SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: cobas® 4800 BRAF V600 Mutation Test

Applicant's Name and Address: Roche Molecular Systems, Inc. (RMS)
4300 Hacienda Drive
Pleasanton, CA 94588-2722

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P110020

Date of FDA Notice of Approval: August 17, 2011

Expedited: Not applicable

II. INDICATIONS FOR USE

The cobas® 4800 BRAF V600 Mutation Test is an in vitro diagnostic device intended for the qualitative detection of the BRAF V600E mutation in DNA extracted from formalin-fixed, paraffin-embedded human melanoma tissue. The cobas® 4800 BRAF V600 Mutation Test is a real-time PCR test on the cobas 4800 system, and is intended to be used as an aid in selecting melanoma patients whose tumors carry the BRAF V600E mutation for treatment with vemurafenib.

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the cobas® 4800 BRAF V600 Mutation Test labeling.

V. DEVICE DESCRIPTION

The cobas® 4800 BRAF V600 Mutation Test is based on two processes:

1. The cobas® 4800 DNA Sample Preparation kit provides reagents for manual specimen preparation to obtain genomic DNA from formalin-fixed, paraffin-embedded tissue (FFPET).
2. The BRAF V600 Mutation Test kit provides reagents for automated real-time PCR amplification and detection of the BRAF target DNA using a

complementary primer pair and two oligonucleotide probes labeled with different fluorescent dyes. One probe is designed to detect the wild-type BRAF V600 sequence and one is designed to detect the V600E mutation sequence.

Two external run controls are provided and the wild-type allele serves as an internal, full process control.

Specimen Preparation

FFPET specimens are processed and genomic DNA is isolated using the cobas® DNA Sample Preparation Kit. A deparaffinized 5 µm section of an FFPET specimen is lysed by incubation at an elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released genomic DNA from DNases. Subsequently, isopropanol is added to the lysis mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the genomic DNA is bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other cellular impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and then eluted with an aqueous solution. The amount of genomic DNA is spectrophotometrically determined and adjusted to a fixed concentration of 5 ng/µL with 25 µL used in the amplification and detection mixture.

PCR Amplification and Detection

Target Selection and Amplification

The cobas® 4800 BRAF V600 Mutation Test uses primers that define a 116-base pair sequence of human genomic DNA containing the BRAF codon 600 site in exon 15. The test is designed to detect the nucleotide 1799 T>A change in the BRAF gene which results in a valine-to-glutamic acid substitution at codon 600 (V600E). BRAF wild-type and mutant DNA target-specific, fluorescent dye-labeled TaqMan® probes bind to the wild-type and mutant sequences, respectively. Thermus species Z05 DNA polymerase is utilized for target amplification. First, the PCR reaction mixture is heated to denature the genomic DNA and expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences. The DNA polymerase, in the presence of divalent metal ion and excess dNTPs, extends each annealed primer, thus synthesizing a second DNA strand. This completes the first cycle of PCR, yielding a double-stranded DNA copy of the targeted 116-base pair region of the BRAF gene. This process is repeated for a number of cycles, with each cycle effectively doubling the amount of amplicon DNA. Amplification occurs only in the region of the BRAF gene between the primers.

Selective amplification of target nucleic acid from the specimen is achieved in the cobas® 4800 BRAF V600 Mutation Test by the use of AmpErase® (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP) which are included in the Reaction Mix reagent. The AmpErase® enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing thymidine. Deoxyuridine is always present in amplicon due to the use of dUTP as one of the nucleotide triphosphates in the Reaction Mix reagent; therefore, only amplicon contains

deoxyuridine. The AmpErase® enzyme is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon.

Automated Real-time Detection

The cobas® 4800 BRAF V600 Mutation Test utilizes real-time PCR technology. Each target-specific, oligonucleotide probe in the reaction is labeled with a fluorescent dye that serves as a reporter, and with a quencher molecule that absorbs (quenches) fluorescent emissions from the reporter dye within an intact probe. During each cycle of amplification, probe complementary to the single-stranded DNA sequence in the amplicon binds and is subsequently cleaved by the 5' to 3' nuclease activity of the Z05 DNA polymerase. Once the reporter dye is separated from the quencher by this nuclease activity, fluorescence of a characteristic wavelength can be measured when the reporter dye is excited by the appropriate spectrum of light. Two different reporter dyes are used to label the target-specific BRAF wild-type (WT) probe and the BRAF V600E mutation (MUT) probe. Amplification of the two BRAF sequences can be detected independently in a single reaction well by measuring fluorescence at the two characteristic wavelengths in dedicated optical channels.

Instrument and Software

The cobas® 4800 system is controlled by the cobas® 4800 system SR2 (v. 2.0) software which provides the core software engines and user interfaces. This core system software was designed to allow multiple assays to be performed on the system using analyte specific analysis package software (ASAP). The cobas z 480 analyzer component of the test system also has its own internal instrument control software which is driven by the core software.

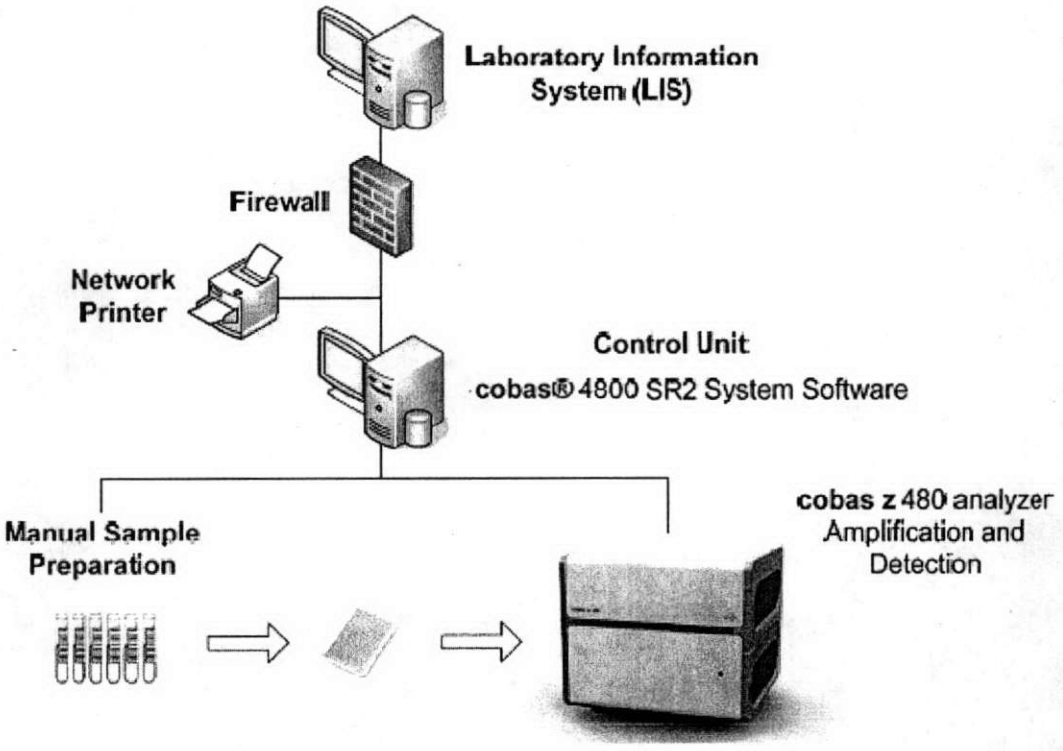
A dedicated Control Unit computer runs the cobas® 4800 system SR2 software and provides an interface to the cobas z 480 and Laboratory Information System (LIS). The computer also processes the fluorescent signals with the analyte specific analysis package and stores the test results in a controlled database. The complete system allows a user to create a test work order for each specimen either manually or automatically when connected to a LIS. A software wizard guides the user through the necessary steps to perform a run which includes z480 maintenance handling, test selection, specimen ID entry, reagent and microwell plate bar code entry, microwell plate loading and run start.

The cobas® 4800 System tracks each specimen during processing and analysis on the cobas z 480 analyzer. Once the thermal run is complete the ASAP software processes the fluorescence data using data analysis algorithms, assesses the validity of the controls and determines the results using the assay specific result interpretation logic. The software then provides the results to the user in three formats; a printable PDF results report, a GUI based result viewer and a result export file that can be exported to the LIS.

The cobas® 4800 System software includes BRAF V600 data analysis algorithm to determine the cycle-to-threshold value — the cycle number where the signal of the accumulating PCR product starts to grow exponentially in each channel. Cycle-to-threshold (Ct) values from each channel (V600E probe in channel 1 and WT probe in

channel 2) for the MUT PC and WT PC reactions are used to determine if the run is valid.

Overall cobas® 4800 System components are shown in the diagram below:



Interpretation of Results

If the run is valid, then the Ct values for each sample will be evaluated against acceptable ranges for each channel. The table below summarizes how each pair of Ct values is translated into a BRAF V600E-mutation status result.

Channel 1 (FAM, V600E probe)	Conditional	Channel 2 (HEX, WT probe)	Mutation Status
No Ct*	and	21.0 ≤ Ct ≤ 35.0	Mutation not detected
21.0 ≤ Ct ≤ 43.0	and	Ct ≥ 21.0	Mutation detected
21.0 ≤ Ct ≤ 43.0	and	No Ct*,†	Mutation detected
Ct <21.0	or	Ct <21.0	Invalid
No Ct*	and	No Ct*	Invalid
No Ct*	and	Ct>35.0	Invalid
Ct >43	and	Ct ≥ 21.0 or No Ct*	Invalid

*No significant amplification detected.

†This result would be observed from 100% mutant sequence, i.e. homozygous specimen for example.

Ct values are not reported to the user. The report results and interpretation are as follows:

Result Interpretation of the cobas® 4800 BRAF V600 Mutation Test

cobas® 4800 BRAF V600 Mutation Test Result	Interpretation
Mutation Detected	V600 E Mutation Detected in the BRAF codon 600 site in exon 15
Mutation Not Detected*	V600E Mutation Not Detected in the BRAF codon 600 site in exon 15
Invalid	Result is invalid. Repeat the testing of specimens with invalid results following the instructions outlined in the "Retesting of Specimens with Invalid Results" section below.
Failed	Failed run due to hardware or software failure

* A Mutation Not Detected result does not preclude the presence of a mutation in the BRAF codon 600 site since results depend on percent mutant sequences, adequate specimen integrity, absence of inhibitors, and sufficient DNA to be detected.

All of the patients in the phase III study NO25026 (BRIM3) sponsored by Hoffmann-La Roche were selected using the cobas® 4800 BRAF V600 Mutation Test. Enrollment in the study was limited to patients whose melanoma tissue tested positive by the test. Therefore it is not known whether patients who test negative by the cobas® 4800 BRAF V600 Mutation Test will benefit from vemurafenib (Zelboraf™) treatment.

Test Controls

Two external positive controls are also provided and the wild-type allele serves as an internal, full process control:

1. *BRAF mutant control:* The MUT Control is a blend of two DNA plasmids containing the BRAF WT and the second containing V600E sequence. This will be included in every run and will serve as a process control for every step except sample preparation. The MUT Control reaction must have BRAF WT and BRAF V600 Ct values within the respective acceptable ranges for the run to be considered valid.

2. *BRAF WT control:* The WT Control consists of a wild-type plasmid and serves to control potential reagent contamination and monitor specimen inhibition. The WT Ct value must be within the pre-established acceptance range for the run to be considered valid.

