SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Real-time PCR test
Device Trade Name:	**cobas**® EGFR Mutation Test v2
Device Procode:	OWD
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:	P120019/S007
Date of FDA Notice of Approval:	November 13, 2015
Expedited:	Granted priority review status on June 2, 2015 because the device addresses an unmet medical need, as demonstrated by significant clinically meaningful advantage.

The original PMA (P120019) for the **cobas**® EGFR Mutation Test (v1) was approved on May 14, 2013. This device is a real-time PCR test for the qualitative detection of exon 19 deletions and exon 21 (L858R) substitution mutations of the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded (FFPET) human non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used as an aid in selecting patients with NSCLC for whom Tarceva® (erlotinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated. The SSED to support the previously approved indication is available on the CDRH website and is incorporated by reference here.

The current panel-track supplement was submitted to expand the intended use and indication for use of the **cobas**® EGFR Mutation Test v2 for the detection of the exon 20 (T790M) substitution mutation in NSCLC patients for whom Tagrisso® (osimertinib) treatment is indicated.

II. INDICATIONS FOR USE

The **cobas**® EGFR Mutation Test v2 is a real-time PCR test for the qualitative detection of defined mutations of the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded tumor tissue (FFPET) from non-small

cell lung cancer (NSCLC) patients. The test is intended to aid in identifying patients with NSCLC whose tumors have defined EGFR mutations and for whom safety and efficacy of a drug have been established as follows:

Tarceva® (erlotinib)	Exon 19 deletions and L858R
Tagrisso® (osimertinib)	T790M

Drug safety and efficacy have not been established for the following EGFR mutations also detected by the **cobas**® EGFR Mutation Test v2:

Tarceva® (erlotinib)	G719X, exon 20 insertions, T790M, S768I and L861Q
Tagrisso® (osimertinib)	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q

For manual sample preparation, FFPET specimens are processed using the **cobas**® DNA Sample Preparation Kit and the **cobas z** 480 analyzer is used for automated amplification and detection.

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the **cobas**® EGFR Mutation Test v2 labeling.

V. DEVICE DESCRIPTION

The **cobas**® EGFR Mutation Test v2 is based on two processes:

1. The **cobas**® DNA Sample Preparation Kit provides reagents for manual specimen preparation to obtain genomic DNA from formalin-fixed, paraffin-embedded tissue (FFPET).
2. The **cobas**® EGFR Mutation Test v2 kit provides reagents for automated real-time PCR amplification and detection of the EGFR mutations.

Two external run controls are provided and the EGFR exon 28 wild-type allele serves as an internal, full process control.

A. 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.

B. PCR Amplification and Detection

Target Selection and Amplification

The **cobas**® EGFR Mutation Test v2 kit uses primers that define specific base-pair sequences for each of the targeted mutations. For the exon 19 deletion mutations, sequences ranging from 125 to 141 base pairs are targeted; for the L858R substitution mutation in exon 21, a 138 base pair sequence is targeted; for the T790M substitution mutation in exon 20, a 118 base pair sequence is targeted; for the G719X substitution mutation in exon 18, sequences ranging from 104 to 106 base pairs are targeted; for the S768I substitution mutation in exon 20, a 133 base pair sequence is targeted; for the exon 20 insertion mutations, sequences ranging from 125 to 143 base pairs are targeted; for the L861Q substitution mutation in exon 21, a 129 base pair sequence is targeted; for the internal control in exon 28, an 87 base pair sequence is targeted. Amplification occurs only in the regions of the EGFR gene between the primers; the entire EGFR gene is not amplified.

The **cobas**® EGFR Mutation Test v2 uses allele-specific PCR (AS-PCR) chemistry for amplification and detection. The selected AS-PCR primers specifically amplify the targeted mutant sequences over the wild-type sequences and/or other human genomic DNA. The **cobas**® EGFR Mutation Test v2 is designed to use three master mix (MMx) reagents which are run in three separate wells. The number and types of primers and probes differ based on the particular target(s). The **cobas**® EGFR Mutation Test v2 detects the following EGFR mutations in exons 18, 19, 20, and 21:

