compared to bi-directional Sanger sequencing. The observed DNA concentrations of eluates for 199 procured clinical specimens ranged from 5ng/µL to 517ng/µL with an average of 122ng/µL. The claimed tumor area for the assay is 20mm^2 to 250mm^2. Smaller tissue (i.e., < 20mm^2) areas cannot ensure reliable results.

b) Thickness

To demonstrate that both 5µm and 10µm sections can be used with the THxID™ BRAF assay, 30 FFPE melanoma specimens were each tested using one 10µm section, and 2 x 5µm sections and testing the eluates of each in parallel. There were 10 V600E (7 lymph and 3 skin), 5 V600K (3 lymph and 2 skin), and 15 wild-type (11 lymph and 4 skin) specimens. Melanin content ranged from 0 to 80%. Acceptance criteria were set to an absolute

mean difference of less than or equal to 1.0 Ct or 1.0 delta Ct between results obtained with the two conditions. Both sections and curls were included in the evaluation depending on tumor content and the requirement for macrodissection. Tumor content ranged from 40-95% prior to macrodissection. The results of the study confirm that the use of one 10 µm section or two 5 µm sections of the same FFPE melanoma sample will give comparable results with the THxID™ BRAF assay. The total surface of tissue should not exceed 250 mm^2 for 1 10 µm, or 500 mm^2 if 2 x 5 µm sections are prepared.

c) Tumor Content and Macrodissection

The instructions for use require the user to macrodissect samples with less than 80% tumor content. To determine whether the THxID BRAF assay specifically fails to detect the V600E and V600K mutant alleles when the tumor content is below 80% and the section has not undergone macrodissection, 40 mutation-positive melanoma FFPE specimens with tumor content ranging from 5% to 70% for each specimen type (skin and lymph node) were tested without macrodissection. Of the 40 samples, 33 were V600E (10 skin and 22 lymph) and 7 V600K (3 skin and 4 lymph). Melanin content ranged from 0 to 50% across the specimens. Results were compared to bi-directional Sanger sequencing. All results were correct.

d) Curl vs. Slide Equivalency

To demonstrate that results generated with the THxID™ BRAF assay starting from FFPE sections on slide versus from FFPE sections in tube (curls) are comparable for samples with ≥ 80% tumor content, a total of 30 FFPE melanoma clinical samples containing ≥ 80% tumor cell were processed in parallel as sections on slides and curls in a tube. The study included 14 V600E (10 lymph node and 4 skin), 2 V600K (both lymph node) and 14 wild-type (13 lymph node and 1 skin) samples. Melanin content ranged from 0- to 50%. Ct and ΔCt values obtained from slides and curls on the same sample were compared. The results demonstrated that 29 of the 30 specimens were concordant with less than a mean difference in ΔCt value of 1.0. One wild-type lymph node, curl was false positive for V600E. Further testing of 10 additional curls from the same specimen were all wild-type suggesting contamination in the original sample. The results met the acceptance criterion.

5. Linearity/Amplification Efficiency as a Function of %Mutant DNA

To demonstrate the linearity of the THxID™ BRAF assay across the range of mutant DNA content, a series of dilutions with varying concentrations of mutant DNA in a background of wild-type DNA at various 3 fixed DNA inputs (20 ng, 350 ng and 700 ng per reaction) from both skin and lymph node specimens for each mutation (V600E and V600K) was evaluated. Mutant to wild-type ratios tested were 100%, 50%, 25%, 10%, 5%, 2.5%, ad 1.25%. Initial samples were presumed to have mutant content of 100% by using a macrodissected specimen with a ΔCt value

<1 (i.e., mutant DNA content at the highest concentration possible). A pool of wild-type DNA was generated from 3 different samples and used to dilute the mutant DNA to create the ratios. The highest 3 concentrations were tested in 6 replicates while the remaining were tested in 7 replicates. Linear regression, second and third order polynomial regressions were performed on the obtained Ct or delta Ct values and the log10 of the mutant to WT ratios. The acceptance criteria for linearity above the claimed LoD of 5% for skin and lymph node were met, however the lymph node high V600K samples was not linear at the high range. This did not impact the correct calls though. At three, fixed DNA input concentrations, amplification efficiency when the mutant DNA concentration ranges from 1.25% to 100% (presumed) is summarized below:

		Intercept (SE)	Intercept (95% CI)	Calculated Slope	Slope (SE)	Two-Sided 95% CI (slope)	Amplification Efficiency
Skin V600E	**high**	31.12 (0.11)	[30.89, 31.34]	-3.13	0.08	[-3.30, -2.96]	109 %
	Med	30.87 (0.08)	[30.72, 31.02]	-2.77	0.06	[-2.90, -2.64]	130 %
	Low	34.64 (0.14)	[34.36, 34.92]	-3.01	0.11	[-3.24, -2.78]	115 %
Lymph V600E	**high**	32.01 (0.08)	[31.86, 32.16]	-3.86	0.06	[-3.98, -3.73]	82 %
	Med	29.88 (0.06)	[29.76, 29.99]	-3.06	0.05	[-3.15, -2.96]	112 %
	Low	35.24 (0.16)	[34.92, 35.56]	-2.92	0.13	[-3.19, -2.66]	120 %
Skin V600K	**high**	31.64 (0.19)	[31.26, 32.02]	-3.14	0.13	[-3.41, -2.87]	108 %
	Med	31.00 (0.08)	[30.83, 31.17]	-3.47	0.07	[-3.61, -3.33]	94 %
	Low	32.93 (0.09)	[32.75, 33.11]	-3.06	0.08	[-3.22, -2.91]	112 %
Lymph V600K	**high**	32.53 (0.10)	[32.33, 32.72]	-4.59	0.08	[-4.74, -4.44]	65 %
	Med	28.97 (0.09)	[28.80, 29.14]	-3.00	0.07	[-3.15, -2.85]	115 %
	Low	35.65 (0.16)	[35.32, 35.98]	-3.07	0.13	[-3.33, -2.81]	112 %

6. **<u>Linearity/Amplification Efficiency as a Function of DNA Input</u>**

The linearity and amplification efficiency of PCR were evaluated using a dilution series of DNA inputs extracted from FFPE cell line blocks and spanning the DNA range for two fixed BRAF mutations (5% to represent the LoD, 100% for V600E and 66% for the V600K). The DNA inputs tested were 700ng, 350 ng, 175ng, 87.5ng, 43.8ng, 21.9 ng, and 10.9 ng. For each condition, 6 replicates per DNA input were tested with one lot. The ΔCt values were used to select the specimens. Linear regression, second and third order polynomial regressions were performed on

the obtained Ct or delta Ct values and the log10 of the DNA. The acceptance criteria were met. The amplification efficiencies are shown below:

V600E FFPE cell lines:

Mutation content	Variable	Intercept (SE)	Intercept 95% CI	Slope	Slope (SE)	Slope 95% CI	Efficiency
5 %	Ct V600E	35.43 (0.05)	[35.34, 35.53]	-3.55	0.02	[-3.59, -3.50]	91 %
5 %	Ct IC V600E	32.50 (0.09)	[32.32, 32.67]	-3.53	0.04	[-3.62, -3.45]	92 %
100 %	Ct V600E	31.78 (0.03)	[31.72, 31.85]	-3.49	0.01	[-3.52, -3.46]	93 %
100 %	Ct IC V600E	31.94 (0.06)	[31.82, 32.06]	-3.41	0.03	[-3.47, -3.36]	96 %

V600K FFPE cell lines:

Mutation content	Variable	Intercept (SE)	Intercept 95% CI	Slope	Slope (SE)	Slope 95% CI	Efficiency
5 %	Ct V600K	35.23 (0.04)	[35.15, 35.31]	-3.44	0.02	[-3.48, -3.41]	95 %
5 %	Ct IC V600K	32.18 (0.05)	[32.07, 32.29]	-3.50	0.03	[-3.55, -3.45]	93 %
66.6 %	Ct V600K	32.23 (0.04)	[32.14, 32.31]	-3.44	0.02	[-3.48, -3.41]	95 %
66.6 %	Ct IC V600K	31.51 (0.06)	[31.39, 31.62]	-3.50	0.03	[-3.55, -3.45]	93 %

The PCR efficiency and linearity were evaluated for each mutation (V600E and V600K) on lymph nodes and skin as well. The DNA inputs tested were 700ng, 350 ng, 175ng, 87.5ng, 43.8ng, 21.9 ng, and 10.9 ng. For each condition, 6 replicates per DNA input were tested with one lot. Linearity was assessed on the range in which all DNA quantities gave consistent results. The results demonstrated for the clinical specimens evaluated. All calls were correct. The linear ranges observed for clinical samples at high (100%) and low (5%) mutant DNA concentrations are summarized below.

	Variable	
Specimen	**CT V600mutant**	**CT ICV600mutant**
Skin V600E Low	10.9 - 350	10.9 - 700
Skin V600 High	10.9 – 350	10.9 - 700
Skin V600K Low	10.9 – 175	10.9 - 350
Skin V600K High	10.9 - 350	10.9 - 700
Lymph node V600E Low	10.9 – 700	10.9 - 700
Lymph node V600E High	10.9 – 700	10.9 - 700
Lymph node V600K Low	10.9 – 87.5	10.9 - 350
Lymph node V600K High	10.9 – 350	10.9 - 700

7. Analytical Specificity

a) Primer and Probe Specificity

An in silico study was performed to evaluate primer and probe specificity using publicly available sequence and mutation databases. Two different types of analysis were performed (1) the potential for cross-reactivity to primer pairs and probe sequences was investigated using public databases and specialized multiple sequence alignment software, and (2) amplification of all homologous sequences in Genbank were predicted in a simulation analysis using specialized bioinformatics software designed for this purpose. The results showed that amplification of non-BRAF genes (including human, fungal, bacterial and viral), BRAF pseudogenes, and ARAF/CRAF genes is not predicted, however, the assay is expected to cross-react to the V600D mutation in BRAF exon 15. The results also showed that other BRAF mutations reported in somatic mutation databases may impact detection, but none of these mutations were reported as occurring in melanoma. The results support the specificity of the assay primer and probes.

b) Inclusivity

The THxID™ BRAF assay is designed to detect the V600E (T1799A) and V600K (GT1798/1799AA) mutations. In addition, the THxID™ BRAF assay was shown to detect a rare form of the V600E mutation (i.e., rare codon GAA) and the V600E/K601E mutation (also referred to as V600E2). using 2 FFPE lymph node specimens and plasmids. Two FFPE samples from lymph node tissues with the rare V600E allele as determined by bi-directional Sanger sequencing were tested with 3 replicates each. Melanin content was 5% and 50%. All results were correctly detected. The sample was then combined with WT DNA to obtain a 5% mutant DNA content in background of wild-type DNA. The sample was tested with 20 replicates. All results were called correctly. The expected results for V600E and V600K mutations are as follows:

	V600E result	V600K result
V600E (GAG)	Mutant detected	Mutant not detected
V600K	Mutant not detected	Mutant detected
V600E rare (GAA)	Mutant detected	Mutant not detected
V600E/K601E	V600E detected	Mutant not detected

c) Cross-Reactivity

i. **Non V600E mutations:** Cross-reactivity of the THxID –BRAF assay was assessed by testing other non-V600E and non-V600K BRAF mutations using plasmids and clinical samples. Plasmids containing the Internal Control PCR target (region of BRAF gene in exon 13) and the

mutant PCR target for the following variants were tested: V600, V600D, V600E/K601E (also referred to as V600E2), V600R, V600L, V600M, V600G, V600A and the BRAF pseudogene (a homologous gene present on the X chromosome). Plasmids were spiked into ATE buffer to mimic a gene copy number equivalent to approximately 700ng of DNA (the maximum claimed range). Two clinical samples (one skin and one lymph node) characterized as V600R by Sanger sequencing were also tested. Three replicates of each variant were tested. The results demonstrated that none of the variants with the exception of V600D and V600E/K601E,, cross-reacted with the V600E or V600K PCR reactions. Cross-reactivity for V600D and V600E/K601E, persisted following dilutions or plasmids with wild-type plasmid at ratios of 50%, 5%, 2%, and 1%. PCR signals were observed for the V600G plasmid, however, the signal was sufficiently high that a false result did not occur.