3. *BRAF WT Internal Control:* The BRAF WT allele from extracted test specimens serves as a full process control. This control ensures that every step of the process from sample preparation to amplification and detection has been successfully completed.

Acceptable Ct Values for Control Reactions

Sample Type	Channel 1 (FAM, V600E probe)	Channel 2 (HEX, WT probe)
MUT Positive Control (Blend of V600E mutant and wild-type plasmids)	Ct between 23.5 and 30.5	Ct between 23.5 and 29.0
WT positive control (Wild-type plasmid alone)	Ct > 43.0	Ct between 31.5 and 38.5

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 mutation status in the selection of patients who are eligible for vemurafenib (Zelboraf™) treatment.

VII. MARKETING HISTORY

The cobas® 4800 BRAF V600 Mutation Test 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 in melanoma treatment.

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

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

For the nonclinical studies described below, tumor characteristics such as % tumor and melanin content were assessed by pathology review. Bi-directional Sanger sequencing was used to select the specimens for testing. The % mutation was determined using a parallel sequencing method.

1. Correlation with Reference Method for Phase III Samples

The primary study objective was to evaluate the performance of the test in the detection of BRAF V600E mutations in clinical samples in malignant melanoma from the Phase III trial with vemurafenib (Zelboraf™) 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. A total of 596 consecutive patients screened for the Phase III trial for which clinical, demographic, and bi-directional sequencing data were collected. Of these cases, 94 were ineligible because of missing

inclusion criteria, 4 cases were without pathology review, 2 cases had invalid cobas test results and 47 specimens had invalid Sanger sequencing results, leaving 449 evaluable cases. Baseline characteristics for the evaluable sample set were provided and representative of Phase III samples. The agreement analysis between the cobas test results and bi-directional sequencing results for the detection of the V600E mutation demonstrated a positive agreement of 97.3%, a negative percent agreement of 84.6% and an overall agreement of 90.9%. There were a total of 35 "Mutation Detected" results by the assay which were not identified as V600E mutations by bi-directional sequencing. Eight (8) of these were wild type, 25 were V600K and 1 was V600E2 and 1 was V600D. Additionally, 6 specimens were identified as mutation not detected by cobas but were identified as V600E by bi-directional sequencing. The cross-reactivity of the cobas® 4800 BRAF V600 Mutation Test for V600K was 65.8% (25 of 38 samples).

BRAF V600 Mutation Test vs. Bi-Directional Sequencing for Phase III Specimens*

cobas® 4800 BRAF V600 Mutation Test Result	Bi-Directional Sequencing (Reference Method)							
	BRAF V600E Mutation Detected	BRAF V600E Mutation Not Detected						
	V600E	V600K	V600E2	V600R	V600D**	V600*** Other	Wild type	Total
Mutation Detected	216	25	1	0	1	0	8	251
Mutation Not Detected	6	13	12	2	1	1	164	198
Total	222	38	13	2	1	1	172	449
		227						
Positive Percent Agreement (95% CI)		216/222 X 100 = **97.3%** (94.2, 98.8)						
Negative Percent Agreement (95% CI)		192/227 X 100 = **84.6%** (79.3, 88.7)						
Overall Percent Agreement (95% CI)		408/449 X 100 = **90.9%** (87.8, 93.2)						

*** Bi-directional sequencing has a limit of detection of approximately 20% of mutant alleles in FFPET specimens DNA. Therefore, bi-directional sequencing may not adequately confirm mutation status at lower percentages of mutant alleles.**

****V600D variant (GTG>GAC)**

*****Mutation not specified in the protocol (ACA insertion immediately before Codon 600)**

2. **Analytical Sensitivity**

a. **Analytical Sensitivity - Limit of Blank (LoB)**
To assess performance of the cobas 4800 BRAF V600 Mutation Test in the absence of template and to ensure that a blank sample, or a sample with an excess of 100% wild type DNA, do not generate an analytical signal that might indicate a low concentration of mutation, samples with no template and 100% BRAF wild type DNA were evaluated.

i. **Limit of Blank (LoB) no template** – a sample with no template was run multiple times and no detectable Ct was identified in either channel. Cts can be measured out to 53 cycles and results indicated as "NaN" for "not-a-number" were outside of the 53 cycles. Therefore, the LoB for no template is >53 and exceeds the threshold of detection.

ii. **Limit of Blank (LoB) FAM channel** – was determined from 24 replicates (8 replicates X 3 lots of reagent) using a 100% BRAF Wild type DNA obtained from a FFPET specimen using 125 ng/25 µL sample. There was no detectable Ct value (>53Ct) for the FAM channel and therefore determined to be "mutation not detected". Additionally, a wild type melanoma cell line (SK-MEL2) was tested in the absence of any mutant DNA using a 5 DNA input concentrations up to approximately 48 times recommended (125 ng). The 5 panel members 200 ng, 1025 ng, 2050 ng, 5975 ng were tested in quadruplicate. There was no Ct value in the mutant channel. The Ct range in the wild-type was 23.1 to 17. A second run using 100% wild-type cell line at 7 different DNA input concentrations (125 ng, 250ng, 375 ng, 500 ng, 625 ng, 750 ng, 875 ng), yielded no detectable Ct in the mutant channel. The Ct range in the wild-type channel was 23.6 to 20.4.

iii. **Limit of Blank (LoB) HEX channel** – A mutant melanoma cell line (SK-MEL 28) was tested up to approximately 40 times the DNA concentration recommended using 5 different DNA concentrations (125 ng, 625 ng, 1250 ng, 2500 ng, 5000 ng). There was no Ct value in the wild-type channel. The Ct range in the mutant channel was 19 to 25.6.

b. **Analytical Sensitivity - Limit of Detection (LoD)**
Replicate cobas® 4800 BRAF V600 Mutation Test measurements were performed on dilution panel members that contained various amounts of genomic DNA and various percentages of the V600E mutant sequence, which bracketed the expected analytical sensitivity of the cobas® 4800 BRAF V600 Mutation Test. The study was performed by testing dilution panels prepared from three types of specimens: cell line blends, FFPET specimen blends, and individual FFPET samples. The studies support the claim that the cobas® 4800 BRAF V600 Mutation Test can detect 5%

V600E mutant alleles in a background of 95% wild type alleles in formalin-fixed, paraffin-embedded tumor samples when 125 ng /25 µL DNA is used. The cobas® BRAF V600E Mutation Test is for the qualitative detection of the BRAF mutation and is not intended to for quantitative measurements.

i. **Cell line blends** – were prepared by mixing DNA stocks obtained from a BRAF melanoma V600E mutant (MUT) cell line and a wild-type (WT) melanoma cell line [SK-MEL 28 (BRAF V600E mutant) and SK-MEL 2 (BRAF wild-type)]. A single cell line blend with a mutation of 5% in a background of wild-type was serially diluted to evaluate different input DNA concentrations. Twenty (20) replicates were tested in 3 lots (60 replicates total). The mean Ct values and %CV for each channel are shown. The lowest amount of genomic DNA that yielded at least a 95% Hit rate for each sample was determined to be 4 ng/25 µL.

V600E Hit Rates Observed for Cell Line Blend

Percent Mutation	Amount of DNA in the Panel Member	No. of Replicates Tested	No. of Replicates "Mutation Detected"	Hit Rate	Mean MUT Ct	% CV (Mean MUT Ct)	Mean WT Ct	% CV (Mean WT Ct)
5%	125 ng/25 µL	60	58	97%	23.9	2.6	23.2	0.9
	31.25 ng/25 µL	60	60	100%	26.2	3.5	25.4	0.9
	15.63 ng/25 µL	60	57	95%	27.2	3.1	26.5	0.9
	7.81 ng/25 µL	60	59	98%	28.1	2.5	27.6	1.0
	3.90 ng/25 µL	60	57	95%	29.3	2.2	28.8	1.0
	1.95 ng/25 µL	60	49	82%	30.2	3.3	29.7	1.1
	0.98 ng/25 µL	60	47	78%	31.3	2.9	30.8	1.0
	0.49 ng/25 µL	60	46	77%	32.6	2.5	31.9	1.5

ii. **Specimen FFPET blends** – were prepared by mixing DNA stocks obtained from 5 V600E mutant FFPET melanoma specimens and wild type FFPET specimens. Specimen blends were made at approximately 10%, 5% and 2.5% mutation. Aliquots from each of the 5 specimen blends with V600E mutation were then diluted to yield additional panel members containing reduced quantities of DNA for estimating analytical sensitivity. Eight (8) replicates of each dilution panel member were tested using each of the 3 lots (24 replicates total). The 2.5% FFPET blend at 125 ng/25 µL was not detected. The study supports the claim that the test can detect the 5% mutant BRAF V600E DNA in a background of wild type DNA when using 125 ng/25 µl DNA from an FFPET blend.

iii. **Individual melanoma FFPET specimens** – were selected based on their mutation status, mutation percentage, pigmentation, and

the amount of tissue in each FFPET block. Two samples were selected for a percent V600E mutation content at the cut-off (approximately 5%). One of these specimens was selected for high pigmentation to assess the impact of endogenous melanin on sensitivity (melanin concentration 6.8 µg/mL). Tumor content was assessed by a pathologist examining two 5 µm sections, 11 slides apart, within the tumor block. DNA was extracted from a total of forty-eight (48) 5 µm sections ("curls") obtained from each of the three V600E mutant FFPET melanoma specimens. Each specimen was then diluted to create a 6-member panel for evaluation. Sixteen (16) replicates of each dilution panel member were prepared and tested per lot. Three lots and 5 instruments were used for testing. The panel member with the lowest amount of DNA that still yielded a V600E mutation detected rate of at least 95% was determined for each percent mutation level. The results demonstrated that all 3 of the FFPET specimens (approximately 4%, 6%, and 12% mutation) were detected ≥98% of the time at the recommended DNA input for the test (125 ng/25 µL). Additionally, it was also demonstrated that a highly pigmented specimen had a similar analytical sensitivity compared to non-highly pigmented specimens used in this study.

c. **Analytical Sensitivity – Ct Range and RFI Validation**

Results obtained with 52 clinical formalin-fixed, paraffin-embedded tumor (FFPET) specimens with various DNA concentrations used for the PCR input [range 1.25 ng (to simulate 99% degradation) and 125 ng (no degradation)] were used for the statistical analysis to establish Ct cutoffs. The Ct cutoffs (21 and 43) have been established so as to contain 99.9% of the populations of Ct values for FFPET specimens. Additionally, the Relative Fluorescence Increase (RFI) Cut-off for the cobas® 4800 BRAF V600 Mutation Test was evaluated by comparing sensitivity and specificity observed for the results of 203 FFPET specimens when Ct values were evaluated at RFI cut-off values of 1.01 to 3. Samples were run in two lots. Receiver Operating Characteristic (ROC) analysis was also performed on the tabulated data to demonstrate how specificity and sensitivity were affected by changes in the RFI cutoff value. The number of correct results obtained at each RFI is shown for each method. The reference method was bi-directional sequencing. Results indicate that specificity and sensitivity are optimized at the current cutoff value of 1.06.