Table 1. EGFR Mutations Detected by the cobas® EGFR Mutation Test v2

Exon	EGFR Mutation	EGFR Nucleic Acid Sequence	COSMIC ID[1]
Exon 18	G719X	2156G>C	6239
		2155G>A	6252
		2155G>T	6253
Exon 19	Ex19Del	2240_2251del12	6210
		2239_2247del9	6218
		2238_2255del18	6220
		2235_2249del15	6223
		2236_2250del15	6225
		2239_2253del15	6254
		2239_2256del18	6255

Exon	EGFR Mutation	EGFR Nucleic Acid Sequence	COSMIC ID[1]
		2237_2254del18	12367
		2240_2254del15	12369
		2240_2257del18	12370
		2239_2248TTAAGAGAAG>C	12382
		2239_2251>C	12383
		2237_2255>T	12384
		2235_2255>AAT	12385
		2237_2252>T	12386
		2239_2258>CA	12387
		2239_2256>CAA	12403
		2237_2253>TTGCT	12416
		2238_2252>GCA	12419
		2238_2248>GC	12422
		2237_2251del15	12678
		2236_2253del18	12728
		2235_2248>AATTC	13550
		2235_2252>AAT	13551
		2235_2251>AATTC	13552
		2253_2276del24	13556
		2237_2257>TCT	18427
		2238_2252del15	23571
		2233_2247del15	26038
Exon 20	S768I	2303G>T	6241
	T790M	2369C>T	6240
	Ex20Ins	2307_2308ins9GCCAGCGTG	12376
		2319_2320insCAC	12377
		2310_2311insGGT	12378
		2311_2312ins9GCGTGGACA	13428
		2309_2310AC>CCAGCGTGGAT	13558
Exon 21	L858R	2573T>G	6224
		2573_2574TG>GT	12429
	L861Q	2582T>A	6213

[1]Catalogue of Somatic Mutations in Cancer (COSMIC), 2011, v.51. http://www.sanger.ac.uk/genetics/CGP/cosmic.

MMx1 (first amplification reaction) contains:

- Fourteen AS-PCR primers, one common primer, and one common probe are used to detect the Exon 19 deletion and complex mutations.
- One AS-PCR primer, one common primer, and one common probe are used to detect the S768I mutation.

MMx2 (second amplification reaction) contains:

- One AS-PCR primer, one common primer, and one common probe are used to detect the L858R mutation.
- One AS-PCR primer, one common primer, and one common probe are used to detect the T790M mutation.

MMx3 v2 (third amplification reaction) contains:

- Three AS-PCR primers, one common primer, and one common probe are used to detect G719X mutations.
- Three AS-PCR primers, one common primer, and one common probe are used to detect Exon 20 insertion mutations.
- One AS-PCR primer, one common primer, and one common probe are used to detect the L861Q mutation.

A derivative of *Thermus* species Z05-AS1 DNA polymerase is utilized for target amplification. Briefly, 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 Z05-AS1 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, which includes the targeted base-pair regions of the EGFR gene. This process is repeated for a number of cycles, with each cycle effectively doubling the amount of amplicon DNA.

Selective amplification of target nucleic acid from the specimen is achieved in the **cobas**® EGFR Mutation Test v2 by the use of AmpErase® (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP), which are included in the Master Mix reagents. The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing thymidine. Deoxyuridine is always present in the amplicons 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 55ºC, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon.

Automated Real-time Detection

The **cobas**® EGFR Mutation Test v2 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, a 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-AS1 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 mutations targeted by the test. Amplification of the

targeted EGFR sequences are detected independently across three reactions by measuring fluorescence at the two characteristic wavelengths in dedicated optical channels.