	V600E result	V600K result	Cross-reactivity detected
BRAF pseudogene	Mutant not detected	Mutant not detected	No
V600D plasmid	V600E detected	Mutant not detected	Yes
V600R plasmid	Mutant not detected	Mutant not detected	No
V600R skin sample	Mutant not detected	Mutant not detected	No
V600R lymph sample	Mutant not detected	Mutant not detected	No
V600L plasmid	Mutant not detected	Mutant not detected	No
V600M plasmid	Mutant not detected	Mutant not detected	No
V600G plasmid	Mutant not detected	Mutant not detected	No
V600A plasmid	Mutant not detected	Mutant not detected	No

ii. **Microorganisms:** A study was conducted to assess whether there are risks of wrong results when a specimen is exposed to microorganism contamination. Five (5) common human microorganisms (*E. coli, S. aureus, P. aeruginosa, A. niger/brasiliensis,* and *C. albicans*) along with 5 of the most prevalent microorganisms (including 3 skin organisms) detected in the manufacturing facilities (*M. luteus, R. picketti, K. vairans, P. fluorescens*, and *S. paucimobilis*) were investigated. The primer and probes of the kit were spiked with a cocktail of the 10 microorganisms to a final concentration of 104 and 102 CFU/mL. No cross detection of THxID -BRAF false results due to contaminants was observed. An *in silico* analysis was also performed to check the risks of cross-detection, including 6 additional skin microorganisms (*S.epidermis, S. aureus, C. xerosis, C. jeikeiu, C. minutissimum*, and *C. ulcerans*). There was no cross-detection predicted.

8. Interference – Melanin

The effect of melanin as an interfering substance on the invalid rate and accuracy of the THxID™ BRAF assay was evaluated using 56 FFPE samples (30 skin and 26 lymph node) containing melanin levels ranging from 50-100% (as determined by pathology review). All three genotypes were represented (i.e., samples were either wild-type (WT), V600E or V600K). Samples with invalid results were tested in accordance with the instructions for use which indicates that first a 1:4 dilution of the extracted sample be tested and if that fails a new section of the sample be re-extracted using higher tissue amount. Specimens were macro-dissected according to protocol. Out of the 56 samples tested, a total of 16 samples had invalid results after the first test; 9 lymph node samples (34.6%) and 7 skin samples (23.3%). Nine (9) of these 16 sample (16.1%) had invalid results not resolved with retesting (4 lymph node and 5 skin). Eight (8) of the 9 samples had melanin content over 80%.

Melanin Content (%)	Invalid Samples	Valid Samples*	Total No. samples
50	1	11	12
60	0	5	5
70	0	8	8
80	2	12	14
90	5	10	15
100	1	1	2
Total : 56			

*After troubleshooting per IFU.

For the 48 samples with Sanger results, there were no false negatives (i.e., all V600E and V600K samples correctly called). There were 5 samples deemed wild-type by Sanger that were called V600E positive by the THxID™ BRAF assay which is attributed to the more sensitive THxID™ BRAF assay. To confirm melanin does not generate false results, an additional 5 highly-pigmented, non-melanoma FFPE skin samples (i.e., BRAF V600 wild-type) from African Americans were evaluated. For four of five samples, the original triplicate results (from independent extractions of each specimen) agreed with the expected WT result determined by bi-directional Sanger sequencing. One specimen gave invalid results for two of three replicates. The instructions for use indicate that samples with high melanin may interfere with the assay.

9. <u>Interference – Effects of Necrotic Tissue</u>

The ability of the THxID™ BRAF Kit to perform correctly when samples have high necrotic tissue content was evaluated. A total of 21 melanoma FFPE specimens (skin and lymph node; V600E, V600K or wild-type) with necrotic tissue concentrations ranging from 15% to 60% were tested with the THxID™ BRAF assay. Necrotic tissue content was determined by pathology review. Each sample was tested in 3 replicates (from 3 extractions) with one lot of THxID™ BRAF Kit. Melanin content ranged from 0 to 90%. For 20 of the 21 samples with necrotic tissue, the initial THxID™ BRAF results agreed with the sequencing result. For one sample (V600K with 50% necrotic tissue and 80% melanin), the original THxID™ BRAF result was invalid for one of three replicates. In accordance with the IFU, the

residual eluates of the three replicates were diluted 1:4. The re-test results agreed with expected result for three of three replicates. The study demonstrates that necrotic tissue does not interfere with the assay.

10. Interference – Endogenous Substances (Hemoglobin and Triglycerides)

To evaluate the impact of high concentrations of hemoglobin and triglycerides (i.e., mimic grossly hemolytic or fatty specimens), two concentrations of hemoglobin (2 mg/mL and 4 mg/mL) or triglycerides (37mM and 74mM) were added to samples during the lysis step (i.e., into lysis buffer prior following deparaffinization and prior to extraction). These concentrations reflect 1x and 2x the CLSI recommended high concentration. Eleven (11) FFPE skin and lymph melanoma tissues were tested with each interferent. Specimens were BRAF V600E, V600K and wild-type and the ΔCt spanned the range to include specimens with low mutant content. Each sample was tested in 3 replicates (from 3 extractions). The results with interferent were compared to the results without interferent. All observed results agreed with the expected result determined by Sanger sequencing across all samples and replicates. There was also no significant change to the ΔCts observed between samples with interferent and without interferent.

11. Precision – Reproducibility

An external study was performed to assess the reproducibility of the THxID™ BRAF assay across 3 external testing sites (2 operators per site), 2 runs per operator and 3 non-consecutive testing days across a 3-week period, un duplicate with two types of samples: (1) a panel consisting of 15 samples using prepared DNA eluates, and (2) FFPE melanoma tissues to evaluate the impact of the extraction method across multiple users on the results. DNA eluates were prepared by blending DNA extracted from FFPE skin or lymph specimens to achieve mutant DNA concentrations near the limit of detection (~5%) or 3x the LoD (~15%-50%) representing V600 wild-type, V600E or V600K. Additionally, three levels of total DNA input were included in the evaluation [low (20-30ng), high (600-700ng/reaction), and medium (approximately 60ng – 400ng/reaction)]. Samples were blinded to users. Three samples had high melanin content. Three reagent lots and three PCR thermocyclers were included in this evaluation. The results of the overall agreement were 100% across all samples in the panel except the sample that was derived from skin with a V600E mutant DNA content blended to be very close to the LoD. Further analysis of this sample revealed the mutant content to be below the LoD of 5%. Overall agreement is shown below.

Overall Agreement Between Three Sites for Reproducibility Panel

Panel Member	Specimen type	DNA input	Percent mutant*	Dilution Step	No. of valid tests all three sites/ Total number of tests (95% CI)
Wild-Type	Skin	Low	n/a	n/a	72/72 (94.9% - 100%)
Wild-Type	Skin	High	n/a	n/a	72/72 (94.9% - 100%)
Wild-Type	Lymph node	Low	n/a	n/a	72/72 (94.9% - 100%)
Wild-Type	Lymph node	High	n/a	n/a	72/72 (94.9% - 100%)
Wild-Type	Skin –	Med	n/a	Diluted	72/72 (94.9% - 100%)

	high melanin				
V600E	Skin – high melanin	Med	-	Diluted	72/72 (94.9% - 100%)
V600E	Skin	Low	Close to LoD	n/a	60/72 (73.1% -90.2%)
V600E	Lymph node	High	Close to LoD	n/a	72/72 (94.9% - 100%)
V600E	Skin	Med	Med- high	-	72/72 (94.9% - 100%)
V600E	Lymph node	Med	Med- high	-	72/72 (94.9% - 100%)
V600K	Lymph node – high melanin	Med	-	Diluted	72/72 (94.9% - 100%)
V600K	Skin	Low	Close to LoD	-	72/72 (94.9% - 100%)
V600K	Lymph node	High	Close to LoD	-	72/72 (94.9% - 100%)
V600K	Skin	Med	Med-high	-	72/72 (94.9% - 100%)
V600K	Lymph node	Med	Med-high	-	72/72 (94.9% - 100%)

An estimate of the within-run precision, between-run (operators), between-days, between lots, between sites/instruments, and the total precision using restricted maximum likelihood method (REML) was conducted. The standard deviation and %CV for Ct and ΔCt results for V600E samples, V600K samples, internal control (IC) Ct values for the WT samples, and positive and negative controls, were investigated as a measure of the variability of the assay. For the Wild-type panel samples, the internal control Ct for the V600E multiplexes ranged from 24.4 to 28.7 9 with % CV range 0-3.3% and the V600K multiplexes IC ranged from 24.2 to 28.5 with %CV range 0-2.8%). The mean ΔCt ranged from 1.9 to 6.1 for the V600E mutation positive samples with associated %CV values ranging from 0 to 16.7%. The mean ΔCt ranged from 1.2 to 4.9 for the V600K mutation positive samples with associated %CV values ranging from 0 to 25.6%. The higher imprecision was associated with the high melanin content sample. For the V600E positive control the mean ΔCt was 3.7 and the %CV values ranged from 0 to 12.8%. The V600K positive control mean ΔCt value was 3.5 with associated %CV ranging from 0 to 15.1%.

12. <u>Precision – Repeatability</u>

The goal of this study was to determine repeatability (within laboratory) precision of the entire THxID™ BRAF assay (extraction and amplification) by evaluating the repeatability between runs, operators, instruments, days and lots at one testing site. The precision panel was comprised of 8 panel members prepared from FFPE melanoma specimens of skin and lymph node representing the different genotypes (V600E, V600K, and wild-type), DNA input (low, moderate, high), and melanin content (range 0 to 100%) and mutant DNA content (based on ΔCt values). Macrodissection was not necessary. Supplemental testing of an additional V600K samples was conducted for one run per day due to the one failed specimen results bringing total to 9. The panel members were evaluated in house with 2 THxID™ BRAF Kit lots, 4 days per lot, 2 runs per day, 2 instruments (2 days per instrument), 2 operators (each performing 1 run per day) and 2 replicates for each condition in each run. Results were assessed qualitatively for percentage of correct calls across the 32 sections per specimen, and an estimation of variance components for the analysis of quantitative variables was performed using the Restricted Maximum

Likelihood (REML) method. The results demonstrated 100% agreement after retesting with the exception of the V600K specimen with high DNA input and mutant DNA content intended to be close to the LoD. However, the ΔCt value was at the cut-off and the results reflect the fluctuation in mutation content that can occur between sections of the same FFPE specimen. Because the V600K panel member at high DNA content close the claimed LoD of 5% was correct only half the time, an additional V600K panel member was obtained and tested at the same high DNA content (317 ng/µL) at the claimed LoD. Precision estimates for all Ct values and ΔCt values were similar. The results demonstrated 100% agreement after retesting.

Correct Call Rate for Precision Testing Panel

Specimen Type	Genotype	Mean DNA input ng/uL /range	Mean ΔCt (Mutant content)	Number of miscalls/ invalids	Correct results	% correct (95% CI)
Skin*	Wild-type	12.0 (low) (3.8 – 26.4)	(not applicable)	0/0	32/32	100 (89.3 – 100)
Lymph-node	Wild-type	174.5 (mid-range) (109 – 303.9)	(not applicable)	0/0	32/32	100 (89.3 – 100)
Skin	V600E	42.5 (low) 12.7 – 65.8	3.17 (low)	0/0	32/32	100 (89.3 – 100)
Lymph-node	V600E	578.7 (high) (328.4- 831.6)	5.0 (low)	0/0	32/32	100 (89.3 – 100)
Skin (high melanin)	V600E	140.4 (mid-range) 97.1- 252.78	0.84 (high)	0/0	32/32 (after dilution 1:4)	100 (89.3 – 100)
Lymph-node	V600K	44.2 (low) (24.8 – 99.12)	1.74 (high)	0/1	32/32	100 (89.3 – 100)
Lymph-node	V600K	84.8 (mid-range) (51.7- 135.5)	2.55 (mid-range)	0/0	32/32	100 (89.3 – 100)
Lymph-node	V600K	302.83 (high) (44.83 to 491.30)	7.02 (very low)	15 (false negatives) /0	17/32	53.1 (36.4 –69.1)
Lymph-node	V600K	317.4 (high) (173- 427)	4.56 (low)	0/0	16/16	100 (89.3 – 100)

13. Sample Handling Variability

The purpose of the FFPE melanoma tissues was to evaluate the impact of the extraction method across multiple users on the results. For the FFPE specimens, curls cut directly from blocks were placed in microtubes and alternating sections of each specimen were forwarded to 3 labs so that each lab received 3 sections per specimen. Each of the 9 tumor specimens was then prepared according to the IFU. Two operators were used to prepare the extractions from each specimen. The samples evaluated were as follows:

14. Lot-to-Lot Reproducibility

An estimate of between-lot reproducibility was obtained from the three site reproducibility using the restricted maximum likelihood method (REML). Each site utilized kits from two different reagent lots as follows: Site one used lots 4 and 5, Site 2 used lots 5 and 6, and Site 3 used lots 4 and 6. For each specimen the SD and %CV were summarized for each mean Ct value obtained for the internal control for both V600E and V600K reactions, the ΔCt vales and the positive controls. The total imprecision (as a function of %CV) between lots was less than 3.10% for internal control Ct values for each reaction, less than 8.7% and 13.4% for the ΔCt values for the positive controls and specimens, respectively.