Cycle threshold ranges for the cobas® 4800 BRAF V600 Mutation Test

Channel 1 (FAM, V600E probe)	Conditional	Channel 2 (HEX, WT probe)	Mutation Status
No Ct*	and	$21.0 \leq Ct \leq 35.0$	Mutation not detected
$21.0 \leq Ct \leq 43.0$	and	$Ct \geq 21.0$	Mutation detected

21.0 ≤ Ct ≤ 43.0	and	No Ct*,†	Mutation detected
Ct <21.0	or	Ct <21.0	Invalid
No Ct*	and	No Ct*	Invalid
No Ct*	and	Ct>35.0	Invalid
Ct >43	and	Ct ≥ 21.0 or No Ct*	Invalid

3. **Analytical Sensitivity – Genomic DNA Input Range**

The recommended DNA input for the cobas® 4800 BRAF V600 Mutation Test is 125 ng per PCR reaction. Various genomic DNA input amounts may result from DNA quantitation errors and/or variation in the amount of degraded DNA. To evaluate the effects of various genomic DNA input amounts, genomic DNA was extracted from 11 melanoma FFPET specimens using the DNA Specimen Preparation Kit and serially diluted with sample representing 250 ng, 125 ng, 62.5 ng, and 31.2 ng/25 µL. All 4 DNA levels were evaluated using 2 lots. Specimens ranged in tumor stage (II to IV), % tumor content (30-85%), % mutant alleles (11% to 55%) and pigmentation (not highly pigmented to highly pigmented). The expected results were obtained for all genomic DNA input levels for all but one sample (31.2 ng) that required repeat testing following an invalid result in the wild type channel. The genomic range data is also supported by the additional data in the limit of detection studies.

4. **Analytical Sensitivity - Minimum Tumor Content**

A total of 33 Stage III and IV melanoma FFPET specimens with tumor content that ranged from 5% to 50% with various percentages of BRAF V600 mutation (range 1% to 36%), and a genomic DNA input concentration of 125 ng/25 µL, were evaluated in singlicate to determine the minimum percent tumor proportion that was needed to detect the presence of mutant DNA. Twelve (12) adjacent 5 µm "curls" were obtained from each of the specimens. The 1st and 12th sections were mounted on slides, stained and assessed by a pathologist for tumor content. One specimen was excluded due to repeated "invalid" results. The results demonstrated that, at the claimed 5% mutation detection, a minimum tumor proportion of 15% or greater is required for V600E mutant positive tumor samples when 125 ng/25 µL of genomic DNA is tested. An additional 24 wild type samples with tumor content ranging from 5 to 45% were evaluated as well. All wild type samples were correctly called.

cobas® 4800 BRAF V600 Mutation Detection as a Function of Various Tumor Content

Melanoma Specimen	% Tumor Content	Test Result
V600E	5%/5%	Mutation not detected
V600E	5%/5%	Mutation not detected
V600E	5%/5%	Mutation not detected
V600E	10%/10%	Mutation not detected
V600E	10%/10%	Mutation Detected
V600E	15%/10%	Mutation Detected

V600E	15%/15%	Mutation Detected
V600E	15%/15%	Mutation Detected
V600E	15%/15%	Mutation Detected
V600E	15%/15%	Mutation Detected
V600E	15%/15%	Mutation Detected
V600E	15%/20%	Mutation Detected
V600E	20%/20%	Mutation Detected
V600E	20%/20%	Mutation Detected
V600E	25% / 25%	Mutation Detected
V600E	30% / 25%	Mutation Detected
V600E	30% / 30%	Mutation Detected
V600E	30% / 35%	Mutation Detected
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V600E	35% / 40%	Mutation Detected
V600E	40% / 35%	Mutation Detected
V600E	40% / 35%	Mutation Detected
V600E	40% / 40%	Mutation Detected
V600E	40% / 40%	Mutation Detected
V600E	40% / 40%	Mutation Detected
V600E	40% / 45%	Mutation Detected
V600E	45% / 45%	Mutation Detected
V600E	50% / 40%	Mutation Detected

5. **Analytical Specificity**

   a. **Primer and Probe Specificity**

   Sequence information and alignment of the primers and probes with the BRAF gene was provided. A demonstration of alignment of the primers with the BRAF gene and other RAF family members (RAF1 and ARAF) was also provided and demonstrated the specificity of the primers and probes for the BRAF sequence. A sequence homology search using the Basic Local Alignment Search Tool (BLAST, v2.0) was conducted to predict the specificity of the primers used in the cobas® 4800 BRAF V600 Mutation Test. A nucleotide pattern search tool from was then used to assess the likelihood of the V600E probe hybridizing to any of the amplicons identified by the BLAST search, and potentially generating a signal. Six hits were identified, all perfect matches for the BRAF gene and specifically for the wild-type sequence. No probe hybridization was predicted for sequences derived from the Chromosome X pseudogene sequence, ARAF, or RAF1 or unrelated sequences.

   b. **Cross Reactivity**

Cross-reactivity of the cobas® 4800 BRAF V600 Mutation Test for other non-V600E mutations, BRAF homologs and skin-related microorganisms was evaluated. Cross-reactivity was evaluated using non-V600E plasmids, non-V600E melanoma FFPET specimens with different percent mutations, plasmids of BRAF homologs and skin-related microorganisms.

i. **Non-V600E mutation plasmids** – Plasmid dilution panels with percent mutation ranging from 5% to 75% were prepared for each of the nine non-V600E BRAF mutations (D549G, G596R, K601E, L597Q, L597S, V600D, V600E2, V600K, and V600R) by diluting a volume of the non-V600E mutation plasmids with an appropriate volume of the BRAF wild-type plasmid. Three replicates of each member of the 9 dilution panels were tested using the cobas® 4800 BRAF V600 Mutation Test. The amount of plasmid DNA was designed to be reflective of percent mutation in 125 ng of DNA. Cross-reactivity was observed for BRAF V600D at ≥10% mutation, V600K at ≥35% mutation, and V600E2 at ≥65% mutation. No cross-reactivity was observed with plasmids for the V600R, D549G, G596R, K601E, L597Q, and L597S mutations when up to 75% mutation was evaluated.

ii. **Non-V600E melanoma FFPET Specimens with different percent mutations:** To determine the cross-reactivity with actual clinical specimens, 14 non-V600E mutant melanoma FFPET specimens representing V600D, V600E2, V600R and V600K were tested. Specimens varied by tumor stage (IIC, III and IV), tumor content (range 15% to 95%) and percent mutation (range 16-69%). Three replicates of each sample were tested. The results demonstrated that 6 of the 9 V600K samples were cross reactivity when percent mutation was ≥31%, 1 of the 3 V600E2 samples was cross reactive when the percent mutation was at 68% and one of the V600D samples was cross-reactive at 18% mutation. No cross reactivity was seen for the V600R specimen with 23% mutation.

Cross- Reactivity of the cobas® 4800 BRAF V600 Mutation Test Detection Observed for BRAF non-V600E mutations

MUTATION	% Tumor by pathologist	% Mutation	No of replicates "Mutation Detected"	Cross-reactivity
V600D	30% / 30%	18%	3	100%
V600E2	75% / 75%	16%	0	0%
	75% / 80%	36%	0	0%
	75% / 75%	68%	3	100%
V600R	15% / 15%	23%	0	0%
V600K	25% / 25%	16%	0	0%
	35% / 40%	22%	0	0%
	40% / 40%	23%	0	0%

MUTATION	% Tumor by pathologist	% Mutation	No of replicates "Mutation Detected"	Cross-reactivity
	60% / 60%	31%	3	100%
	75% / 75%	35%	3	100%
	80% / 80%	39%	3	100%
	95% / 95%	36%	3	100%
	75% / 75%	62%	3	100%
	80% / 80%	69%	3	100%

While the cobas® 4800 BRAF V600 Mutation Test demonstrated cross-reactivity, the ability to reliably detect these non-V600E mutations at these percent mutations has not been demonstrated.

iii. **BRAF Homolog Panels: Samples -** Samples were prepared for three BRAF homolog plasmids (BRAF pseudogene, ARAF, RAF1), BRAF V600E mutant plasmid, and BRAF Wild Type plasmid as outlined in the Table below. Three to six replicates of each panel member were tested using the cobas® 4800 BRAF Mutation Test. None of the BRAF homolog plasmids tested were detected by the cobas test when tested alone, indicating that the BRAF homolog plasmids do not cross-react with the test.

BRAF Homolog Plasmid Samples

Panel		Composition by Volume	
Name	**Member**	**Component 1**	**Component 2**
BRAF Pseudogene	1	95% BRAF Pseudogene	5% V600E Mutant
	2	100% BRAF Pseudogene	- - -
ARAF	1	95% ARAF	5% V600E Mutant
	2	100% ARAF	- - -
RAF1	1	95% RAF1	5% V600E Mutant
	2	100% RAF1	- - -
Control	1	95% BRAF Wild-type	5% V600E Mutant
	2	100% BRAF Wild-type	- - -
	3	95% DNA Elution Buffer	5% V600E Mutant

iv. **Testing of microorganisms –** DNA was isolated from twenty-one 5 µM sections from two melanoma FFPET specimens; one V600E with approximately 50% tumor and 8% mutation, and one V600 wild-type with 90% tumor content. After the addition of Lysis Buffer, 1x 10^6 colony forming units (CFU) of 6 different skin-related microorganisms were added to each of three replicates for each specimen, leaving 3 replicates of each specimen without added microorganism (test control). All test results for the V600E mutant specimen with 8% mutation were "Mutation Detected" in

the presence of the 6 microorganisms tested. All test results for the Wild-type specimen were "Mutation Not Detected" in the presence of 6 microorganisms tested. The results demonstrated that the 6 skin-related microorganisms did not interfere or cross-react with the detection of an FFPET specimen with a low-level of the BRAF V600E mutation when 1 x 10^6 colony forming units were added during the tissue lysis step. The skin related organisms evaluated were:

- Staphylococcus epidermis
- Staphylococcus aureus
- Corynbacterium xerosis
- Corynebacterium jeikeiu
- Corynbacterium minutissimum
- Corynbacterium ulcerans

6. **Interference – Effects of Necrotic Tissue**

The ability of the cobas® 4800 BRAF V600 Mutation Test to perform correctly when samples have high necrotic tissue content was evaluated. Eleven (11) V600E specimens were selected for necrotic tumor content (range 15% to 95%) and percent mutation (range 4 to 65%). Sixteen (16) V600 wild type melanoma FFPET specimens with necrotic tumor (range 10% to 90%) were also evaluated. The tumor content and percentage of necrotic tissue in each specimen was reviewed by a pathologist. Samples were run in duplicate using 1 lot of reagents. "Mutation Detected" results were obtained for all 11 BRAF V600E specimens, except for one sample with 30% necrotic tumor. However, this sample had mutant tumor content below the threshold of detection (approximately 4%). The specimen with 95% necrotic tissue had a mutant tumor content of approximately 10% and was correctly called mutation detected. Mutation not detected results were obtained for all wild type specimens. MD = Mutation Detected, MND = Mutation Not Detected.