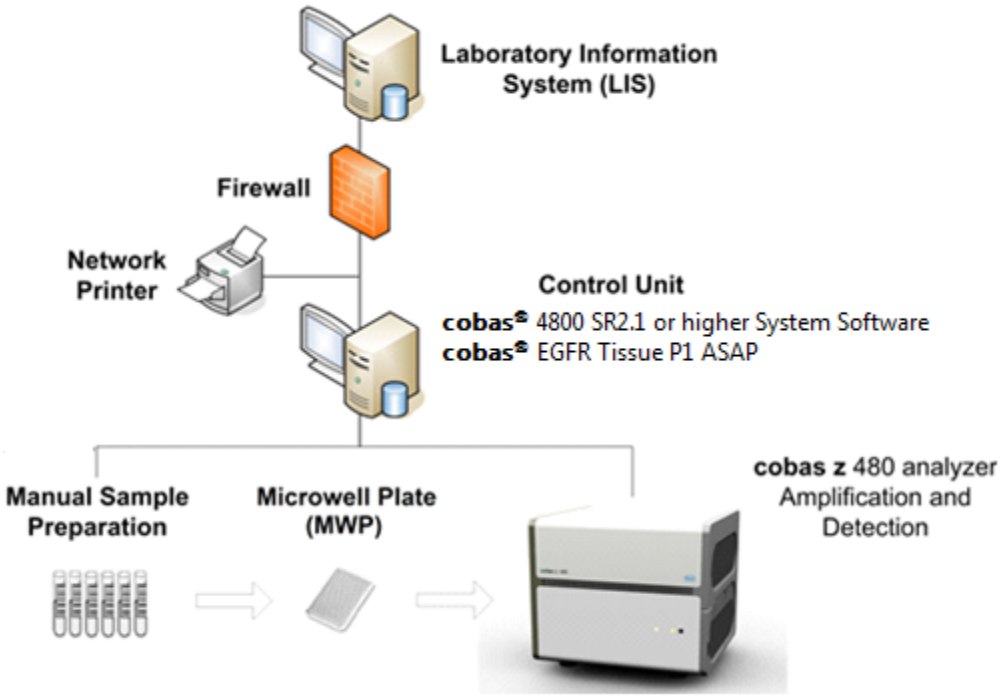
Instrument and Software

The **cobas**® 4800 system is controlled by the **cobas**® 4800 system 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 assay 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 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 **cobas z** 480 maintenance handling, test selection, specimen ID entry, reagent and microwell plate barcode 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 the **cobas**® 4800 EGFR Analysis Package (AP) software, which contains an algorithm to determine sample results and run validity. The overall **cobas**® 4800 system components are shown in the diagram below:



The final version of the ASAP software used to analyze all studies in this panel-track supplement is EGFR Tissue P1 AP v1.0.0.1560.

Interpretation of Results

If the run is valid, then the cycle threshold (Ct) and CtR (relative cycle threshold) values for each sample will be evaluated against acceptable ranges for each channel. The CtR value is determined by calculating the difference between the mutation's observed Ct and the corresponding Internal Control (IC) Ct value from the same Master Mix. Ct values are not available to the user. Tables 2 and 3 summarize how the individual amplification Master Mix results are combined to provide an overall result.

Table 2. Individual Amplification Master Mix Results to Overall Results.

Master Mix 1 Result	Master Mix 2 Result	Master Mix 3 v2 Result	Reported Result
Valid, No Mutation Detected	Valid, No Mutation Detected	Valid, No Mutation Detected	Valid, No Mutation Detected
Valid, No Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected
Valid, No Mutation Detected	Valid, No Mutation Detected	Invalid	Invalid
Valid, No Mutation Detected	Valid, Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected
Valid, No Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected

Master Mix 1 Result	Master Mix 2 Result	Master Mix 3 v2 Result	Reported Result
Valid, No Mutation Detected	Valid, Mutation Detected	Invalid	Invalid
Valid, No Mutation Detected	Invalid	Valid, No Mutation Detected	Invalid
Valid, No Mutation Detected	Invalid	Valid, Mutation Detected	Invalid
Valid, No Mutation Detected	Invalid	Invalid	Invalid
Valid, Mutation Detected	Valid, No Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected
Valid, Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected
Valid, Mutation Detected	Valid, No Mutation Detected	Invalid	Invalid
Valid, Mutation Detected	Valid, Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected
Valid, Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected
Valid, Mutation Detected	Valid, Mutation Detected	Invalid	Invalid
Valid, Mutation Detected	Invalid	Valid, No Mutation Detected	Invalid
Valid, Mutation Detected	Invalid	Valid, Mutation Detected	Invalid
Valid, Mutation Detected	Invalid	Invalid	Invalid
Invalid	Valid, No Mutation Detected	Valid, No Mutation Detected	Invalid
Invalid	Valid, No Mutation Detected	Valid, Mutation Detected	Invalid
Invalid	Valid, No Mutation Detected	Invalid	Invalid
Invalid	Valid, Mutation Detected	Valid, No Mutation Detected	Invalid
Invalid	Valid, Mutation Detected	Valid, Mutation Detected	Invalid
Invalid	Valid, Mutation Detected	Invalid	Invalid
Invalid	Invalid	Valid, No Mutation Detected	Invalid
Invalid	Invalid	Valid, Mutation Detected	Invalid
Invalid	Invalid	Invalid	Invalid