15. Guard Band Studies

The objective of the guard band studies was to establish the robustness of the PCR conditions for the THxID™ BRAF assay. The following parameters were assessed in three different studies: (1) varying thermal cycling profile (alterations to cycling times and temperatures), and (2) varying volumes (±25%) of the Reagent Mix (Primers and probes V600K and V600E solutions and Master Mix) and (3) varying Proteinase K digestion times. An analysis of variance was carried out to evaluate the impact of the studied parameters, as well as their interaction, on the Ct and Delta Ct values. For the first two condition, 3 V600E replicates at 15% mutant content and 3 WT replicates (V600E PCR), and3 V600K replicates at 15% mutant content and 3 WT replicates (V600K PCR) (generated using genomic DNA extracted from melanoma cell lines) were tested at both 20 and 700 ng DNA input per reaction, three replicates per run. For the evaluation of proteinase K digestion, a lymph node V600E specimen, a skin V600K specimen and a lymph node wild-type specimen were assessed at three incubation times (30 minutes, one hour and 2 hours). The results were satisfactory to within 0.7ºC, ±1second, ±14.5% master mix volume, ±18.5% Reagent sphere volume.

16. Cross-Contamination

A study to evaluate the incidence of false positive results related to cross-contamination of specimens during the entire work flow of the THxID™ BRAF assay was conducted by alternating BRAF mutation negative (WT) specimens and BRAF mutation positive (V600E and V600K) having high mutation content. Specimens were selected to increase the risk of carryover, i.e., low DNA content for WT specimens (35ng input), and high mutant DNA input (606 ng input) and concentration for mutation-positive specimens (one V600E and one V600K). WT FFPE clinical specimens were processed in alternating order with V600E and V600K FFPE clinical specimens. A total of five PCR runs were performed generating 115 WT sample amplification results for evaluation of carryover with each V600E and V600K PCR reaction. No unexpected amplification or false positive results were observed for wild-type samples during the study, demonstrating that carry-over contamination does not occur under tested conditions.

17. Comparison of Amplification if Plate vs. Strip Formats

The ABI 7500Fast dx Real-time PCR Instrument allows a user to perform the test with either plates or strips. The goal of this study was to confirm that results of the

THxID™ BRAF assay generated using plates for PCR amplification are comparable to results obtained when using strips for PCR amplification. Mutant V600E or V600K DNA extracted from cell lines were diluted with wild-type (WT) DNA to obtain a 3% mutant to WT ratio and a total DNA concentration of 10 ng/µl for WT, V600E and V600K samples. There were a total of 12 runs; 6 with plates and 6 with strips. Forty-four (44) replicates of WT DNA were tested for both V600E reaction and V600K reaction; 92 replicates of mutant DNA were tested for the corresponding PCR mutation reaction (i.e. V600E or V600K). The study was performed with one lot of assay reagent, one instrument and one operator. Each DNA solution was tested in both strips and plates. Mean values of Ct and ΔCt values were obtained for each sample type. Results showed an absolute difference of less than 0.6 Ct and less than 0.3 ΔCt between results obtained for the plate and strips. The study confirms that results generated using plates for PCR amplification are comparable to those obtained with strips.

18. Stability – Specimen

a) Clinical Specimen (blocks, slide-mounted, and sections) The stability of FFPE specimens (skin and lymph) used to perform the THxID™ BRAF assay was evaluated for FFPE blocks, slides and sections under a variety of temperatures and time points up to 24 months. The studies assessed specimen stability using released lots of the ThxID ™ BRAF products, and were designed to evaluate regular storage conditions, transport storage conditions, prolonged freezing, and freeze-thaw (3 cycles). Melanin content of specimens ranged from 0 to 100% and represented WT, V600E and V600K mutations. Data was available for three months. The acceptance criteria were that all results passed and there was no degradation in Ct or ΔCt values when compared to the baseline time point. The data demonstrated that:

- The clinical skin and lymph node FFPE blocks stored at 18-25°C are stable 3 months.
- The clinical FFPE 10µm thick sections in tube stored at 18-25°C or at 2-8°C or at –31°C/ -19°C are stable 3 months.
- The clinical FFPE 10µm thick sections on slides (after mimicking a shipment condition at 18-25°C and 3 cycles of freeze-thaw) are stable 3 months at 18-25°C
- The clinical FFPE 10µm thick section on slide stored at 18-25°C or at 2-8°C or at –31°C/ -19°C are stable 3 months.

b) Extracted Clinical Specimen DNA The stability of DNA extract eluate generated from twelve different FFPE skin (2 WT, 2 V600E, and 2 V600K) and lymph node (2 WT, 2 V600E, and 2 V600K) specimens was evaluated. Storage and freeze thaw conditions were evaluated to mimic potential sample conditions as follows: storage at room temperature (18-25°C) for 2 hours, storage at 2-8°C for 24 and 48 hours, storage at -31°C to -19°C including 4 cycles of freezing/ thawing, for 1, 2, 6 and 7 months, and storage at ≤ -60°C including 4 cycles of freezing/ thawing, for 3, 6 and 7 months. A

trend analysis of the Ct Internal Control (IC) and ΔCt values were compared over time. The data met the predefined acceptance criteria in that any difference from baseline was always below 1 Ct of 1 ΔCt values, and no change in the qualitative result occurred. The data supports stability to 7 months at -31°C to -19°C

19. Stability- Reagents

Real-Time stability and shipping: The shelf life of the THxID™ BRAF Kit was determined by real-time stability studies performed on 3 lots of THxID™ BRAF (upright and inverted) over a period of 24 months under two conditions (1) storage at 2-8ºC to mimic the storage in the warehouse, and during shipment; and (2) customer storage conductions in which 2 lots have undergone thermal shocks to mimic transport conditions. The customer storage conditions for THxID™ BRAF PUR reagents (except THxID™ BRAF columns) are stored at 18-25°C,and THxID-BRAF column from the THxID™ BRAF PUR and THxID™ BRAF AMP reagents stored at 2-8°C. The procedure to perform stability studies consists of 4 positive controls (PC), 4 negative controls (NC), 4 WT samples (2 extractions x 2 replicates of amplification), 8 V600E samples and 8 V600K samples (4 extractions x 2 replicates). To date the stability testing supports expiration dating of 6 months when stored at stored at 2-8°C, and at 6 months for the THxID BRAF PUR kit at 18-25°C except for the kit columns which are stored at 2-8°C.

20. Open Vial

Multiple-Use Stability of Reagent Solutions: A multiple-use stability study was evaluated at 3 different time points: Reagents, Master Mix, and reconstituted extraction buffers were stored 1 month at -31C to -19C and included 2 freeze/thawing cycles. Result obtained on solution prepared with reagents coming from 2 lots stored 2 months at 2-8°C allow support the following multiple-use stability clams in the package insert:

i. V600E Primers solution (PRM dil + PRM V600E) can be use twice in 1 month when stored at -31°C to -19°C,
ii. V600K Primers solution (PRM dil + PRM V600K) can be use twice in 1 month when stored at -31°C to -19°C,
iii. Positive Control Solution (CONT+dil + CONT+) can be use twice in 1 month when stored at -31°C to -19°C,
iv. Master Mix can be use twice in 1 month when stored at 2-8°C.

In-Use Stability of Reagent Solutions: An in-use stability study was conducted in order to evaluate the stability of reagents, master mix and reconstituted extraction buffers after 1 hour storage at room temperature (18-25°C):

The stability testing was done at 2 time points on two lots stored under customer conditions (2-8°C). The procedure to perform stability point is based on the QC release test. Results obtained on solution prepared with reagents coming from 2 lots stored 2 months at 2-8°C allow the following in-use stability claim in the package insert:

i. V600E Primers solution (PRM dil + PRM V600E) can be stored 30 minutes on the bench at 18-25°C,

ii. V600K Primers solution (PRM dil + PRM V600K) can be stored 30 minutes on the bench at 18-25°C,
iii. Reagent Mix solution (Master Mix + V600E or V600K solution) can be stored 30 minutes on the bench at 18-25°C. Remaining reagent Mix solution cannot be re-used.
iv. Positive Control Solution (CONT+dil + CONT+) can be stored 30 minutes on the bench at 18-25°C
v. Master Mix can be stored 30 minutes on the bench at 18-25°C.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The diagnostic manufacturer bioMérieux (BMX) performed retrospective studies to establish a reasonable assurance of safety and effectiveness of the THxID™ BRAF kit to select patients who may benefit from treatment with dabrafenib (Tafinlar®) on the basis of a V600E positive result with their melanoma tissue; and to select patients who may benefit from treatment with tramatenib (Mekinist™) on the basis of a V600E or V600K positive test result with their melanoma specimen. Data from these studies were the basis for the PMA approval decision.

GlaxoSmithKline (GSK) is the manufacturer of dabrafenib (Tafinlar®) which is a selective inhibitor of BRAF kinase activity in melanoma patients whose tumors encode the BRAF T1799A (V600E) mutation; and tramatenib (Mekinist™) which is a kinase that inhibits the mitogen activated protein kinase (MEK) kinase activity in melanoma patients whose tumors encode either the V600E mutation or the BRAF mutation GT1798-1799AA (V600K)[1].

B-RAF is a serine/threonine kinase that belongs to a phosphorylation cascade called the MAPK pathway (Mitogen Activated Protein Kinase). This pathway is activated by extra-cellular signals such as growth factors, and modulates key cellular processes such as cell division. In this cascade, RAS (a membrane-anchored GTPase) phosphorylates and activates B-RAF, which then phosphorylates and activates MEK1/2 kinase. Approximately 40-60% of melanomas have BRAF mutations, and 80-90% of these mutations consist of the T to A substitution at base 1799 in exon 15 of the BRAF oncogene resulting in a glutamate amino acid at codon 600 rather than a valine amino acid (i.e., V600E mutations). An additional 10-15% of these mutations result in a lysine substitution (i.e., V600K mutations) due to GT to AA base substitutions at bases 1798 and 1799. The THxID™ BRAF test is designed to detect the V600E and V600K mutations which represent approximately 97% of the total mutations at this codon.

1 Refer to drug label available on the web at DRUGS@fda for specific drug indications.

A summary of the studies conducted to support a dabrafenib (Tafinlar®) selection claim is presented in Section (1) and a summary of the studies conducted to support a tramatenib (Mekinist™) selection claim are presented in Section (2) below.

(1) Summary of Primary Clinical Studies - Dabrafenib (TAFINLAR®)

A summary of the clinical studies for Dabrafenib are presented below.