Results of Testing BRAF V600E Mutant Specimens with Necrotic Tumor

Specimen	% Necrosis	Test Results (n = 2)
V600E	15%	MD / MD
	15%	MD / MD
	20%	MD / MD
	25%	MD / MD
	25%	MD / MD
	30%	MD / MD
	30%	MND / MND
	55%	MD / MD
	55%	MD / MD
	60%	MD / MD
	95%	MD / MD

7. **Interference – Melanin**

Melanin is an inhibitor of DNA polymerases and therefore could be a potential interfering substance in a PCR-based assay such as the cobas® 4800 BRAF V600 Mutation Test. The impact of high concentrations of endogenous melanin was evaluated using highly pigmented melanoma FFPET samples. A total of 41 unique FFPE melanoma tumor specimens were selected based upon their level of pigmentation: 33 were highly pigmented, 3 were from African Americans and 5 were lightly pigmented for comparison. DNA was extracted from the tissue and melanin concentration was determined for each sample. A single replicate of the DNA stock from each of the two sections obtained from each of the 41 specimens was tested. Results were considered acceptable for a specimen if both calls corresponded to the expected results for the specimen. Results of testing the 14 BRAF V600E mutant specimens yielded a "Mutation Not Detected" result for one of the two replicates and three specimens yielded a result of "Invalid" on at least one replicate. The specimen with the false negative result had a mutation percentage that was below the expected analytical sensitivity of 5% for the cobas® 4800 BRAF V600E Mutation Test. The results for V600E samples are shown below. Mutation Detected, MND = Mutation Not Detected, INV = Invalid.

Impact of Melanin on Mutation Detection by the cobas® 4800 BRAF V600 Mutation Test for V600E FFPET

	Tissue Appearance	% mutation	BRAF results of two replicates	Melanin concentration in sample
V600E	Not highly pigmented	65%	MD / MD	0.087 ± 0.008 µg
		11%	MD / MD	0.109 ± 0.001 µg
		7%	MD / MD	0.181 ± 0.008 µg
		10%	MD / MD	0.247 ± 0.045 µg
	Highly pigmented	2%	MD / MND	0.057 ± 0.005 µg
		48%	MD / MD	0.104 ± 0.010 µg
		13%	MD / MD	0.105 ± 0.053 µg
		26%	MD / MD	0.126 ± 0.027 µg
		22%	INV / INV	0.153 ± 0.012 µg
		10%	MD / MD	0.166 ± 0.069 µg
		58%	MD / MD	0.205 ± 0.024 µg
		58%	MD / INV	0.237 ± 0.006 µg
		25%	INV / INV	0.337 ± 0.031 µg
		39%	MD / MD	0.501 ± 0.248 µg

For specimens with an "Invalid" result, an additional aliquot was obtained from the DNA stock for that specimen and used to prepare the recommended concentration of DNA, as well as two-fold, four-fold, and eight-fold dilutions. The resulting diluted DNA samples (containing a total of 125 ng, 61.5 ng, 31.25

ng, or 15.6 ng DNA in the 25 µL) were retested to determine if the corresponding reduction in melanin by dilution allowed valid results to be obtained. Both undiluted replicated of all three specimens and all diluted specimens yielded the expected results when diluted two fold. Mutation Detected, MND = Mutation Not Detected, INV = Invalid.

Test Results Following Dilution of Specimens with Invalid Test Results

Specimen ID	Dilution	Amount Melanin in sample (µg)	Result
1	None	0.15 µg	INV / INV
	Two-fold	0.08 µg	MD / MD
	Four-fold	0.04 µg	MD / MD
	Eight-fold	0.02 µg	MD / MD
2	None	0.24 µg	INV / INV
	Two-fold	0.12 µg	MD / MD
	Four-fold	0.06 µg	MD / MND
	Eight-fold	0.03 µg	MND / INV
3	None	0.34 µg	INV / INV
	Two-fold	0.17 µg	MD / MD
	Four-fold	0.08 µg	MD / MD
	Eight-fold	0.04 µg	MD / MD

Results of testing the 17 V600 wild type specimens showed that all of the samples were correctly assigned a "mutation not detected" result with the exception of 2 highly pigmented samples which were false positive for mutation detected. Mutation Detected, MND = Mutation Not Detected, INV = Invalid.

Impact of Melanin on Mutation Detection by the cobas® 4800 BRAF V600 Mutation Test for Wild Type FFPET

V600 Wild type	BRAF results of two replicates	Melanin concentration in sample (µg)
Not highly pigmented	MND / MND	0.110 ± 0.074
African American	MND / MND	0.042 ± 0.018
	MND / MND	0.153 ± 0.011
	MND / MND	0.186 ± 0.016
Highly Pigmented	MND / MND	0.074 ± 0.008
	MND / MND	0.085 ± 0.011
	MND / MND	0.086 ± 0.009
	MND / MND	0.108 ± 0.033
	MND / MND	0.115 ± 0.049
	MND / MND	0.145 ± 0.037

V600 Wild type	BRAF results of two replicates	Melanin concentration in sample (μg)
	MND / MND	0.156 ± 0.007
	MND / MND	0.163 ± 0.014
	MND / MND	0.176 ± 0.057
	MD / MD	0.210 ±0.007
	MD / MND	0.222 ±0.053
	MND / MND	0.223 ±0.042
	MND / MND	0.294 ±0.172

8. **Interference – Triglycerides or Hemoglobin**

Two different concentrations of hemoglobin (2 mg/mL and 4 mg/mL) or triglycerides (37 mM and 74 mM) were added to 5 μm sections obtained from 10 melanoma FFPET specimens after the sections had been deparaffinized but prior to extraction. Specimens were BRAF V600E mutant and Wild-type. Two specimens had approximately 30% tumor content, and approximately 4% and 10% mutation. These percent mutations were used in this study to assess the impact of the interference at approximately 1X to 2X analytical sensitivity. Two solutions of triglycerides in phosphate-buffered saline and two solutions of hemoglobin, in phosphate-buffered saline were prepared and used to spike 3 replicate tissue pellet suspensions for each specimen, resulting in 15 replicate suspensions that contained either added hemoglobin (3 replicates, at a final concentration of either 2 mg/mL or 4 mg/mL), triglycerides (3 replicates at a final concentration of either 37 mM or 74 mM), or 3 replicates of phosphate-buffered saline for each specimen. (These levels of triglycerides and hemoglobin were equal to and double the levels of concentrations recommended to be tested by the Clinical and Laboratory Standards Institute (CLSI) EP7-A2, Appendix D 2005.) All observed results matched the expected results when each level of triglycerides was tested, indicating that triglycerides do not interfere. For hemoglobin, observed results matched the expected results when 2 mg/mL of hemoglobin was tested, and one replicate from each of two specimens yielded unexpected "Mutation Not Detected" results when 4 mg/mL of hemoglobin was tested. One replicate without any added interferent also yielded an unexpected "Mutation Not Detected" results.

9. **Repeatability**

To evaluate the repeatability of the cobas test, a separate internal study was conducted. Duplicate results were obtained on five different melanoma FFPET specimens, tested on four different days by two operators, using two different reagent lots and two cobas 4800 instruments. Four BRAF V600E-mutant specimens, two with tumor content less than 35%, two with mutation percentages less than 12% (~2X of the analytical sensitivity of the Test of 5% mutation detection), and one Wild-type specimen, were selected for this study. On each of the four days, each of two operators tested four sections from each of the 5 FFPET melanoma specimens, two sections were tested per lot of DNA

Specimen Preparation Kit and cobas®4800 BRAF V600 Mutation Test kit. A total of 32 replicates were evaluated per sample. Repeatability was considered acceptable if at least 95% of the calls corresponded to sequencing results for the specimens. A single replicate of one BRAF V600E mutant specimen (10% mutation) and a single replicate of another BRAF V600E mutant specimen (10% mutation) had results of "Mutation Not Detected." All other replicates for those specimens and all replicates for the remaining three specimens matched the expected results, corresponding to a correct call accuracy of 98.75% across the entire study.

Repeatability of the cobas® 4800 BRAF V600 Mutation Test

Panel Member (% mutation)	% Tumor Content	Tumor Stage	Total no. replicates tested	Total no incorrect calls	% Accuracy
V600E (10%)	60% / 55%	IV	32	1	96.9
V600E (12%)	40% / 35%	IIIB	32	0	100
V600E (10%)	30% / 30%	IV	32	1	96.9
V600E (17%)	30% / 35%	II	32	0	100
WT	60% / 60%	IV	32	0	100

10. **Reproducibility**

An external study was performed to assess the reproducibility of the cobas® 4800 BRAF V600 Mutation Test across 3 external testing sites (2 operators per site), 3 reagent lots, and 5 non-consecutive testing days, with an 8-member panel consisting of DNA samples derived from FFPE sections of malignant melanomas. This panel included both pigmented and non-pigmented samples and a range of percent tumor content (50% to 90%) and percent mutant alleles, including one sample at the claimed limit of detection (LOD) of 5%. Out of 94 runs, 92 (97.9%) were valid. Of 1442 samples tested, 2 samples (0.14%) gave invalid results. For all of the panel members except for the LOD samples, the correct call was made for 100% of valid tests, including samples panel members with 20% mutation, and two panel members determined to be highly pigmented. For the 5% mutation panel member, the V600E mutation was detected in 90% (162/180) of samples. There were no false positives for any WT sample tested. Results by overall agreement are presented below.

Overall Agreement Estimates by Panel Member in the cobas® 4800 BRAF V60) Mutation Test Reproducibility study

Panel Member	No. of Valid Tests	Agreement (%)	95% CI for Agreement (%)
Wild Type – 75% Tumor	180	100% (180/180)	98.0, 100.0
Wild Type – 75% Tumor, Pigmented	180	100% (180/180)	98.0, 100.0
V600E – 5% Mutation	180	90% (162/180)	98.0, 100.0
V600E – 20% Mutation	180	100% (180/180)	98.0, 100.0

V600E Mutant – 50% Tumor	180	100% (180/180)	98.0, 100.0
V600E Mutant – 50% Tumor, Pigmented	180	100% (180/180)	98.0, 100.0
V600E Mutant – 75% Tumor	180	100% (180/180)	98.0, 100.0
V600E Mutant – 90% Tumor	180	100% (180/180)	98.0, 100.0
Note: Results are included as agreement when a valid test of Mutant Type panel member has a result of "mutation detected" or when a valid test result of Wild Type panel member has a result of "mutation not detected".			
95% CI = 95% exact binomial confidence interval			

The total precision standard deviation and total variance of the mutant Ct values attributed to Lot, Site/Instrument, Operator, Day, and Within Run by Panel Member were calculated and presented in the table below. Across all components, the CV (%) was <3% for all panel members.

Reproducibility for Tumor Specimens – Mutant Channel

			Percentage of Total Variance (CV (%))					
Panel Member	N	Total SD[a]	Lot	Site/Inst.	Operator	Day	Within-Run	Total CV[a] (%)
V600 E-5% Mutation	162	0.78	2.8% (0.5)	27.0% (1.5)	3.5% (0.5)	2.6% (0.5)	64.1% (2.3)	2.9
V600 E-20% Mutation	180	0.43	52.9% (1.2)	18.2% (0.7)	2.3% (0.2)	4.1% (0.3)	22.5% (0.8)	1.6
V600 E Mutant-50% Tumor	180	0.41	40.6% (1.0)	12.6% (0.5)	15.9% (0.6)	15.8% (0.6)	15.1% (0.6)	1.5
V600 E Mutant-50% Tumor Pigmented	180	0.53	36.0% (1.1)	18.7% (0.8)	4.7% (0.4)	16.0% (0.7)	24.5% (0.9)	1.8
V600 E Mutant-75% Tumor	180	0.41	36.7% (0.9)	21.3% (0.7)	5.3% (0.4)	0.0% (0.0)	36.6% (0.9)	1.5
V600 E Mutant-90% Tumor	180	0.37	38.2% (0.8)	2.8% (0.2)	26.9% (0.7)	7.9% (0.4)	24.1% (0.7)	1.4

[a] SD = Standard Deviation, CV = Coefficient of Variation.