Table 3. Result Interpretation of the cobas® EGFR Mutation Test v2

Test Result	Mutation Result**	Interpretation
Mutation Detected (MD)	*G719X* Ex19Del *S768I* *T790M* *Ex20Ins* L858R *L861Q* (More than one mutation may be present)	Mutation detected in specified targeted EGFR region.
No Mutation Detected* (NMD)	N/A	No mutation detected in targeted EGFR regions
Invalid	N/A	Specimen result is invalid. Repeat the testing of specimens with invalid results following the instructions outlined in the "Retesting of Specimens with Invalid Results" section.
Failed	N/A	Failed run due to hardware or software failure. Contact your local Roche office for technical assistance

*A No Mutation Detected (NMD) result does not preclude the presence of a mutation in the targeted EGFR regions, because results depend on percent mutant sequences, adequate specimen integrity, absence of inhibitors, and sufficient DNA to be detected.
**Italicized mutation results consist of new mutations included in this device version based on data in this submission. Mutations other than one exon 19 deletions (Ex19Del), L858R, and T790M will be intended for analytical detection only.

Test Controls

One EGFR mutant control and one EGFR negative control are provided. The EGFR wild-type allele on exon 28 serves as an internal, full process control.

1. *EGFR Mutant Control:* The Mutant Control is a blend of six DNA plasmids containing specified EGFR mutation sequences and cell line DNA that is wild-type for EGFR. The Mutant Control is composed of plasmids representing the most frequently observed mutation for each mutation class detected by the test. The Mutant Control will be included in every run and will serve as a process control for amplification and detection. The Mutant Control must yield Cycle threshold (Ct) values for the Internal Control (IC), exon 19 deletion mutations, and L858R mutation within the respective acceptable ranges for the run to be considered valid.

2. *EGFR Negative Control:* The Negative Control is a full process contamination control for a given test batch of specimens. The Negative Control consists of a blank vial containing no specimen (specimen diluent only) is processed through specimen preparation and the resulting eluate is subsequently diluted, amplified and detected. The Negative Control Ct values must be either not detected or

greater than the pre-established Ct maximum value for the exon 19 deletion and L858R mutation groups and the IC for the run to be considered valid.

3. *EGFR WT Internal Control (IC):* The Internal Control in EGFR exon 28 from test specimens serves as a full process control. This control ensures that every step of the process from specimen preparation to amplification and detection has been completed successfully.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA-cleared or -approved alternatives for the testing of formalin-fixed, paraffin-embedded NSCLC tissue for EGFR mutation status in the selection of patients who are eligible for treatment with Tarceva® (erlotinib) or Tagrisso® (osimertinib).

VII. MARKETING HISTORY

The **cobas**® EGFR Mutation Test (v1) was introduced into the United States and globally starting on May 14, 2013. The **cobas**® EGFR Mutation Test (v1) is commercially available in the following countries: Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Croatia, Cyprus, Czech Republic, Denmark, Ecuador, Estonia, Finland, France, Germany, Greece, Guatemala, Hong Kong, Hungary, Iceland, India, Indonesia, Ireland, Italy, Japan, Korea, Latvia, Liechtenstein, Lithuania, Luxembourg, Malaysia, Malta, Mexico, Netherlands, New Zealand, Nicaragua, Norway, Pakistan, Panama, Peru, Philippines, Poland, Portugal, Romania, Singapore, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, United Arab Emirates, United Kingdom, United States, Uruguay, Venezuela, Vietnam.

The **cobas**® EGFR Mutation Test v2 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 **cobas**®EGFR Mutation Test v2 results and subsequently improper patient management decisions in NSCLC treatment. For the specific adverse events that occurred in the clinical studies, please see Section X below.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

For the non-clinical studies described below, percentage of tumor was assessed by pathology review. Bi-directional Sanger sequencing and next generation sequencing (NGS) methods were used to select the specimens for testing. Percentage of mutation of NSCLC FFPET specimen was determined using an NGS method.