A. Study Design - Dabrafenib

A phase 3 [BREAK-3 (BRF11683); NCT01227889] clinical trial was conducted to determine the safety and efficacy of a new investigational drug, dabrafenib (Tafinlar®). BREAK-3 was an international two-arm, open-label, randomized (3:1) Phase 3 study comparing the efficacy and safety of dabrafenib to dacarbazine in patients with advanced (Stage III) or metastatic (Stage IV) melanoma whose tumor tissue harbors a V600E mutation. The BREAK-3 study was sponsored by GlaxoSmithKline (GSK) and the data was submitted to the FDA in New Drug Application (NDA 202806). Patients were screened for enrollment into this trial using an investigational clinical trial assay (CTA) designed to detect the V600E and V600K mutations. Enrollment commenced in January 2011 completed September 2011. A total of 734 patients were screened for eligibility, and 250 were enrolled into either the dabrafenib treatment arm (n=187) or to dacarbazine (n-63). Randomization was stratified for disease stage at baseline. Treatment continued until disease progression, death or withdrawal. The main efficacy measure was progression free survival (PFS) as assessed by the investigator. Specimens from patients were banked and retested in retrospective studies designed to establish the analytical and clinical concordance between the THxID -BRAF assay and the CTA (i.e., bridging studies) to clinically validate the test as safe and effective for selecting patients who may benefit with dabrafenib (Tafinlar®).

1. Inclusion and Exclusion Criteria for Specimen Testing –Dabrafenib

Formalin fixed, paraffin embedded (FFPE), tissue blocks from a biopsy in the metastatic setting were required for the assessment of BRAF mutation status to determine trial eligibility. Each block contained 5-10 mm^2 of tumor tissue submitted in standard 4x3 cm cassettes. If sites were unable to send a tissue block, tissue slides were submitted from a single 5 micron thick section. If archived biopsy tissue was not available, an FFPE core biopsy from a metastatic site was required. One section was stained with hematoxylin and eosin (H&E stain) to determine tumor presence. Tumors were required to be macrodissected if tumor content was below 80% of the section. For retesting with the THxID™ BRAF Kit, tissue sections from the same block were used first. In the rare absence of the availability of tissue sections, archived DNA eluate from the original extraction was used. All specimens from patients whose eligibility for the trial was based on their tumor mutations status were retested (i.e., includes mutation positives, mutation negatives, and invalids). Reasons for missing samples were accounted. For all studies, repeat testing was performed according to the trouble shooting section of the

test labeling [i.e., Instructions for Use (IFU)]. No more than two repeat tests were performed for a sample when attempting to obtain a valid result.

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

Inclusion Criteria - Subjects eligible for enrollment must meet all the following criteria

a. Has provided signed informed consent.
b. Histologically confirmed advanced (unresectable Stage III) or metastatic melanoma (Stage IV) and BRAF mutation-positive (V600 E) melanoma as determined via central testing with a BRAF mutation assay.
c. Are treatment naïve for advanced (unresectable) or metastatic melanoma, with the exception of IL-2.
d. Measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1).
e. Age ≥18 years of age
f. Able to swallow and retain oral medication.
g. Women with child-bearing potential and men with reproductive potential must be willing to practice acceptable methods of birth control during the study. Additionally, women of childbearing potential must have a negative serum pregnancy test within 14 days prior to the first dose of study treatment.
h. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0-1[Oken, 1982].
i. Must have adequate organ function as defined by a set of screening values described in the clinical protocol.

Exclusion Criteria – Subjects meeting any of the following criteria must not be enrolled in the study

a. Previous treatment for metastatic melanoma, including treatment with a BRAF or MEK inhibitor.
b. Known ocular or primary mucosal melanoma.
c. Currently receiving cancer therapy (chemotherapy, radiation therapy, immunotherapy, biologic therapy, or surgery).
d. Use of any investigational anti-cancer or other drug within 28 days or 5 half-lives, whichever is longer, preceding the first dose of dabrafenib.
e. Current use of a prohibited medication or is expected to require any of these medications during treatment with dabrafenib.
f. Any major surgery, radiotherapy, or immunotherapy within the last 4 weeks.
g. Presence of active gastrointestinal disease or other condition that will interfere significantly with the absorption of drugs. If clarification is needed as to whether a condition will significantly affect absorption of drugs, contact the GSK medical monitor for permission to enroll the subject.
h. A history of Human Immunodeficiency Virus (HIV) infection
i. A history of glucose-6-phosphate dehydrogenase (G6PD) deficiency.

j. A history of other malignancy. Subjects who have been disease-free for 5 years, or subjects with a history of completely resected non-melanoma skin cancer or successfully treated in situ carcinoma are eligible.
k. Evidence of active CNS disease (radiographically unstable, symptomatic lesions). However prior treatment with stereotactic radiosurgery (SRS) or surgical resection is allowed if the subject remains without evidence of disease progression in the brain ≥3 months, and has been off corticosteroids for ≥3 weeks. Whole brain radiotherapy is not allowed except in those subjects who have had definitive resection or SRS of all radiographically detectable parenchymal lesions.
l. History of alcohol or drug abuse within 6 months prior to Screening.
m. Psychological, familial, sociological, or geographical conditions that do not permit compliance with the protocol, or unwillingness or inability to follow the procedures required in the protocol.
n. Cardiac abnormalities as defined in the clinical protocol.

3. <u>Follow-up Schedule – Dabrafenib</u>

***Patients were followed for efficacy*:**
Patients in each treatment arm underwent scheduled clinical and tumor assessments at baseline and then every 3 weeks thereafter until confirmation of disease progression. Patients who were removed from treatment were assessed thereafter every 12 weeks until death or study completion. All subjects who permanently discontinue study treatment were followed for survival and additional anti-cancer therapies [including radiotherapy] every 12 weeks until death or study completion. In addition, those subjects who permanently discontinued study treatment without progressive disease were to have radiographic disease assessments performed on the same assessment schedule noted protocol every 9 weeks until Week 27 and then every 12 weeks thereafter until disease progression, start of new anti-cancer therapy, or death.

Patients were followed for safety:
Information related to the adverse events experienced by patients treated with dabrafenib in the BREAK-3 trial were collected until disease progression or unacceptable toxicity for at least 6 months. Adverse events (AEs) were collected from the time the first dose of study treatment was administered until 28 days after discontinuation of study treatment. Serious adverse events (SAEs) were collected over the same time period. Abnormal laboratory and safety assessments reported as abnormal were outlined and evaluated according to schedule. After discontinuation of treatment, the investigator monitored all AEs and SAEs that were ongoing until resolution or stabilization of the event or until the subject was lost to follow-up. The investigator and site staff were responsible for detecting, documenting and reporting events that meet the definition of an adverse event (AE) or serious adverse event (SAE) as described in the study protocol.

4. Clinical Endpoints - Dabrafenib

The primary efficacy evaluation was performed on the intent to treat (ITT) population. The Intent-to-Treat (ITT) population comprised all randomized subjects regardless of whether or not treatment was administered. This population was based on the treatment to which the subject was randomized. Any subject who received a treatment randomization number was considered to have been randomized.

With regards to safety: The safety objectives for this study were to assess the rate of non-melanoma skin lesions and other second malignancies in both treatment groups; to further characterize the safety and tolerability of dabrafenib administered as a single agent for BRAF mutation positive metastatic melanoma; and to evaluate the qualitative and quantitative toxicities between treatment arms. The safety endpoints of this study were (1) the frequency and severity of treatment-emergent adverse events and laboratory abnormalities; and (2) rate of treatment-emergent non-melanoma skin lesions and second malignancies in each treatment group defined as the percentage of subjects with non-melanoma skin lesions reported.

With regards to efficacy:

i. ***Based on patient specimen testing by the central laboratory with the clinical trial assay:*** Efficacy of dabrafenib was based on therapeutic response observed with a target population identified as having BRAF V600E mutation positive melanoma by a clinical trial assay (CTA) conducted at a central testing laboratory. The primary objective of this study was to evaluate and compare investigator-assessed PFS in subjects treated with dabrafenib with those treated with dacarbazine. PFS, defined as the time from randomization to the earliest date of radiographic/photographical disease progression or death due to any cause, was based on the investigator's assessment. Disease progression and response evaluations were determined according to the definitions established in the Response Evaluation Criteria in Solid Tumors (RECIST 1.1).

ii. ***Based on the THxID –BRAF assay (BREAK-3 Bridging Study):*** The purpose of the bridging study was to demonstrate the ability of the THxID™ BRAF test to detect the V600E mutation with significant agreement to the CTA to support the efficacy conclusions observed in the BREAK-3 trial. There were two objectives of the bridging assay. The first was to evaluate the agreement between the THxID-BRAF Kit and the CTA for detection of BRAF V600E mutations. The second was to assess the clinical outcome (investigator assessed PFS) of the patients enrolled in the BREAK-3 study whose tumor specimens were V600E positive as detected by the THx-ID assay.

B. Accountability of PMA Cohort - Dabrafenib

A total of 734 patients were screened for the BREAK-3 trial. There were 744 specimens for the 734 patients because there were 5 cases where there were 2 specimens for each subject. Of those, 109 patients were tested but were not included in the trial for failing

eligibility criteria unrelated to testing. Patients were eligible for the trial if their melanoma specimens were V600E positive by the CTA. 315 patients were not eligible on the basis of a non-V600E result (i.e., V600K or WT). An additional 60 patients were not eligible due to CTA invalid results or inadequate sample. Therefore, a total of 584 specimens warranted retesting with the THxID™ BRAF assay. Invalids were included in the retest set because they represent challenging specimens for which the THxID™ BRAF test may provide a result. A total of 576 or 98.6% of specimens were available for retesting (8 specimens were unavailable for various reasons due to lack of informed consent, or lack of samples). Following removal of 6 duplicate specimens and 1 sample that had insufficient quantity, there were 565 specimens (=565 subjects) available for testing. The BREAK-3 trial randomized a total of 250 patients identified as V600E mutation positive by the CTA to the treatment arm (n = 187) or dacarbazine arm (n = 63). Of these, a total of 243 or 97.2% were available for retesting. Because some samples produced invalid results by the THxID™ BRAF assay or were unavailable for retesting due to exhausted sample, a final total of 232 specimens were available (n =177; 55, respectively) or 92.8% for the reanalysis of efficacy on the basis of a THxID result. The following table summarizes the number and distribution of specimens from patients screened in the BREAK-3 study and those retested by the THxID -BRAF test.

Specimen Accountability Table for Specimens from Patients Screened for Eligibility into BREAK-3 trial

Characteristic	Total Patients	Total Specimens[1]	Total Available for retesting with THxID[4]
BREAK-3 Phase III Patients Screened for Eligibility	734	744	576
Reported as BRAF V600E mutation-positive by CTA and treated in the dafrafenib arm	187	253	184[2]
Reported as BRAF V600E mutation-positive by CTA and treated in the dacarbazine arm	63		59[2]
Not treated based on V600K mutation-positive (trial enrolled V600E)	50	50	50
Not treated based on mutation-negative CTA result (Reported as BRAF V600 Wild-type)	265	267	264
Not treated based on failed Inclusion criteria for reasons other than test result These specimens were not retested because these samples did not meet the GSK inclusion criteria of the drug trial (i.e., these patients would not have come forward for testing)	109	110	n/a
Reported as Invalid [Out of Detectable Range" (OODR)]	19	20	19[3]

Reported as "Quantity Not Sufficient" (QNS)	28	29	n/a
Reported as No Tumor indicated	12	14	n/a
Reported as Specimen received, but not tested	1	1	n/a
Total percentage of retest population	(584 patients with CTA results)		98.6% 576/584

[1]There were a greater number of specimens than subjects due to the submission of multiple specimens per subject for BRAF testing during the trial. n/a = not applicable

[2] A total of 243 specimens from the trial were available for retesting but some results were invalid by THxID™ BRAF Assay. A total of 232 specimens representative of the 250 subjects enrolled in the trial were available for the efficacy analysis

[3]Patients with specimens whose samples were invalid by the CTA, were not enrolled due to no result. These samples were therefore retested with the THxID –BRAF assay as they represent challenging specimens.

[4]After removal of duplicates.

C. Study Population Demographics and Baseline Parameters –Dabrafenib

The demographics of the study population are shown in the table below and demonstrate the distribution of the baseline parameters for clinically relevant variables important for understanding the treatment effect. The median age of tramatenib patients was 53 years, 60% were male, 97% non-hispanic, and 67% had an ECOG performance status of 0, normal LDH (62%), and M1c disease. Twenty-eight (44%) patients crossed over from the dacarbazine arm at the time of disease progression to receive dabrafenib.