The total precision standard deviation and total variance of the wild type Ct values attributed to Lot, Site/Instrument, Operator, Day, and Within Run by Panel Member were calculated and are summarized in the table below. The Across all components, the CV (%) was <1.8% for all panel members.

Reproducibility for Tumor Specimens – Wild Type Channel

Panel Member	N	Total SD[a]	Percentage of Total Variance (CV [%])					Total CV (%)
			Lot	Site/Inst.	Operator	Day	Within-Run	
Wild Type-75% Tumor	180	0.46	43.4% (1.1)	15.1% (0.7)	19.3% (0.8)	9.2% (0.5)	12.9% (0.6)	1.7
Wild Type-75% Tumor Pigmented	180	0.29	37.1% (0.7)	1.6% (0.1)	40.5% (0.7)	8.4% (0.3)	12.4% (0.4)	1.1
V600 E-5% Mutation	180	0.44	37.1% (1.0)	16.3% (0.7)	13.2% (0.6)	14.6% (0.6)	18.8% (0.7)	1.7
V600 E-20% Mutation	180	0.42	64.2% (1.3)	13.9% (0.6)	5.0% (0.4)	6.9% (0.4)	10.0% (0.5)	1.6
V600 E Mutant-50% Tumor	180	0.40	36.2% (0.9)	13.7% (0.6)	16.4% (0.6)	19.9% (0.7)	13.8% (0.6)	1.5
V600 E Mutant-50% Tumor Pigmented	180	0.48	34.5% (1.0)	18.5% (0.7)	5.0% (0.4)	18.7% (0.7)	23.3% (0.8)	1.7
V600 E Mutant-75% Tumor	180	0.39	52.9% (1.1)	10.4% (0.5)	9.2% (0.5)	7.0% (0.4)	20.4% (0.7)	1.5
V600 E Mutant-90% Tumor	180	0.39	40.7% (0.9)	6.2% (0.4)	21.7% (0.7)	7.8% (0.4)	23.6% (0.7)	1.5

Note: Channel 580 (wild type channel) was used for the determination of CV(%) and SD.
[a] SD = Standard Deviation, CV = Coefficient of Variance.

11. Lot-to-Lot Reproducibility

The cobas® 4800 BRAF V600 Mutation Test utilizes two separate kits: (1) The DNA Specimen Preparation kit for isolation of DNA from melanoma FFPET specimens, and (2) the cobas® 4800 BRAF V600 Mutation Test kit for the amplification and detection of the isolated DNA for its V600E mutation status. Ten melanoma FFPET specimens; 7 V600E mutant and 3 wild-type were selected for the study based upon their V600E mutation status, tumor content (range 55% to 90%) and mutation percentage (range 10% to 65%). One specimen with 10% mutation had percent mutation of approximately 2X the analytical sensitivity of mutation detection for the test (5%). Eighteen (18) 5 µm sections ("curls") were obtained from each of the ten FFPET melanoma specimens. Two sections were used for testing with each of the nine unique combinations of the DNA Sample Preparation Kit and cobas® 4800 BRAF V600 Mutation Test Kit. Results of the cobas® 4800 BRAF V600 Mutation Test ("Mutation Detected" or "Mutation Not Detected") and the Ct values for mutant and wild-type targets were tabulated for each reagent combination, for each of the ten specimens. Observed results matched expected results when 9 lot combinations of reagents (three lots of the cobas® DNA Specimen Preparation Kit and three lots of the cobas® 4800 BRAF V600 Mutation Test) and 6 specimens per lot combination, were used. The mean CT and % CV values for each channel were summarized across lots by DNA specimen preparation kit

and the BRAF V600E Mutation Test Kit. No trend in Ct values was observed and the acceptance criterion (100% correct calls) was met.

12. **Specimen Handling - Curl Versus Slide Equivalency:**
To evaluate the equivalency between DNA extracted from 5 µm unmounted melanoma FFPET sections (FFPET "curls") and DNA extracted from melanoma FFPET sections mounted on slide (FFPET "slides"), 50 melanoma FFPET specimens (10 wild-type and 40 V600E mutant specimens) were tested. Two (2) sections were sliced from each melanoma FFPET specimen; one section was mounted on a slide and the other section placed into a microfuge tube, and prepared according to directions. Tumor content ranged from 50 – 80%. The results demonstrated 98% (49/50) equivalency between unmounted melanoma FFPET sections and FFPET sections mounted on slides. To investigate the inconsistent result for the one discordant specimen, sequencing of the specimen demonstrated that the mutation is a V600K specimen with ~ 31% mutation. The detection of the V600K in 2 out of 6 runs was due to the limited cross-reactivity which the test exhibits toward V600K specimens.

13. **Specimen Handling – Macrodissection**
The accuracy of samples following macrodissection was evaluated with specimens that had less than 50% tumor content. Observed results matched expected results.

14. **Guard banding**
The objective of the guard banding studies was to establish the robustness of the PCR conditions for the cobas® 4800 BRAF V600 Mutation Test. Guard banding studies were performed on the cobas® 4800 BRAF V600 Mutation Test Thermal Cycling Profile, components of the Working Master Mix (i.e., when the BRAF oligo mixture and magnesium acetate are added to mixture) and Proteinase K concentration (for DNA isolation procedure). Genomic DNA from 100% mutant melanoma cell line (SK-MEL-28) and a 0% mutant wild-type CRC cell line (HCT116) were used. Experiments were conducted with 5ng of a 50% mutant blend of the 2 cell lines was used to mimic an average FFPET sample at 125ng genomic DNA input. Additional experiments used 2.5ng of a 25% mutant blend and 1.25ng of a 12.5% mutant blend to represent poor quality FFPET specimens and challenge the assay. To determine the acceptance criteria for guard banding studies, inter- and intra-plate variability was established for 5ng of 50% mutant DNA input by running 94 replicates per plate on 2 plates in 2 instruments. Variability was defined as the maximum Mutant and Wild-type Ct difference across runs. The total variability across 376 replicates for Mutant Ct values was 2.1 cycles and for Wild-type Ct values was 1.7 cycles. The guard band parameters were considered acceptable if the Ct values were within 3.1 cycles of the control condition for the Mutant channel and within 2.7 cycles of the control condition for the Wild-type channel for all guard band studies described below. All replicates at all conditions passed the acceptance criteria for each channel as described above, with the exception of the working master mix

studies. For this study all conditions passed except for the conditions in which the reaction mix (RXNMX) was increased by 25% in combination with a decrease in magnesium acetate (MGAC) by 25%. All workflow guard banding however passed at ±15% volumetrically of each component in combination.

15. **Cross-Contamination**

AmpErase® (Uracil- N-glycosylase) is used in the test to avoid carryover contamination. Studies were performed to evaluate the incidence of false-positive results observed when replicates of a V600E mutant FFPET specimen with high V600E mutation percentage were run adjacent to replicates of a melanoma Wild-Type FFPET specimen. Two FFPE melanoma tumor tissue specimens were selected for the study based upon their V600E mutation status (V600 Mutant or Wild-Type), and BRAF V600E mutation percentage. Sixty (60) 5 µm sections were obtained from each of the 2 melanoma FFPET specimens. Each of the 60 sections was processed to isolate DNA using 1 lot of kits. One replicate of the DNA eluate from each of the 120 samples was tested with one lot of the cobas® 4800 BRAF V600 Mutation Test over five consecutive runs, each with 12 V600E-mutant replicates alternated with 12 Wild-Type replicates on the plate. The Ct values were examined for apparent trends over the course of the 5 consecutive runs. All 60 replicates of the BRAF Wild-type specimen yielded the expected result of "Mutation Not Detected." All 60 replicates of the V600E-mutant specimen yielded the expected result of "Mutation Detected." The Ct values for the 120 replicates showed no trend over the five consecutive runs. Additional studies evaluated whether 100% mutant genomic DNA isolated from cell lines and amplified and sampled using the cobas test created false positive in neighboring blank wells. Results from 5 cross-contamination runs using 125 ng/PCR reaction of extracted BRAF V600E mutant cell line DNA interspersed with elution buffer were evaluated. Sixty total replicates of cell line and Specimen Diluent were extracted and amplified/detected in a checkerboard layout (12 replicates each per run). Expected results were observed.

16. **Stability Studies**

a. **Extracted Specimens –** DNA extracts obtained from each of 10 melanoma FFPET specimens were tested as follows:

i. after storage at -20°C for 31 or 61 days,
ii. after storage at 2 - 8° C for 0, 8, 15, 31, or 61 days,
iii. after storage at 32° C for 1, 4, or 8 days,
iv. after one, two, three, or four freeze-thaw cycles consisting of storage at -20°C for at least 24 hours, thawing and sampling, and then re-freezing.

After storage at –20°C, 2 – 8°C, or 32°C, results from the ten specimens matched the expected result (based on sequencing results) at all time points, indicating that extracted DNA used with the cobas® 4800 BRAF V600 Mutation Test is stable for at least 61 days when stored at -20°C or at 2 – 8°C. The results also indicated that the extracted DNA is stable for at

least eight days when stored at 32°C. No trend in Ct values was detected over these storage periods. In addition, all results from the ten specimens also matched the expected result (based on sequencing) when the extracted DNA was subjected from one to four freeze-thaw cycles.

b. **Clinical FFPET Specimens**–The stability of clinical FFPET specimens at room-temperature (15°C – 30°C), the recommended storage condition for the clinical FFPET specimens, was evaluated. Two µm5 µm sections were obtained from each of the ten specimens with either BRAF V600E mutation or wild type and varying percent tumor content. For all specimens tested, the observed results matched the expected results and no trend in Ct values was detected for the time points tested to date. The results support the stability of clinical specimens to 9 months.

c. **Clinical Specimen, Slide-Mounted**– To evaluate the stability of 5 µm slide-mounted sections obtained from melanoma FFPET specimens, multiple µm5 µm section slides obtained from each of ten melanoma FFPET specimens seven V600E mutant and three wild-type, were tested on Day 0 and after storage at 32°C for 31, 61 and 91 days. One specimen had percent mutation less than three times the analytical sensitivity of ~5% mutation detection for the test. Sixteen (16) 5 µm sections were obtained from each specimen and mounted on separate slide (one section per slide). Each of the slide-mounted sections was then air-dried at ambient temperature for 30 minutes. The DNA from 2 slide-mounted sections of each specimen was immediately isolated and tested (one replicate for each slide). The remaining 14 slide-mounted sections from each specimen were stored at 32°C for 31, 61 and 91 days. Observed results matched the expected results for eight of ten specimens. One specimen had a single invalid result that was corrected upon retesting and the other produced a valid result after a 1:2 dilution. This specimen had a high melanin concentration suggesting melanin inhibition. Slide-mounted 5 µm sections of melanoma FFPET specimens that were stored at 32°C are stable for up to 91 days.

d. **Prepared Specimen Plus Activated Master Mix**–To evaluate room temperature stability of extracted DNA after it was added to working Master Mix, duplicate samples of DNA eluate from each of 10 melanoma FFPET specimens were each mixed with working Master Mix, stored at 32°C for 0, 60, 90 and 120 minutes prior to amplification and detection. A single lot of reagents was used for the study. Seven (7) specimens were BRAF V600E Mutant and three specimens were BRAF V600 Wild-type. Two specimens had a percent mutation of approximately 2X the analytical sensitivity of mutation detection for the test (5%). Observed results matched the expected results when the sample plus working Master Mix was stored for 0, 60, 90, or 120 minutes at 32°C prior to amplification and detection. In addition, no trend in Ct values was detected over these storage periods. Master Mix is stable for at least two hours when stored at 32°C.

e. **Working Master Mix**–To evaluate the stability of cobas® 4800 BRAF V600 Mutation Test working Master Mix at 32°C over 120 minutes, duplicate samples of DNA eluate from each of ten melanoma FFPET specimens were each mixed with working Master Mix, stored at 32°C for 0, 30, 60, 90 and 120 minutes, and then used to test melanoma FFPET specimens. One lot of reagents and seven V600E mutant and three Wild-type specimens. Two specimens had a percent mutation approximately 2X the analytical sensitivity of mutation detection for the test (5%). Bulk working Master Mix reagent was prepared according to directions, then distributed to 5 tubes representing the 5 testing time points (0, 30, 60, 90, or 120 minutes). The DNA eluate from each sample was then tested in duplicate using each of the five working Master Mix reagents. All valid specimens' results matched the expected result at all time points, indicating that cobas® 4800 BRAF V600 Mutation Test working Master Mix is stable for at least two hours when stored at 32°C.

f. **Open Vial DNA sample Preparation Kit Reagents**–Twelve 5 µm sections were obtained from each of six melanoma FFPET specimens. One 5 µm section from each specimen was processed with freshly-opened and reconstituted reagents from the DNA Sample Preparation Kit and tested at Day 0 and after 16, 31, 46, 63, 71, 81, and 91 days of storage at 32°C (kit normally stored at 15°C-30°C). Four specimens were BRAF V600E-mutant and two specimens were BRAF V600 wild-type. Two of the four BRAF V600E mutant specimens had a low percentage (12-14%) of BRAF V600E mutation. Observed results matched the expected results for all time points or for 8 uses of the reagents over 91 days when stored at 32°C between uses. These results indicate the generic DNA Sample Preparation Kit reagents can be used up to 8 times over at least 91 days when stored at 15°C-30°C between each use.

g. **Open Vial, cobas® 4800 BRAF V600 Mutation Test Reagents**–Two sets of kits were used in the study. Opened kits 1 & 2 were tested on days 0, 14, 21 and 31. Opened kits 3 & 4 were tested on days 0, 31, 46 and 61. This was done to test the open vial stability over 30 and 60 days with up to 4 uses per kit. Multiple 5 µm sections were obtained from each of seven melanoma FFPET specimens at the beginning of the study. One section from each specimen was tested in duplicate with opened reagents of one cobas® 4800 BRAF V600 Mutation Test kit per time point. Six BRAF V600E mutant specimens and one BRAF Wild-type specimen were selected for this study. One specimen with approximately 10% BRAF V600E mutation was 2X the analytical sensitivity of mutation detection for the test (5%). All results from the seven specimens matched the expected result when the cobas® 4800 BRAF V600 Mutation Test kit reagents were used 4 times over 61 days when stored at 2 – 8°C between uses. These results indicate that the open-vial stability of the cobas® 4800 BRAF V600 Mutation Test Kit reagents is at least 61 days.

h. **Cobas® 4800 BRAF V600 Mutation Kit** – Stability of the cobas® 4800 BRAF V600 Mutation Test Kit and its components were assessed at

various time points after storage at 4 °C (real time) in upright and inverted orientations using 3 lots of the kit reagents and controls. Stability was evaluated by performing functional testing before and after each storage interval. Testing was done at 4 weeks, 8 weeks, 3 months, 6 months, 9 months, 12 months, and 13 months.

i. *Real Time Functional testing:* Functional testing consisted of testing: 1) twenty replicates of BRAF mutant Control that had been diluted to 4000 copies/mL (100 copies per PCR reaction), 2) two replicates of undiluted BRAF Mutant Control, and 3) two replicated of undiluted BRAF Wild-Type Control. The results were acceptable if "Mutation Detected" results were obtained for 19 of the 20 diluted BRAF Mutant Control replicates, and Mutant Ct and Wild-Type Ct values obtained for the two replicates of the undiluted BRAF Mutant Control and undiluted BRAF Wild-Type Control were all within specified control ranges. The results indicate that the 3 lots of test kit reagents and controls are stable for the period of time each lot has been tested to date. Real-time stability at 4°C: Thirteen-month stability has been demonstrated. Stability studies are ongoing to support 24 months.

i. **DNA Sample Preparation Kit**–Stability of the DNA Isolation Kit and its components were assessed at various time points after storage at 32°C (real time) in upright and inverted orientations using three lots of kit reagents at specified time points for three lots.

i. *Functional testing:* Functional testing consisted of processing nine replicates of a Wild-Type cell line (K562) DNA using DNA Isolation Kit reagents that had been stored as specified. These nine replicates are tested and compared to the Ct of the unprocessed DNA using the cobas® 4800 BRAF V600 Mutation Test. Functional testing also consisted of five melanoma

ii. *Chemical testing:* Chemical Testing consisted of five kit reagents; tissue lysis buffer, DNA binding buffer, wash buffer I, wash buffer II and DNA elution buffer. Appearance, color, PH, (all 5 reagents) and determination of guanidine hydrochloride concentration (for wash buffer 1 and DNA Binding Buffer) were evaluated.

iii. *Real-Time testing:* Real-time stability was evaluated by performing functional and chemical testing on the 3 lots of reagents during storage at 32°C in upright and inverted orientations for various periods of time. Additional Testing with the DNA Binding Buffer was assessed due to failure of several time points for pH specifications. Five melanoma FFPE specimens were selected based upon their V600E mutation status. The results indicate that the three lots of DNA Isolation Kit reagent are stable for the period of time over which each lot has been tested to date. Real time stability for 6 months has been demonstrated.

The DNA Sample Preparation Kit has a recommended storage temperature of 15-30°C.

j. **Shipping**–To evaluate the tolerance of the cobas® 4800 BRAF V600 Mutation Test Kit to temperature extremes that can occur during shipping of the product under each of three different shipping categories were tested. The cumulative thermal stress that can occur during shipping of the of the cobas® 4800 BRAF V600 Mutation Test Kit under each of three different shipping conditions: exposure to 40 hours at 32°C, 15 days at 15°C, and 5 days at 37°C; exposure to 40 hours at 32°C, 15 days at 15°C, and 5 days at -20°C; and exposure to 160 hours at 25°C and 15 days at 15°C. The results of this study indicate that the reagents and controls in the cobas® 4800 BRAF V600 Mutation Test Kits are not affected by the temperature extremes that can occur during shipping of the product under any of the three shipping conditions that were studied.

17. **Antimicrobial Effectiveness Testing of the Test Kit**
To assess the effectiveness of the preservatives, a total of five microorganisms (*Staphylococcus aureus, Candida albicans, Aspergillus niger, Pseudomonas aeruginosa and Escherichia coli*) were individually spiked in each of the kit reagents on Day 0, and the colony forming units (cfu) count in log10 was determined on Day 14 and Day 28. There is no impact of microbial contamination on the functional performance of the cobas® 4800 BRAF V600 test when stored for up to 28 days at 25°C.

18. **Controls – Value Assignment Process**
The value assignment for the controls is performed using the calibrator bracketing method. The high and low calibrators are prepared from a mutant or wild-type plasmid reference standard and represent the copy number limits of the controls. Twenty replicates each of the formulated controls and calibrators are tested within the same run. The average Ct value of the formulated controls must fall within the average Ct values of the calibrators. A linear trend line is calculated between the low and high calibrators using log of copy number vs. the average Ct value. The average Ct value of each formulated control is then used with the linear trend line equation to calculate the copy number.

B. Animal Studies
None.

C. Additional Studies
None.

X. SUMMARY OF PRIMARY CLINICAL STUDY

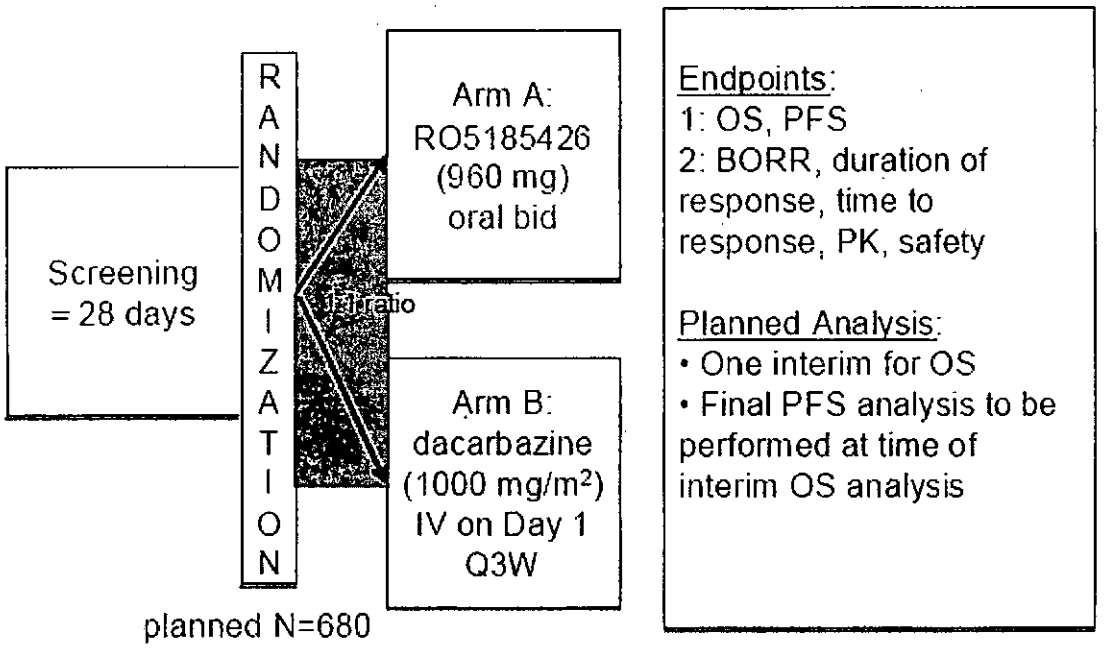
The cobas® 4800 BRAF V600 Mutation Test was used as a companion diagnostic test for selecting patients for treatment with vemurafenib (Zelboraf™) in the clinical study NO25026 (BRIM3). The results of this clinical study described in NDA 202429 support approval of vemurafenib (Zelboraf™) and therefore establish the safety and effectiveness of the cobas® 4800 BRAF V600 Mutation Test for its indicated use, as well. Formalin-

fixed paraffin-embedded tissue (FFPET) samples from all melanoma patients being considered for treatment were tested with the cobas® 4800 BRAF V600 Mutation Test. Patients with a positive test result were eligible for enrollment in the drug trials if they met other eligibility criteria. Patients with a negative test result were ineligible for drug trial enrollment. The use of the cobas® 4800 BRAF V600 Mutation Test in both the Phase II and Phase III clinical trials were conducted under IDE G070126 for US enrollment sites. The N025026 study was sponsored by Hoffmann La Roche, Inc., and was conducted at approximately 104 sites [US (22 centers), Germany (17 centers), UK (14 centers), Australia (11 centers), France (10 centers), Italy (7 centers), Canada (7 centers), New Zealand and Sweden (4 centers each), Netherlands and Israel (3 centers each) and Switzerland (2 centers)]. The study dates were from January 4, 2010 (first patient randomized) to December 30, 2010 (clinical cut-off date). The BRAF V600 mutation status of tumor tissue was assessed for patient eligibility using the cobas® 4800 BRAF V600 Mutation Test by the following laboratories: Esoterix Clinical Trial Services, Research Triangle Park, North Carolina; Clarient, Inc., Aliso Viejo, California; TMD - A Quintiles Central Laboratory, Westmont, Illinois; Targos Molecular Pathology, Kassel, Germany; and the Peter MacCallum Cancer Centre, Melbourne, Australia. A summary of the clinical study is presented below.