BREAK-3 Demographics (ITT Population)

		Number of Subjects (%)
		Total (N=229)
Age (years)	Mean (SD)	52.7 (13.64)
	Median (min, max)	52.0 (21, 93)
Age Group	<65	181 (79)
	>=65	48 (21)
	>=75	13 (6)
Gender	Female	93 (41)
	Male	136 (59)
Ethnicity	Hispanic or Latino	7 (3)
	Not Hispanic or Latino	222 (97)
Height (cm)[1]	Mean (SD)	172.4 (8.80)
	Median (min, max)	173.0 (150, 196)
Weight (kg)[2]	Mean (SD)	80.1 (17.5)
	Median (min, max)	79.30 (36.2, 130.0)

	Dabrafenib N= 187	DTIC N= 63
Age: Median (Range)	53 (22-93)	50 (21-82)
Male	112 (60%)	37 (59%)
White	186 (100%)	63 (100%)

Baseline LDH equal to or below ULN	116 (62%)	40 (63%)
IVM1c at baseline	124 (66%)	40 (63%)
ECOG =0	124 (66%)	44 (70%)
Visceral disease at baseline	137 (73%)	43 (68%)

Specimen Characteristics - Dabrafenib:

The following table summarizes the tissue type characteristics for specimens with available information as pertains to the tumor cell concentration, tumor area, requirement for macrodissection, and other specimen characteristics based on pathological review.

Summary of Sample Characteristics for All Testing Sites

		Lymph Node (162)	Skin (163)	Other sites (112)	Unknown (6)	All (443)
≥80% Tumor cells in the sample section		160 (98.8%)	163 (100.0%)	112 (100.0%)	6 (100.0%)	441
<80% Tumor cells in the sample section		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
Tumor cells in the sample section**		2 (1.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2
Selected Tissue area* ≥ 20mm^2		159 (98.1%)	157 (96.3%)	106 (94.6%)	6 (100.0%)	428
Selected Tissue area* < 20mm^2		3 (1.9%)	6 (3.7%)	6 (5.4%)	0 (0.0%)	15
Macro-dissection performed		84 (51.9%)	128 (78.5%)	75 (67.0%)	5 (83.3%)	292
Melanin Content	**None**	40 (24.7%)	39 (23.9%)	21 (18.8%)	1 (16.7%)	101
	Low^	63 (38.9%)	57 (35.0%)	53 (47.3%)	3 (50.0%)	176
	Medium^	16 (9.9%)	38 (23.3%)	15 (13.4%)	0 (0.0%)	69
	High^	43 (26.5%)	29 (17.8%)	23 (20.5%)	2 (33.3%)	97
Necrosis		107 (66.0%)	69 (42.3%)	62 (55.4%)	2 (33.3%)	240
Fatty Tissue		41 (25.3%)	62 (38.0%)	21 (18.8%)	2 (33.3%)	126
Hemorrhage		105 (64.8%)	66 (40.5%)	54 (48.2%)	1 (16.7%)	226

(*) represents tissue area that includes tumor cells and the surrounding matrix

(**) indicates % tumor cells in sample selection; when the section contained less than 80% tumor cells, the tumor cells might not be specified (e.g., NA)

(^) The values for the melanin content are: Low (≤10% melanin in tumor area); Medium (11-24% melanin in tumor area); High (≥25% melanin of tumor area

D. Safety and Effectiveness Results - Dabrafenib

1. Safety Results

The safety of the THxID™ BRAF 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 therapeutic. A false positive result would potentially expose the patient to a therapeutic that may not be beneficial as well as any possible side effects associated with the therapeutic. Overall, the most commonly occurring adverse events (≥20%) in patients treated with dabrafenib were hyperkeratosis, pyrexia, arthralgia, papilloma, alopecia, and palmar-plantar erythrodysesthesia syndrome (PPE). Cutaneous squamous cell carcinomas and keratoacanthomas (cuSCC) occurred in 11% (64/586) of patients treated with dabrafenib. The most frequent (≥2%) adverse reactions leading to

dose reduction of dabrafenib were pyrexia (9%), PPES (3%), chills (3%), fatigue (2%), and headache (2%). Refer to the drug label for more information about adverse events associated with dabrafenib (Tafinlar®). The THxID™ BRAF test was shown to have high accuracy when compared to Sanger bi-directional sequencing (overall agreement >95%) indicating the possibility of false results is very low.

2. **Effectiveness Results – based on mutation detection with CTA**

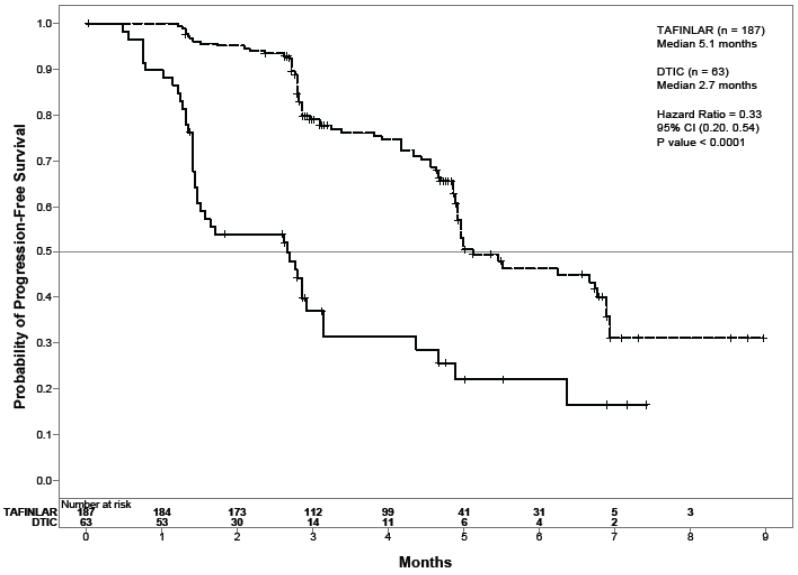
The safety and efficacy of dabrafenib were evaluated in 250 patients with BRAF V600E mutation-positive, unresectable or metastatic melanoma as assessed by a clinical trial assay. The primary efficacy outcome measure was progression-free survival (PFS). Patients were randomized to receive dabrafenib (n = 187) or dacarbazine (n = 63). The BREAK-3 study demonstrated a statistically significant increase in PFS observed for patients treated with tramatenib (median 5.1 months) when compared to dacarbazine (median 2.7 months). The table below and graph (upper line dabrafenib arm) summarize the PFS results.

Investigator-Assessed Progression-Free Survival based on CTA

	TAFINLAR® N = 187	**Dacarbazine N = 63**
Progression-free Survival Median, months (95% CI)	5.1 (4.9, 6.9)	2.7 (1.5, 3.2)
HR [a] (95% CI)	0.33 (0.20, 0.54)	
p-value [b]	*P* <0.001	

[a] Pike estimator, stratified by disease stage. [b] stratified log-rank test; CI = Confidence interval; HR = hazard ratio

Kaplan-Meier Curves of Investigator –Assessed Progression-Free Survival (ITT Population Based on CTA)



Refer to the drug label available at DRUGS@FDA available on the web for the most up to date information about Dabrafenib.

3. **Effectiveness Results – based on mutation detection with THxID™ BRAF Kit**

DABRAFENIB BRIDGING STUDY

The purpose of the bridging study was to demonstrate the analytical and clinical concordance of the THxID™ BRAF assay to the CTA to support the efficacy conclusions observed in the BREAK-3 trial.

Analytical Concordance:

Melanoma Specimens tested for V600E mutations to determine trial eligibility with the CTA were retested with the THxID™ BRAF test. The analytical agreement between the THxID™ BRAF Kit and the CTA was evaluated for both mutation-positive and mutation negative specimens. A total of 734 patients were screened for the trial. Of these, a total of 584 specimens had CTA results (including invalids). Of the 584 specimens, 565 were available for retesting (96.7%). Table 1 shows the analytical concordance between the CTA results and the THxID™ BRAF results with specimens available for retesting. Dabrafenib is indicated for patients whose melanoma harbor V600E mutations. The agreement for the V600E mutation was 96.7% (95% CI: 93.6% to 98.3%) when including the test invalids. Agreement with non-V600E alleles was 95% (95% CI 92.7% to 97.0%) Overall agreement between the assays was approximately 95% (95% CI: 92.7% to 96.4%). The invalid rate for the retrospective testing of specimens from the BREAK-3 trial was 3.4%). The results demonstrate acceptable agreement between the CTA and the THxID™ BRAF assay.

Agreement between the THxID™ BRAF Assay and CTA for all subjects (All Testing Sites)

		Clinical Trial Assay (CTA)					
		V600E	**V600K**	**V600E&K**	**WT**	**Invalid**	**Total**
THxID BRAF Assay	**V600E**	232	1	0	8	1	242
	V600K	0	45	0	1	0	46
	V600 E&K	0	0	0	0	0	0
	WT	4	2	0	245	2	253
	Invalid	4	2	0	4	14	24
	Total	240	50	0	258	17	565

	Agreements (All, 5x5)			Agreements (without THxID & CTA Invalids)		
Agreement	**No. of concordance / No of tests**	**Agreement rate (%)**	**95% score CI**	**No. of concordance / No of tests**	**Agreement rate (%)**	**95% score CI**
for V600E	232/240	96.70%	[93.6%;98.3%]	232/236	98.30%	[95.7%;99.3%]

	Agreements (All, 5x5)			Agreements (without THxID & CTA Invalids)		
Agreement	**No. of concordance / No of tests**	**Agreement rate (%)**	**95% score CI**	**No. of concordance / No of tests**	**Agreement rate (%)**	**95% score CI**
for V600K	45/50	90.00%	[78.6%;95.7%]	45/48	93.80%	[83.2%;97.9%]
Mutation-negative	245/258	95.00%	[91.6%;97.0%]	245/254	96.50%	[93.4%;98.1%]
Overall	536/565	94.90%	[92.7%;96.4%]	522/538	97.00%	[95.2%;98.2%]

Clinical Concordance:

The second objective was to assess the concordance on clinical outcome (investigator assessed PFS) in patients enrolled in the BREAK-3 trial whose tumors were V600E positive as detected by the THxID™ BRAF assay. A total of 250 BRAF V600E mutation positive patients were randomized to the BREAK-3 Trial (187 to the dabrafenib arm and 63 to dacarbazine). Among these patients, a total of 232 had specimens testing positive by the THxID™ BRAF. This subset of patients test was used to calculate the hazard ratio [HR =0.34, 95% CI (0.20, 0.57)]. The HR of dabrafenib to dacarbazine for the subset of THxID™ BRAF tested subjects was similar to that from the randomized population [HR 0.33, 95% CI (0.20, 0.54)]. The estimate for improved median PFS time was similar regardless of which test was used (approximately 2.3 months).

Summary of Progression Free Survival for THxID™ BRAF Assay V600E Mutation Positive Subjects Using Investigator Assessed PFS.

	THxID –BRAF		CTA	
	Dabrafenib	**DTIC**	**Dabrafenib**	**Dacarbazine**
Number of Subjects	**177**	55	187	63
Hazard Ratio Estimate	0.34		0.33	
95% Confidence Interval	(0.20,0.57)		(0.20, 0.54)	
P-Value	<0.0001			
Estimates for PFS (months) l				
Median	5.0	2.7	5.1	2.7
95% Confidence Interval	(4.9,6.8)	(1.5,3.2)	(4.9, 6.9)	(1.5, 3.2)

The expected PFS hazard ratio for all patients who are V600E mutation positive using the THxID BRAF assay was estimated. An array of possible hazard ratios was considered for those patients who could be labeled mutation- positive by the THxID™ BRAF assay yet wild-type by the CTA (i.e., those excluded from the trial). Conditional probability was used to combine a postulated hazard ratio with the hazard ratio estimate in the trial for patients who are mutation-positive by both

assays. The PFS hazard ratio for those patients positive by THxID™ BRAF assay yet negative by CTA was allowed to range from 0.32 to 1.0. For these values, the expected estimate of the PFS hazard ratio for THxID™ BRAF mutation-positive patients was between 0.32 and 0.34, indicating consistency with the PFS hazard ratio in the randomized population. These analyses were also conducted separately on the basis of specimen type (skin vs. Lymph node vs. other) and the results were consistent showing that specimen type does not impact the conclusions (data not shown).