A. Study Design

Trial NO25026 (BRIM 3) was an international, randomized, open-label, controlled, multicenter, Phase III study in previously untreated patients with unresectable stage IIIC or stage IV melanoma with V600E BRAF mutation to evaluate the efficacy of vemurafenib (Zelboraf™) versus dacarbazine. The database for this PMA reflected data collected through January 04, 2010 and December 30, 2010. A total of 1998 patients were screened by the cobas® 4800 BRAF V600 Mutation Test to determine eligibility of enrollment. The study design is shown in Figure 1 below.

Figure 1 Study Design and Endpoint Summary



Stratification factors at randomization included: metastatic disease stage classification, ECOG performance status, LDH level, and geographic region.
BORR = best overall response rate; ECOG = Eastern Cooperative Oncology Group; LDH = lactate dehydrogenase; OS = overall survival; PFS = progression-free survival; PK = pharmacokinetics.

The trial enrolled 675 patients; 337 were allocated to receive vemurafenib and 338 to receive dacarbazine. Randomization was stratified according to disease stage, lactate dehydrogenase (LDH), ECOG performance status and geographic region. Treatment continued until disease progression, unacceptable toxicity, and/or consent withdrawal. The major efficacy outcome measures of the trial were overall survival (OS) and progression-free survival (PFS). Other outcome measures included confirmed investigator-assessed best overall response rate.

Baseline characteristics were balanced between treatment groups. Most patients were male (56%) and Caucasian (99%), the median age was 54 years (24% were ≥ 65 years), all patients had ECOG performance status of 0 or 1, and the majority of patients had metastatic disease (95%).

There are two co-primary efficacy endpoints for this study: overall survival (OS) and progression-free survival (PFS). To maintain the alpha level of 0.05 (two-sided) while accounting for two co-primary endpoints, statistical significance for the comparison of OS was based on an alpha level of 0.045 (two-sided), and statistical significance for the comparison of PFS was based on an alpha level of 0.005 (two-sided). The primary analysis population for efficacy was the intent-to-treat (ITT) population. All safety analyses were performed on the safety population.

To assess analytical performance of the cobas® 4800 BRAF V600 Mutation Test for the detection of V600E mutations, DNA from tumor specimens of subjects screened

for participation in the clinical trial were retained for subsequent comparative testing with bi-directional sequencing of V600E mutation detection. The percent agreements (positive, negative and overall) with bi-directional Sanger sequencing as the primary reference method were determined.

Data from the NO25026 study were monitored by an external Data Safety Monitoring Board (DSMB). The DSMB, which reviewed safety data from all vemurafenib trials, consisted of clinicians who are experts in the disease area and one statistician. The DSMB reviewed available safety data from this trial at regularly scheduled intervals specified in the DSMB charter. In addition, for this study the DSMB reviewed the results of the interim analysis of OS and the prespecified final analysis for PFS performed at the time of the interim analysis for OS.

Data from the cobas® 4800 BRAF V600 Mutation Test were managed by using a clinical database developed by an external vendor. Data quality assurance within Data Management were performed according to established internal RMS procedures and applicable regulatory requirements.

1. Clinical Inclusion and Exclusion Criteria
   a. *Inclusion and Exclusion Criteria for Specimen Testing:* The melanoma tumor samples used in this study were obtained from Phase II, conducted at 15 sites in the U.S. and Australia, and Phase III, conducted at 103 sites within the European Union, Australia, New Zealand, and North America (U.S. and Canada).

      Inclusion Criteria
      1. Male or female patients ≥18 years of age
      2. Patients with histologically confirmed metastatic melanoma
      3. Must provide archival or recently obtained melanoma tumor biopsy for mutation analysis
      4. Written informed consent of patient or legal guardian

      Exclusion Criteria
      Unwillingness or inability to provide written informed consent

   b. *Inclusion and Exclusion Criteria for Patient Enrollment into Phase III Study:* The target population was male or female patients ≥ 18 years of age with histologically confirmed metastatic melanoma who had not received prior systemic anti-cancer treatment and whose melanoma was confirmed to bear the *BRAF* V600E mutation by the cobas® 4800 BRAF V600 Mutation Test.

      Inclusion Criteria
      Patients had to meet all of the following criteria to be included in the study:
      1. Male or female patients ≥ 18 years of age
      2. Histologically confirmed metastatic melanoma (surgically incurable and unresectable Stage IIIC or Stage IV (American Joint Committee

on Cancer [AJCC]). Unresectable Stage IIIC disease needed confirmation from a surgical oncologist.

3. Treatment-naïve, i.e., no prior systemic anti-cancer therapy for advanced disease (Stage IIIC and IV). Only prior adjuvant immunotherapy was allowed.
4. Must have had a *BRAF* V600-positive mutation (by Roche cobas test) prior to administration of study treatment
5. ECOG performance status of 0 or 1
6. Life expectancy > 3 months
7. Measurable disease by RECIST criteria (version 1.1) prior to the administration of study treatment
8. Must have recovered from effects of any major surgery or significant traumatic injury at least 14 days before the first dose of study treatment
9. Cutaneous SCC lesions identified at baseline must be excised. Adequate wound healing was required prior to study entry. Baseline skin exam was required for all patients.
10. Adequate hematologic, renal, and liver function as defined by laboratory values performed within 28 days prior to initiation of dosing.
11. For premenopausal women, negative serum pregnancy test within 10 days prior to commencement of dosing; women of non-childbearing potential were included if they were either surgically sterile or postmenopausal for ≥ 1 year.
12. For fertile men and women, the use of an effective method of contraception during treatment and for at least 6 months after completion of treatment as directed by their physician, in accordance with local requirements.
13. Absence of any psychological, familial, sociological or geographical condition that would potentially hamper compliance with the study protocol and follow-up schedule; such conditions were discussed with the patient before trial entry
14. A signed informed consent form (ICF) obtained prior to study entry and prior to performing any study-related procedures

Exclusion Criteria

Patients meeting any of the following criteria were excluded from the study:

1. Any active central nervous system (CNS) lesion (i.e., those with radiographically unstable, symptomatic lesions). However, patients treated with stereotactic therapy or surgeries were eligible if patient remained without evidence of disease progression in brain ≥ 3 months. Patients were also required to be off corticosteroid therapy for ≥ 3 weeks. Whole brain radiotherapy was not allowed with the exception of patients who had definitive resection or stereotactic therapy of all radiologically detectable parenchymal lesions.
2. History of carcinomatous meningitis

3. Regional limb infusion or perfusion therapy
4. Anticipated or ongoing administration of anti-cancer therapies other than those administered in this study
5. Pregnant or lactating women
6. Refractory nausea and vomiting, malabsorption, external biliary shunt, or significant small bowel resection that would preclude adequate RO5185426 absorption (patients had to be able to swallow pills)
7. Mean QTc interval $\geq$ 450 msec at screening
8. NCI CTCAE Version 4.0 grade 3 hemorrhage within 4 weeks of starting the study treatment
9. Any of the following within the 6 months prior to study drug administration: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, symptomatic congestive heart failure, serious cardiac arrhythmia requiring medication, uncontrolled hypertension, cerebrovascular accident or transient ischemic attack, or symptomatic pulmonary embolism
10. Known clinically significant active infection
11. History of allogeneic bone marrow transplantation or organ transplantation
12. Other severe, acute or chronic medical or psychiatric condition or laboratory abnormality that could increase the risk associated with study participation or study drug administration, or could interfere with the interpretation of study results, which in the judgment of the investigator would make the patient inappropriate for entry into this study
13. Previous malignancy within the past 5 years, except for basal or squamous cell carcinoma of the skin, melanoma in-situ, and carcinoma in-situ of the cervix (an isolated elevation in prostate-specific antigen in the absence of radiographic evidence of metastatic prostate cancer was allowed)
14. Previous treatment with a BRAF inhibitor
15. Known human immunodeficiency virus (HIV) positivity, AIDS-related illness, active hepatitis B virus, or active hepatitis C virus
16. Randomization to this trial at another participating site

2. Follow-up Schedule

Patients were followed for efficacy:

Tumor assessments were done at screening, every six weeks for the first 12 weeks, every nine weeks subsequently, and at the final visit. Patients were followed for adverse events (AEs) (with exception of SCC) up to 28 days after the last dose in all patients. All SCC events occurring at any time during the study or follow-up period (every three months until patient death, withdrawal of consent, or lost to follow-up) were collected and reported as a serious adverse event (SAE) to the sponsor.

Patients were followed for safety:
The Phase III trial NO252026 included safety assessments at baseline, on day 1 ± four days of every 21-day cycle, and at the end of treatment (within 28 days of last dose). Serious adverse events that had not recovered completely by the end of treatment were to be followed until resolution.

At baseline, safety assessments included medical, oncologic, and surgical history, vital signs, physical exam, laboratories (hematology, chemistries, liver function), assessment of ECOG PS, ECG, and dermatology evaluation. Safety assessments performed at the start of each cycle were the same as at baseline, except ECGs were required prior to every other cycle and dermatology evaluation occurred at cycle 2 and every four cycles thereafter. Post-treatment follow-up was to occur every 3 months and included dermatology evaluation.

3. Clinical Endpoints
There were two co-primary efficacy endpoints for this study: overall survival (OS) and progression-free survival (PFS). To maintain the alpha level of 0.05 (two-sided) while accounting for two co-primary endpoints, statistical significance for the comparison of OS was based on an alpha level of 0.045 (two-sided), and statistical significance for the comparison of PFS was based on an alpha level of 0.005 (two-sided). The primary analysis population for efficacy was the intent-to-treat (ITT) population. All safety analyses were performed on the safety population.

B. Accountability of PMA Cohort

Between January 4, 2010 and December 30, 2010 a total of 2107 were screened for enrollment based on trial inclusion criteria, and 1998 patients were tested by the cobas® 4800 BRAF V600 Mutation Test. Of these 675 patients who tested positive were randomized to one of two arms, 337 were randomized to the vemurafenib arm and 338 were randomized to the dacarbazine arm in the Phase III trial.

Initially bi-directional sequencing was performed on all available tumor specimens obtained in the Phase III trial for patients screened as of June 15, 2010 and enrolled into the trial. For the intent to treat population there were 220 samples whose cobas test results were compared to Sanger; 109 in the Dacarbazine arm and 111 in the vemurafenib arm. The mutations are shown below:

Cobas® 4800 BRAF V600 Mutation test "Mutation Detected" [1]	Dacarbazine N = 338	Vemurafenib N= 337
Total tested by Sanger:	**109**	**111**
BRAF V600E	76	88
BRAF V600K	9	10
BRAF V600E2	-	1
Other	1	-
Wild type[2]	6	2
No Sanger result	17	10

[1] Two samples were test negative by the cobas test but enrolled into the trial.
[2] Sanger sequencing is not as sensitive as the cobas® 4800 BRAF V600 Mutation test.

C. Study Population Demographics and Baseline Parameters

The table below summarizes patient demographic and baseline characteristics for all randomized patients. Demographic characteristics were generally similar between treatment groups.
The proportions of males in the vemurafenib arm and Dacarbazine groups were 59% and 54%, respectively. A total of 99% of patients were White. The age range was 17 to 86 years old.

	DTIC N = 338	RO5185426 N = 337
Sex (num,%)		
FEMALE	157 (46%)	137 (41%)
MALE	181 (54%)	200 (59%)
n	338	337
Race (num,%)		
WHITE	338 (100%)	333 (99%)
HISPANIC	-	2 (<1%)
OTHER *	-	2 (<1%)
n	338	337
Age in years		
Mean	52.6	55.2
SD	13.89	13.80
SEM	0.76	0.75
Median	52.5	56.0
Min-Max	17 - 86	21 - 86
n	338	337
Age in years		
<65yrs	270 (90%)	244 (72%)
>=65yrs	68 (20%)	93 (28%)
n	338	337
Age in years		
<=40yrs	70 (21%)	48 (14%)
41-54yrs	114 (34%)	111 (33%)
55-64yrs	86 (25%)	85 (25%)
65-74yrs	46 (14%)	65 (19%)
>=75yrs	22 (7%)	28 (8%)
n	338	337
Weight in kg		
Mean	78.44	79.15
SD	17.678	18.098
SEM	0.966	0.992
Median	77.10	78.60
Min-Max	35.0 - 143.5	37.0 - 151.4
n	335	333

n represents number of patients contributing to summary statistics.
Percentages are based on n (number of valid values). Percentages not calculated if n < 10.
EM11 28MAR2011:23:05:57 (1 of 1)
* "Other" race was recorded by the investigator as Syrian in 1 patient and non-Hispanic in 1 patient.

D. Safety and Effectiveness Results

1. Safety Results

   The safety with respect to treatment with vemurafenib (Zelboraf™) will not be addressed in the SSED for the cobas® 4800 BRAF V600 Mutation Test. Overall, the most common adverse events (AEs) with vemurafenib were in the body system skin and subcutaneous tissue disorders, where 90% of patients had at least one AE (vs. 19% in the dacarbazine group). The most commonly occurring AEs in the body system were rash, alopecia, and photosensitivity reaction. There were several safety signals that emerged from the randomized clinical trial, including cutaneous squamous cell carcinomas, new primary malignant melanomas, liver toxicity, ophthalmologic adverse events, joint-related adverse events and cardiac events. Refer to the drug label for more information.

2. Effectiveness Results

   The cobas® 4800 BRAF V600 Mutation Test was used as a companion test for selecting patients for treatment with vemurafenib (Zelboraf™) study (BRIM3). Formalin-fixed paraffin-embedded tissue (FFPET) samples from all melanoma patients being considered for treatment were tested with the cobas® 4800 BRAF V600 Mutation Test. Patients with a test positive result were eligible for enrollment in the drug trials if they met other eligibility criteria. Patients with a negative test result were ineligible for drug trial enrollment.

   The cobas® 4800 BRAF V600 Mutation Test was designed to detect the V600E mutation in the BRAF gene. The test is cross-reactive will several other V600 mutations including V600D, V600E2, and V600K. Based on correlation data with bi-directional sequencing using a subset of samples from the Phase III trial, the sensitivity for the V600E mutation was greater than 97% however the specificity was lower as the test detected approximately two-thirds of the V600K samples evaluated in the trial.

   Treatment Naive Patients

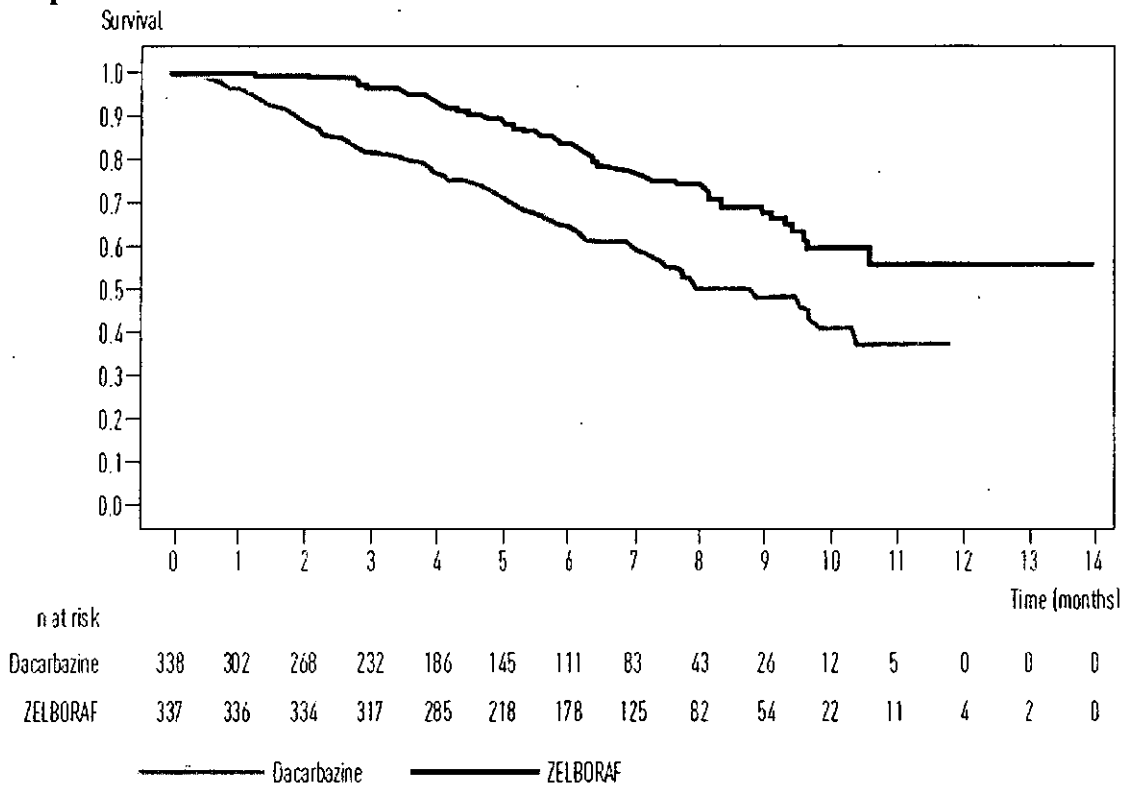
   The efficacy and safety of vemurafenib in patients with treatment-naïve, BRAFV600Emutation-positive unresectable or metastatic melanoma as detected by the cobas® 4800 BRAF V600 Mutation Test were assessed in an international, randomized, open-label trial (Trial 1). The trial enrolled 675 patients; 337 were allocated to receive vemurafenib and 338 to receive dacarbazine. The major efficacy outcome measures of the trial were overall survival (OS) and investigator-assessed progression-free survival (PFS). Other outcome measures included confirmed investigator-assessed best overall response rate.

Efficacy of Vemurafenib in Treatment-Naive Patients with $BRAF^{V600E}$ Mutation-Positive Melanoma[a]

	Vemurafenib (N=337)	Dacarbazine (N=338)	p-value[d]
Overall Survival			
Number of Deaths	78 (23%)	121 (36%)	
Hazard Ratio (95% CI)[b]	0.44 (0.33, 0.59)		<0.0001
Median Survival (months) (95 % CI)[c]	Not Reached (9.6, Not Reached)	7.9 (7.3, 9.6)	-
Median Follow-up (months) (range)	6.2 (0.4, 13.9)	4.5 (<0.1, 11.7)	
Progression-free survival Hazard Ratio (95% CI)[b]	0.26 (0.20, 0.33)		<0.0001
Median PFS (months)[c]	5.3 (4.9, 6.6)	1.6 (1.6, 1.7)	-

[a] As detected by the cobas® 4800 BRAF V600 Mutation Test
[b] Hazard ratio estimated using Cox model; a hazard ratio of < 1 favors vemurafenib
[c] Kaplan-Meier estimate
[d] Unstratified log-rank test

Kaplan-Meier Curves of Overall Survival – Treatment Naive Patients



The confirmed, investigator-assessed best overall response rate was 48.4% (95% CI: 41.6%, 55.2%) in the vemurafenib arm compared to 5.5% (95% CI: 2.8%, 9.3%) in the dacarbazine arm.

All of the patients in the phase III study NO25026 (BRIM3) sponsored by Hoffmann La Roche were selected using the cobas® 4800 BRAF V600 Mutation Test. Enrollment in the study was limited to patients whose melanoma tissue tested positive by the test. Therefore it is not known whether patients who test negative by the cobas® 4800 BRAF V600 Mutation Test will benefit from vemurafenib (Zelboraf™) treatment.

3. Subgroup Analyses
   Overall Survival (OS), progression-free survival (PFS) and best overall response rate (BORR) (confirmed) were examined by subgroups as exploratory analyses in order to assess generalizability of results. These results were generally consistent across subgroups as described in the studies used to support Zelboraf™ approval. Refer to clinical review of NDA 202429 for more information.

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. Safety Conclusions

The adverse effects of the device are based on data collected in the clinical study conducted to support PMA approval as described above. As an in vitro diagnostic test, the cobas® BRAF V600E Mutation Test involves testing on formalin-fixed, paraffin embedded human melanoma tissue sections. These tissue sections are routinely removed as part of the diagnosis of melanoma by pathologists. The test, therefore, presents no additional safety hazard to the patient being tested.

B. Effectiveness Conclusions

Correlation to the reference method (bidirectional Sanger Sequencing) in the detection of BRAF V600E Mutations demonstrated that the PPA with Sanger sequencing was high (97.3%). The NPA between the cobas® 4800 BRAF V600 Mutation Test and Sanger sequencing was lower (84.6) than the PPA. The lower NPA may be accounted for by (1) the higher sensitivity of the cobas® 4800 BRAF V600 Mutation Test for the V600E mutation, and (2) detection of a sizeable number of V600K mutations by the cobas® 4800 BRAF V600 Mutation Test (which was designed for the detection of V600E mutations).

Analytical performance studies with the cobas® 4800 BRAF Mutation Test demonstrated an ability to detect the V600E mutation with an analytical sensitivity of 5% mutation when 125 ng /25 µL DNA is used. Very good agreement in the reproducibility studies was shown. Several studies assessed the impact of melanin on test results. Results demonstrated that samples with high concentrations of melanin may result in invalid results but a two-fold dilution improves detection.

C. **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. Metastatic melanoma has a grim prognosis. Less than 10% of those that are diagnosed with metastatic melanoma will live beyond 5 years from diagnosis and since the disease occurs at a younger age compared to other cancers such as prostate cancer, the number of years of life lost per person is amongst the highest of all malignancies. BRAF mutations are common in melanoma and frequently result in a valine-to-glutamic acid substitution at codon 600 (V600E). The V600E-Mutant BRAF was evaluated as a therapeutic target in melanoma cancer using vemurafenib (Zelboraf™) in a clinical study as described in the sections above. Vemurafenib was shown to be superior to dacarbazine in this population. Vemurafenib represents an important new treatment option with a favorable risk-benefit profile for patients with BRAF V600E mutation-positive unresectable or metastatic melanoma, as identified by The cobas® 4800 BRAF V600 Test, when compared to available treatments.

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.

XIII. CDRH DECISION

CDRH issued an approval order on August 17, 2011. The final conditions of approval 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) on April 15, 2011.

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.
Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Limitations in the device labeling. Refer to the drug label for Zelboraf™ for additional information related to use of the drug.

Post-approval Requirements and Restrictions: See approval order.