The results from the bridging study provide a demonstration of the clinical utility of the THxID™ BRAF test to support the selection of patients whose melanoma tissue is BRAF V600E positive for treatment with dabrafenib (Tafinlar®).

Summary of Expected Progression Free-Survival Hazard Ratios for THxID™ BRAF Assay Mutation Positive Subjects Using FDA investigator Assessed PFS

Estimated PFS Hazard Ratio in Subjects who are V600E Mutation Positive by Both Assays	Probability of being V600E Mutation Positive by Both Assays	Postulated Expected PFS Hazard Ratio In Subjects who are THxID™ BRAF V600E Mutation Positive and CTA Wild Type	Probability of being THxID™ BRAF V600E Mutation Positive and CTA Wild Type	Expected Estimated PFS Hazard Ratio for THxID™ BRAF Assay V600E Mutation Positive Subjects (95% CI)
0.3246	0.963	0.32	0.037	0.32 (0.22,0.47)
0.3246	0.963	0.33	0.037	0.32 (0.22,0.47)
0.3246	0.963	0.35	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.37	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.39	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.41	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.43	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.45	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.47	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.50	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.52	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.55	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.58	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.61	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.64	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.67	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.70	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.74	0.037	0.33 (0.23,0.48)
0.3246	0.963	0.78	0.037	0.34 (0.23,0.50)

Estimated PFS Hazard Ratio in Subjects who are V600E Mutation Positive by Both Assays	Probability of being V600E Mutation Positive by Both Assays	Postulated Expected PFS Hazard Ratio In Subjects who are THxID™ BRAF V600E Mutation Positive and CTA Wild Type	Probability of being THxID™ BRAF V600E Mutation Positive and CTA Wild Type	Expected Estimated PFS Hazard Ratio for THxID™ BRAF Assay V600E Mutation Positive Subjects (95% CI)
0.3246	0.963	0.82	0.037	0.34 (0.23,0.50)
0.3246	0.963	0.86	0.037	0.34 (0.23,0.50)
0.3246	0.963	0.90	0.037	0.34 (0.23,0.50)
0.3246	0.963	0.95	0.037	0.34 (0.23,0.50)
0.3246	0.963	1.00	0.037	0.34 (0.23,0.50)

(2) SUMMARY OF PRIMARY CLINICAL STUDIES – TRAMATENIB (MEKINIST™)

A summary of the clinical studies for tramatenib are presented below.

A. Study Design – Tramatenib (Mekinist™)

The Phase 3 [MEK114267 (METRIC); NCT01245062] clinical trial and the associated bridging study were conducted to determine the safety and efficacy of THxID™ BRAF test to select patients for tramatenib (Mekinist™). MEK114267 (METRIC) was an international, multi-center, two-arm, open-label, randomized (2:1) Phase III study comparing the efficacy, safety and tolerability of tramatenib to dacarbazine or paclitaxel (chemotherapy) in patients with advanced (stage III) or metastatic (Stage IV) melanoma whose melanoma tissue harbor a BRAF V600E or V600K mutation. The METRIC study was sponsored by GlaxoSmithKline (GSK) and the data was submitted to the FDA in New Drug Application (NDA 204114). Enrollment into this trial was limited to patients whose melanoma tissue tested positive for V600E or V600K mutations using an investigational clinical trial assay (CTA) designed to detect the V600E and V600K mutations. Enrollment commenced in December 2010, and was completed July, 2011. A total of n=1108 patients were screened for eligibility, and a total n= 322 were enrolled. Of those, 214 patients received treatment with tramatenib. In the chemotherapy arm n = 108 received dacarbazine or paclitaxel. Randomization was stratified according to prior use of chemotherapy for advanced or metastatic disease (yes or no) and LDH level (upper or lower). Treatment continued until disease progression, death or withdrawal. Patients who were randomized to chemotherapy and whose disease progressed were allowed to crossover and receive tramatenib. A total of 51 patients crossed-over after independent confirmation of progression. The primary endpoint of METRIC was progression free survival (PFS). Retrospective bridging studies to establish the analytical and clinical concordance between the THxID™ BRAF assay and the CTA were conducted to support of the use of this test with tramatenib (Mekinist™).

1. **Inclusion and Exclusion Criteria for Specimen Testing - Tramatenib**

Formalin fixed, paraffin embedded (FFPE), tissue blocks from a biopsy in the metastatic setting were required for the assessment of BRAF mutation status to determine trial eligibility. Each block contained 5-10 mm^2 of tumor tissue submitted in standard 4x3 cm cassettes. If sites were unable to send a tissue block, tissue slides were submitted from a single 5 micron thick section. If archived biopsy tissue was not available, an FFPE core biopsy from a metastatic site was required. One section was stained with hematoxylin and eosin (H&E stain) to determine tumor presence. Tumors were required to be macrodissected if tumor content was below 80% of the section. For retesting with the THxID™ BRAF Kit, tissue sections from the same block were used first. In the rare absence of the availability of tissue sections, archived DNA eluate from the original extraction was used. All specimens from patients whose eligibility for the trial was based on their tumor mutations status were retested (i.e., includes mutation positives, mutation negatives, and invalids). Reasons for missing samples were accounted. For all studies, repeat testing was performed according to the trouble shooting section of the test labeling [i.e., Instructions for Use (IFU)]. No more than two repeat tests were performed for a sample when attempting to obtain a valid result..

2. **Clinical Inclusion and Exclusion Criteria for Patient Enrollment - Tramatenib**

Inclusion Criteria - Subjects eligible for enrollment must meet all the following criteria

a. Has provided signed informed consent.
b. Age ≥18 years of age
c. Histologically confirmed advanced (unresectable Stage III) or metastatic melanoma (Stage IV) and BRAF mutation-positive (V600 E/K) melanoma as determined via central testing with a BRAF mutation assay.
d. Subjects may have received no prior treatment or up to one prior regimen of chemotherapy for advanced or metastatic melanoma. Subjects having received one prior regimen of chemotherapy must have had documented disease progression prior to randomization. Prior treatment with immunotherapy (with the exception of prior ipilimumab, which is only allowed if given in the adjuvant setting), cytokine therapy, biological or vaccine regimen is permitted. Prior use of sorafenib is allowed. Disease progression must be documented for any anti-cancer therapy (i.e., immunotherapy or biologic therapy), if given as a most recent treatment prior to randomization.
e. Measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1).
f. All prior treatment- related toxicities must be CTCAE (Version 4.0) ≤ Grade 1 (except alopecia) at the time of randomization.
g. Able to swallow and retain oral medication.
h. Women with child-bearing potential and men with reproductive potential must be willing to practice acceptable methods of birth control during the study. Additionally, women of childbearing potential must have a negative

serum pregnancy test within 14 days prior to the first dose of study treatment.

i. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0-1.
j. Must have adequate organ function as defined by a set of screening values.

Exclusion Criteria – Subjects meeting any of the following criteria must not be enrolled in the study

a. Women who were pregnant or in the period of lactation.
b. Any prior use of:
    - BRAF inhibitors or MEK inhibitors.
    - Ipilimumab in the advanced or metastatic setting.
c. Subjects who have received dacarbazine or paclitaxel prior to randomization will not be eligible to receive the same chemotherapy as study medication (i.e. a subject who received prior dacarbazine cannot receive dacarbazine on this trial and would thus receive paclitaxel if randomized to the control arm).
d. Any major surgery, extensive radiotherapy, chemotherapy with delayed toxicity, biologic therapy or immunotherapy within the last 21 days. Chemotherapy given daily or weekly without the potential for delayed toxicity within the last 14 days.
e. Administration of an investigational drug within 28 days or 5 half-lives, (whichever is shorter), prior to randomization – at least 14 days must have passed between the last dose of the prior investigational anti-cancer drug and randomization.
f. Current use of any prohibited medication.
    - Use of anticoagulants such as warfarin and low molecular weight heparin is permitted, however INR must be monitored in accordance with local institutional practice.
g. History of another malignancy.
   Exception: Subjects who have been disease-free for 3 years (i.e., subjects with second malignancies that are indolent or definitively treated at least 3 years ago) or subjects with a history of completely resected non-melanoma skin cancer. Consult GSK Medical Monitor if unsure whether second malignancies meet requirements specified above.
h. Any serious and/or unstable pre-existing medical (aside from malignancy exception above), psychiatric disorder, or other conditions that could interfere with subject's safety, obtaining informed consent or compliance to the study procedures.
i. Known Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), or Hepatitis C Virus (HCV) infection (with the exception of chronic or cleared HBV and HCV infection which will be allowed).
j. Brain metastases with the a few exceptions that are ALL confirmed by the GSK Medical Monitor:
k. History or evidence of cardiovascular risk
l. History of interstitial lung disease or pneumonitis.
m. History or current evidence / risk of retinal vein occlusion (RVO) or central serous retinopathy (CSR):

n. Known immediate or delayed hypersensitivity reaction or idiosyncrasy to drugs chemically related to the study drug, or excipients or to dimethyl sulfoxide (DMSO) or to Cremophor EL (polyoxyethylated castor oil).
o. Lactating female.

3. **Follow-up Schedule - Tramatenib**

***Patients were followed for efficacy*:**
Patients in each treatment arm underwent scheduled clinical and tumor assessments at baseline and then every 3 weeks thereafter until confirmation of disease progression and thereafter every 12 weeks until death or study completion. Survival follow-up continued until 80% of the subjects on the study died or were lost to follow-up.

Patients were followed for safety:
Information related to the adverse events (AE) experienced by patients treated with tramatenib in the METRIC trial was collected until disease progression or unacceptable toxicity for at least 6 months. Adverse events were collected from the time the first dose of study treatment was administered until 30 days after discontinuation of study treatment. Abnormal laboratory and safety assessments reported as abnormal were outlined and evaluated according to schedule. After discontinuation of treatment, the investigator monitored all AEs and SAEs that were ongoing until resolution or stabilization of the event or until the subject was lost to follow-up.

4. Clinical Endpoints - Tramatenib
The Intent-to-Treat (ITT) population comprised all randomized subjects regardless of whether or not treatment was administered. This population was based on the treatment to which the subject was randomized. Any subject who received a treatment randomization number was considered to have been randomized.

With regards to safety: The Safety population (SAFETY) comprised all randomized subjects who received at least one dose of study medication and will be based on the actual treatment received if this differs from that to which the subject was randomized. This population was used for the analysis of clinical safety data.
The primary efficacy population of interest, BRAF V600E /V600K subjects without a prior history of brain metastases, will be a subset of the ITT population. Additionally any other subpopulations of interest explored in the analyses of efficacy data will be subsets of the ITT population. The Crossover population comprised the subset of subjects who were randomized to chemotherapy and who elect to crossover to trametenib treatment arm following progression on chemotherapy.

With regards to efficacy:
i. ***Based on clinical trial assay:*** The primary objective for this study was to establish superiority of tramatenib over chemotherapy with respect to PFS for subjects with advanced/metastatic BRAF V600E/V600K mutation-

positive melanoma without a prior history of brain metastases. Investigator assessments were used as it serves as a direct measure of the effect of randomized treatment. A blinded, independent, central review was also performed. Disease progression and response evaluations were determined according to the definitions established in the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1).

i. ***Based on the THxID™ BRAF assay (METRIC Bridging Study):*** Efficacy of tramatenib was based on therapeutic response observed with a target population identified as having BRAF V600E or V600K mutation positive melanoma by a clinical trial assay (CTA) conducted at a central testing laboratory. The purpose of the bridging study was to demonstrate the ability of the THxID™ BRAF test to detect the V600E and V600K mutations with significant agreement to the CTA to support the efficacy conclusions observed in the METRIC trial. There were two objectives of the bridging assay. The first was to evaluate the agreement (PPA, NPA, and OPA) between the THxID™ BRAF Kit and the CTA for detection of BRAF V600E and BRAF 600K mutations. The second was to assess the clinical outcome (investigator assessed PFS) of the patients enrolled in the METRIC trial whose tumor specimens were V600E or V600K positive as detected by the THxID™ BRAF assay.

B. Accountability of PMA Cohort

A total of 1108 patients were screened for the METRIC trial. There were 1119 specimens for the 1108 patients because there were cases where there were duplicate specimens for each subject. Of those, 230 patients were tested but were not included in the trial for failing eligibility criteria unrelated to testing. Patients were eligible for the trial if their melanoma specimens were V600E or V600K positive by the CTA. 455 patients were not eligible on the basis of a non-V600E result (i.e., generally WT for V600). An additional 76 patients were not eligible due to CTA invalid results or inadequate sample. Invalids were included in the retest set because they represent challenging specimens for which the THxID™ BRAF test may provide a result. Therefore, a total of 817 specimens warranted retesting with the THxID™ BRAF assay. A total of 793 or 97.6% of specimens were available for retesting (specimens were unavailable for various reasons due to lack of informed consent, or inadequate tissue). After removal of duplicates there were 766 specimens (=2ubjects) available for testing. The METRIC trial randomized a total of 322 patients identified as V600E or V600K mutation positive by the CTA to the treatment arm (n = 214) or dacarbazine arm (n = 108). Of these, a total of 307 (95.3%) were available for retesting. Because some samples produced invalid results by the THxID™ BRAF assay or were unavailable for retesting due to exhausted sample, a final total of 289 or 89.8% specimens were available (n =196; 93, respectively) or 92.8% for the reanalysis of efficacy on the basis of a THxID™ BRAF result. The following table summarizes the number and distribution of specimens from patients screened in the METRIC study and those retested by the THxID™ BRAF test.

Specimen Accountability Table for Specimens from Patients Screened for Eligibility into METRIC trial

	Total Patients	Total Specimens[1]	Total available for retesting with THxID
METRIC Phase III Patients Screened for Eligibility	1108	1119	793
Reported as either BRAF V600E or V600K mutation-positive by CTA and treated in the trametenib arm	214	214	208
Reported as either BRAF V600E or V600K mutation-positive by CTA and treated in the chemotherapy arm	108	108	99
Not treated based on mutation-negative CTA result (Reported as BRAF V600 Wild-type)	455	459	455
Not treated: Failed Inclusion criteria for reasons other than test result These specimens were not retested because these samples did not meet the GSK inclusion criteria of the drug trial (i.e., these patients would not have come forward for testing had the	230	230	n/a
Reported as Invalid [Out of Detectable Range" (OODR)]*	31	36	31
Reported as "Quantity Not Sufficient" (QNS)	31	n/a	n/a
Reported as No Tumor indicated	8	n/a	n/a
Reported as Specimen received, but not tested	6	n/a	n/a
Total percentage of retest population	(808 patients who had CTA results)		98.1% 793/808

[1] There were a greater number of specimens than subjects due to the submission of multiple specimens per subject for BRAF testing during the trial. n/a = not applicable

C. Study Population Demographics and Baseline Parameters

The demographics of the study population are shown in the table below and demonstrate the distribution of the baseline parameters for clinically relevant variables important for understanding the treatment effect. The median age for randomized patients was 54 years, 54% were male, 100% were Caucasian, and all patients had baseline ECOG performance status of 0 or 1. All patients had tumor

tissue with mutations in BRAF V600E (87%), V600K (12%), or both (<1%) on centralized testing. Most patients had metastatic disease (94%), were Stage M1c (65%), had elevated LDH (37%), no history of brain metastasis (97%), and received no prior chemotherapy for advanced or metastatic disease (66%).

METRIC Demographics and Disease Characteristics (ITT Population)

Category	**Tramatenib N= 214**	**Chemotherapy N = 108**
Age: Median (Range)	54.5 (23-85)	54 (21-77)
Male	120 (56%)	53 (49%)
White	214 (100%)	108 (100%)
History of Brain Mets*	9 (4%)	2 (2%)
Prior Immunotherapy	68 (32%)	30 (28%)
Prior Chemotherapy	71 (33%)	38 (35%)
Baseline LDH above ULN	77 (36%)	42 (39%)
Stage IVM1c	144 (67%)	63 (58%)
ECOG 0	136 (64%)	69 (64%)
V600E	184 (86%)	97 (90%)
V600K	29 (14%)	11 (10%)
V600E/K	1 (<1%)	0

Specimen Characteristics:

The following table summarizes the tissue type characteristics as pertains to the tumor cell concentration, tumor area, requirement for macrodissection, and other sample characteristics based on pathological review.

Summary of Sample Characteristics for All Testing Sites

		Lymph Node (229)	Skin (281)	Other sites (154)	Unknown (1)	All (665)
≥80% Tumor cells in the sample section		229 (100.0%)	280 (99.6%)	154 (100.0%)	1 (100.0%)	664
<80% Tumor cells in the sample section		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
NA** Tumor cells in the sample section		0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1
Selected Tissue area* ≥ 20mm^2		228 (99.6%)	279 (99.3%)	153 (99.4%)	1 (100.0%)	661
Selected Tissue area* < 20mm^2		1 (0.4%)	2 (0.7%)	1 (0.6%)	0 (0.0%)	4
Macro-dissection performed		128 (55.9%)	155 (55.2%)	78 (50.6%)	0 (0.0%)	361
Melanin Content^	None	96 (41.9%)	104 (37%)	61 (39.6%)	0 (0.0%)	261
	Low	69 (30.1%)	98 (34.9%)	56 (36.4%)	1 (100.0%)	224
	Medium	17 (7.4%)	21 (7.5%)	12 (7.8%)	0 (0.0%)	50
	High	47 (20.5%)	58 (20.6%)	25 (16.2%)	0 (0.0%)	130
Necrosis		126 (55.0%)	107 (38.1%)	91 (59.1%)	0 (0.0%)	324
Fatty Tissue		114 (49.8%)	99 (35.2%)	32 (20.8%)	0 (0.0%)	245
Hemorrhage		101 (44.1%)	97 (34.5%)	83 (53.9%)	1 (100.0%)	282

(*) represents tissue area that includes tumor cells and the surrounding matrix
(**) indicates % tumor cells in sample section; when the section contained less than 80% tumor cells, the % tumor cells might not be specified (e.g., NA)
(^)The values for the melanin content are: Low: ≤10% melanin of tumor area; Medium: 11% - 24% melanin of the tumor area; High: ≥25% melanin of tumor area.

D. Safety and Effectiveness Results - Tramatenib

1. Safety Results

The safety of the THxID™ BRAF 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 therapeutic. A false positive result would potentially expose the patient to a therapeutic that may not be beneficial as well as any possible side effects associated with the therapeutic. Overall, the most commonly occurring adverse events (≥20%) in patients treated with dabrafenib were rash, diarrhea, fatigue, peripheral edema, nausea, dermatitis acneiform, and vomiting. The incidence of adverse reactions for patients treated with tramatenib resulting in permanent discontinuation of tramatenib was 9%. Refer to the drug label for more information for adverse events linked to tramatenib (Mekinist™). The THxID™ BRAF test was shown to have high accuracy when compared to Sanger bi-directional sequencing (overall agreement >95%) indicating the possibility of false results is very low.

2. Effectiveness Results – based on mutation detection with CTA

The safety and efficacy of tramatenib were evaluated in 322 patients with BRAF V600E or V600K mutation-positive, unresectable or metastatic melanoma as

assessed by a clinical trial assay. The primary efficacy outcome measure was progression-free survival (PFS). Patients were randomized to receive tramatenib (n = 214) or chemotherapy (n = 108). The METRIC study demonstrated a statistically significant increase in progression-free survival in the patients treated with tramatenib with a 3.3 month improvement in progression-free survival observed for patients treated with tramatenib. The table below and graph (upper line is tramatenib) summarize the PFS results.

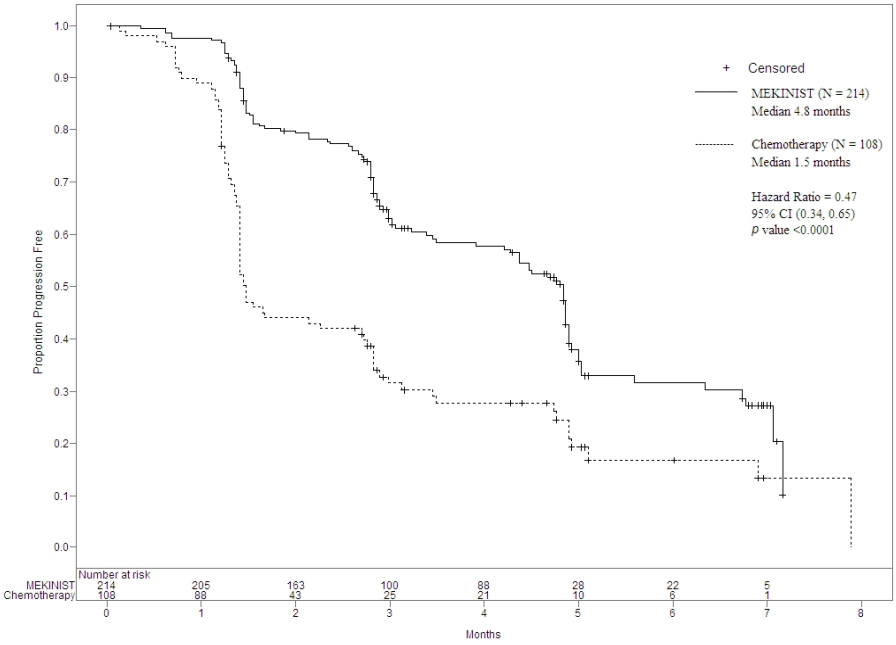
Investigator-Assessed Progression-Free Survival Results Based on CTA

	MEKINIST™ N = 214	**Chemotherapy N = 108**
PFS		
Median, months (95% CI)	4.8 (4.3, 4.9)	1.5 (1.4, 2.7)
HR (95% CI)	0.47 (0.34, 0.65)	
P value (log rank)[a]	*P*<0.001	

CI = confidence interval; HR = Hazard Ratio; PFS = Progression-free Survival; PR=partial response

[a] Pike estimator, unstratified analysis

Kaplan-Meier Curves of Investigator-Assessed Progression-Free Survival (ITT population) based on CTA



Refer to the drug label available at DRUGS@FDA available on the web for the most up to date information about Tramatenib.

3. Effectiveness results – based on mutation detection with THxID™ BRAF Assay

TRAMATENIB BRIDGING STUDY

The purpose of the bridging study was to demonstrate the analytical and clinical concordance of the THxID™ BRAF test to the CTA to support the efficacy conclusions observed in the METRIC trial. There were two objectives of the bridging assay: analytical concordance and clinical concordance.

Analytical Concordance:

Melanoma Specimens tested for V600E and V600K mutations to determine trial eligibility with the CTA were retested with the THxID™ BRAF test. The first objective was to evaluate the analytical agreement (PPA, NPA, and OPA) between the THxID™ BRAF Kit and the CTA for both mutation-positive and mutation negative specimens. For the purposes of calculating agreement, mutation positive specimens are defined as those with either the BRAF V600E or BRAF V600K mutation. Mutation negative is any result that is not BRAF V600E or V600K. Consistent with results observed in the dabrafenib trial, data demonstrate acceptable agreement between the CTA and the THxID- BRAF assay with overall agreement after excluding invalids was > 97%.

The patient specimens that were retested represent those patients for whom the test result determined enrollment (i.e., includes test positives, test negatives, and test failures). For all studies, repeat testing was performed according to the trouble shooting section of the test labeling [i.e., Instructions for Use (IFU)]. No more than two repeat tests were performed for a sample when attempting to obtain a valid result. The invalid rate for the retrospective testing of specimens from the METRIC trial was 3.2%)

Analytical Agreement between the CTA and the THxID- BRAF assay

The total number is larger than the specimen accountability table because there were duplicates for a single patient.

Agreement between the THxID™ BRAF Assay and CTA for all Subjects (All Testing Sites)

		Clinical Trial Assay (CTA)					
		V600E	V600K	V600E&K	WT	Invalid	Total
THxID BRAF Assay	V600E	252	1	0	11	0	264
	V600K	0	33	0	0	0	33
	V600 E&K	0	2	1	0	0	3
	WT	5	0	0	434	0	439
	Invalid	10	2	0	7	8	27
	Total	267	38	1	452	8	766

Agreements (All, 5x5)	Agreements (without THxID™ BRAF & CTA Invalids)

	No. of concordance/ No of tests	Agreement rate (%)	95% CI	No. of concordance/ No of tests	Agreement rate (%)	95% CI
PPA	286/306	93.50%	[90.1%;95.7%]	286/294	97.30%	[94.7%;98.6%]
PPA for V600E	252/267	94.40%	[90.9%;96.6%]	252/257	98.10%	[95.5%;99.2%]
PPA for V600K	33/38	86.80%	[72.7%;94.2%]	33/36	91.70%	[78.2%;97.1%]
NPA	434/452	96.00%	[93.8%;97.5%]	434/445	97.50%	[95.6%;98.6%]
OPA	728/766	95.00%	[93.3%;96.4%]	720/739	97.40%	[96.0%;98.3%]

*The V600E &K double mutation results are excluded from this subgroup analysis.

Clinical Concordance:

The second was to assess the clinical outcome (investigator assessed PFS) of the patients enrolled in the METRIC trial whose tumor specimens were V600E or V600K positive as detected by the THxID™ BRAF assay. A total of 322 BRAF V600E or K positive patients were randomized to the METRIC trial (214 received treatment with tramatenib, and 108 received chemotherapy). A total of 289 specimens tested positive by the THxID™ BRAF test and were used to calculate the Hazard Ratio (HR = 0.48 95% CI 0.34, 0.68). The HR of tramatenib to chemotherapy for the subset of THxID™ BRAF tested subjects was similar to that from the randomized population (HR .47 95% CI 0.34, 0.65). The estimates for PFS (3.3 months) were the same regardless of the assay (4.8 months for tramatenib treated subjects and 1.5 months for chemotherapy subjects).

Summary of Progression Free Survival for THxID™ BRAF Assay V600E Mutation Positive Subjects Using Investigator Assessed PFS.

	THxID™ BRAF		**CTA**	
	Tramatenib	**Chemotherapy**	**Tramatenib**	**Chemotherapy**
Number of Subjects	196	93	214	108
Adjusted Hazard Ratio [a] Estimate	0.48		0.47	
95% Confidence Interval	(0.34,0.68)		(0.34, 0.65)	
P-Value	<0.001		<0.001	
Estimates for PFS (Months)				
Median	4.8	1.5	4.8	1.5
95% Confidence Interval	(4.2,4.9)	(1.4, 2.7)	(4.3, 4.9)	(1.4, 2.7)

[a] Pike estimator; CI = confidence interval; HR = Hazard Ratio; PFS = Progression-free Survival

To estimate the expected PFS hazard ratio from subjects who are mutation positive using the THxID™ BRAF assay, conditional probabilities were calculated for an array of possible hazard ratios for those subjects who could be labeled mutation-positive by the THxID™ BRAF assay yet wild-type by the CTA (i.e., those excluded from the trial). The estimated PFS hazard ratio for those positive by THxID™ BRAF assay yet negative by CTA was allowed to range from 0.45 to 1.0. The results show that for these values, the estimated PFS hazard ratio for the subjects with concordance on both assay was between 0.47 and 0.48 showing the results to be consistent with the randomized population.

The results from the bridging study provide a demonstration of the clinical utility of the THxID™ BRAF test to support the selection of patients whose melanoma tissue is BRAF V600E and V600K positive for treatment with tramatenib (Mekinist™).

Summary of Expected Progression Free-Survival Hazard Ratios for THxID-BRAF Assay Mutation Positive Subjects Using FDA investigator assessed PFS

Estimated PFS Hazard Ratio in Subjects who are Mutation Positive by Both Assays	Probability of being Mutation Positive by Both Assays	Postulated Expected PFS Hazard Ratio In Subjects who are THxID™ BRAF Mutation Positive and CTA Wild Type	Probability of being THxID™ BRAF Mutation Positive and CTA Wild Type	Expected Estimated PFS Hazard Ratio for THxID™ BRAF Assay Mutation Positive Subjects (95% CI)
0.4685	0.963	0.47	0.037	0.47 (0.35,0.63)
0.4685	0.963	0.50	0.037	0.47 (0.35,0.63)
0.4685	0.963	0.52	0.037	0.47 (0.35,0.63)
0.4685	0.963	0.55	0.037	0.47 (0.35,0.63)
0.4685	0.963	0.58	0.037	0.47 (0.35,0.63)
0.4685	0.963	0.61	0.037	0.47 (0.35,0.63)
0.4685	0.963	0.64	0.037	0.47 (0.35,0.63)
0.4685	0.963	0.67	0.037	0.47 (0.35,0.63)
0.4685	0.963	0.70	0.037	0.48 (0.35,0.63)
0.4685	0.963	0.74	0.037	0.48 (0.35,0.63)
0.4685	0.963	0.78	0.037	0.48 (0.35,0.63)
0.4685	0.963	0.82	0.037	0.48 (0.35,0.63)
0.4685	0.963	0.86	0.037	0.48 (0.35,0.63)
0.4685	0.963	0.90	0.037	0.48 (0.35,0.63)
0.4685	0.963	0.95	0.037	0.48 (0.35,0.63)
0.4685	0.963	1.00	0.037	0.48 (0.35,0.63)

E. Financial Disclosures

The BREAK-3 and METRIC bridging studies were conducted retrospectively at three and five testing sites, respectively in the US, and are exempt from the requirements for Investigational Device Exemption as defined in Title 21 of the Code of Federal Regulations (21 CFR), 812.2(c)(3). The investigational product was not used in the diagnosis or treatment of patients. The applicant has adequately disclosed the financial interest/arrangements with clinical investigator. 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)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Devices Panel, an FDA advisory committee, for review and recommendation.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of the THxID™ BRAF Kit was demonstrated in retrospective analyses for patients enrolled in in the Phase-3 BREAK-3 study and the Phase 3 METRIC study, in which the BRAF mutation status of the patient specimen was originally determined by a CTA. The effectiveness of the THxID™ BRAF Kit is supported by both analytical and clinical validation data. The bridging study between the CTA and the THxID™ BRAF Kit consisted of two components: an assessment of analytical concordance between results obtained with the CTA and results obtained with the THxID™ BRAF Kit, and analysis of PFS based on the mutation positive subset(s) identified by the THxID™ BRAF Kit. Based on overall analysis of specimens for patients in the BREAK-3 and METRIC studies, the THxID™ BRAF assay demonstrates high analytical concordance with the CTA for detecting the mutations for BRAF V600E and BRAF V600K (approximately 94.9 and 93.8% for overall agreement when considering invalids, for BREAK-3 and METRIC, respectively. The retrospective testing of 232 specimens from patients enrolled in the BREAK-3 study demonstrated that the estimates for PFS (median 2.4 months) and hazard ratios with 95% confidence intervals (0.33; 0.20, 0.54) were the same when specimens were tested by the THxID™ BRAF Kit (median 2.3 months; hazard ratio 0.34 [0.20, 0.57). The retrospective testing of 289 specimens from patients enrolled in the METRIC study demonstrated that the estimates for PFS (median 3.3 months) and hazard ratios with 95% confidence intervals (0.47; 0.34, 0.65) were the same when specimens were tested by the THxID™ BRAF Kit (median 3.3 months; hazard ratio 0.48 [0.34, 0.68]). The observed clinical benefit in the subset of the patients tested with the THxID™ BRAF Test was comparable to that observed in the full study population. Additional sensitivity analyses addressing the discordant results between the CTA and the THxID™ BRAF test are consistent with the PFS results observed in these patients.

B. Safety Conclusions

The safety of the device is based on data collected in the clinical and non-clinical

studies conducted to support PMA approval and described above. As an in vitro diagnostic test, the THxID™ BRAF Kit involves testing on formalin-fixed, paraffin-embedded (FFPE) human melanoma tissue sections. These tissue sections are routinely removed as part of the diagnosis of melanoma by pathologies and pose no additional safety hazard to the patient. The risks of the THxID™ BRAF Kit are associated with the potential mismanagement of patients resulting from failure of the device to perform as expected, or failure to user to correctly interpret test results. A patient with a false negative result may not be presented the option for treatment with dabrafenib or tramatenib. A patient with a false positive result may undergo treatment with dabrafenib or tramatenib with inappropriate expectation of therapeutic benefit and experience side effects. Overall, the safety profile of the therapeutics was acceptable in relationship to their potential for benefit in the population of patients for which the therapeutics is indicated. The safety of the THxID™ BRAF Kit was demonstrated in an assessment of accuracy of the THxID™ BRAF Kit result when compared to Sanger bi-directional sequencing as the reference method: agreement for the detection of V600E and V600K mutations was 98.1% ; negative agreement was 93.9% and overall agreement 95.9% when excluding the THxID™ BRAF invalid results. The THxID™ BRAF test was shown to have high accuracy indicating the safety of this device is acceptable.

C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in a clinical studies conducted to support PMA approval of the THxID™ BRAF Kit as described above and are shown to outweigh the probable risks. Cutaneous melanoma is the most aggressive form of all skin cancers. The median overall survival time for subjects with stage IV melanoma remains short at approximately 6 months. Fewer than 10% of patients are alive at 5 years. Dabrafenib and Tramatenib are two drugs that showed statistically significant improvement in progression free survival (3.3 months, and 2.4 months respectively) in melanoma patients with unresectable or metastatic melanoma.

The risks of the THxID™ BRAF Kit are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial and has adverse events. A false negative result may prevent a patient access to a potentially beneficial drug. The likelihood of false results was assessed in the analytical and clinical performance evaluations and showed acceptable analytical performance with overall agreement to Sanger bi-directional sequencing 92.3% when considering test invalids and 95.9% when excluding test invalids. The agreement for detecting the V600E and V600K mutations when combined (i.e., PPA) in clinical specimens from the trial was 98.1% when excluding test invalids. The agreement for detecting wild-type and non-V600E/K mutants (NPA) was ≥ 93.9%.

Treatment with dabrafenib or tramatenib provide meaningful clinical benefit measured by progression-free survival and overall response rate, with an acceptable safety profile

when patients are selected for treatment on the basis of their FFPE melanoma tissue testing positive by the THxID™ BRAF Kit. The THxID™ BRAF assay was evaluated for its ability to support the dabrafenib and trametinib efficacy claims. The bMx THxID-BRAF assay demonstrates high analytical concordance with the CTA for detecting BRAF V600E, V600K and WT forms of the BRAF gene. To this end, subjects enrolled on the dabrafenib and trametinib study demonstrated the same progression free survival when tested with the THxID™ BRAF assay as when they were screened CTA. An evaluation of the impact of discordance between the two test methods showed no effect on the conclusions.

In conclusion, given the available information above, the data support the use of the THxID BRF kit to aid in selecting melanoma patients whose tumors carry the BRAF V600E mutation for treatment with dabrafenib (Tafinlar®) and as an aid in selecting melanoma patients whose tumors carry the BRAF V600E or V600K mutation for treatment with trametinib (MekinistTM).

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 V600E and V600K mutant BRAF was evaluated as therapeutic targets in melanoma cancer using either dabrafenib (Tafinlar®) or tramatenib (Mekinist™).in clinical studies as described in the sections above. Safety and efficacy of dabrafenib (Tafinlar®) and tramatenib (Mekinist™) were shown to be acceptable in this population when patients were selected on the bases of BRAF mutation status selected by a clinical trial assay. Bridging analyses to the bioMérieux THxID™ BRAF test supported the safe and effective use of the treatments. Dabrafenib (Tafinlar®) and tramatenib (Mekinist™) represent important new treatment options with favorable risk-benefit prolife for patients with BRAF mutation positive unresectable or metastatic melanoma as identified by the THxID™ BRAF Kit.

XIII. CDRH DECISION

CDRH issued an approval order on May 29, 2013. The final conditions of approval order can be found in the approval order.

The applicant's manufacturing facilities were 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. Refer to the drug

labels for Tafinlar® and Mekinist™ for additional information related to the use of the drug.

Post-approval Requirements and Restrictions: See approval order.