Software changes occurred after completion of testing of the AURA2 clinical specimens. The revised software changes were appropriately validated with regression testing and the data from all studies were reanalyzed with the revised software versions.

During performance of the clinical studies on patient samples from the AURA2 study, a 3.1% (12/383) discordant rate was observed between the **cobas**® EGFR Mutation Test v2 and the NGS reference method. As a result of this and similar inquiries from customers outside the US, the algorithm for detection of the exon 20 insertion mutations was reassessed. The new cut-off was validated using an independent cohort of specimens and validated against the analytical and clinical study specimens. As a result of the change the ASAP software was updated to EGFR Tissue P1 AP v1.0.0.1560 and the results of all studies were reanalyzed. Upon reanalysis of the study data, the only studies affected by the change were the Limit of Detection, Slide vs. Curl Equivalency, and Reproducibility studies. The changes in these studies are noted in their descriptions below.

1. Correlation with Reference Method

The analytical performance of the **cobas**® EGFR Mutation Test v2 was assessed by comparing it to a validated quantitative NGS method. Percent mutation present in specimens was determined for all specimens used to demonstrate analytical and clinical performance.

Patients were enrolled into the AURA2 study using an Investigative Use Only (IUO) version (v1) of the **cobas**® EGFR Mutation Test which reported results for the additional five mutations which were masked in the version of the test approved under P120019. All specimens subsequently retested using version 2 of the test and the percent of EGFR mutation was identified using a validated NGS method. Table 4 summarizes the calculated positive percent agreement (PPA) and negative percent agreement (NPA) and overall percent agreement (OPA). Thirteen specimens were determined to be invalid upon comparison with NGS and thirty (30) of the 383 samples were identified as T790M+ by NGS but were negative by the **cobas**® EGFR Mutation Test v2. In 11 of the 30 discordant samples, the percent T790M mutation as determined by NGS was below the LoD of the **cobas**® EGFR Mutation Test v2 (2.0%). Of the remaining 19 samples, the T790M mutation was detected by the v1 test in seven samples (7/383 = 1.8%) , but the mutation was not detected by the v2 test, because the Internal Control Ct values were outside of the acceptable range, indicating poor amplifiability of the DNA template. The remaining 12 samples in which T790M was not detected by either version of the test, each of the Internal Control Ct values were moderately delayed, again suggesting poor amplifiability of the DNA template.

Table 4. cobas® EGFR Mutation Test v2 vs. NGS Using AURA2 Specimens

		NGS			
		Exon T790M Deletion			
		MD	MND	Invalid	Total
cobas® EGFR Mutation Test v2 Result	MD	226	3	2	231
	MND	30	109	0	139
	Invalid	5	6	2	13
	Total	261	118	4	383
Without Invalid Results	PPA (95% CI)	226/256 = 88.3% (95% CI: 83.8%, 91.7%)			
	NPA (95% CI)	109/112 = 97.3% (95% CI: 92.4%, 99.1 %)			
	OPA (95% CI)	(226+109)/368 = 91.0% (95% CI: 87.7%, 93.5%)			
With Invalid Result	PPA (95% CI)	226/263 = 85.9% (81.2%, 89.6%)			
	NPA (95% CI)	109/122 = 89.3% (82.6%, 93.7%)			
	OPA (95% CI)	(226+109)/383= 87.5% (83.8%, 90.4%)			

Note: Estimates with invalid results assume that the results invalid by both methods are discordant with the reference method (worst case scenario).

Table 5 below summarizes the ability of the **cobas®** EGFR Mutation Test v2 to accurately identify the four rare EGFR mutations was also established by comparison to the NGS reference method. Diagonal cells (shaded) represent concordance between NGS and the **cobas®** EGFR Mutation Test v2 test results, while the off-diagonal cells represent discordance between NGS and the **cobas®** EGFR Mutation Test v2. Specimens included in the "Other Mutations" column due to the identification of other mutations detected by NGS and the **cobas®** EGFR Mutation Test v2.

Table 5. cobas® EGFR Mutation Test v2 vs. NGS for Rare Mutations

	NGS							
cobas® EGFR Mutation Test v2	**G719X**	**S768I**	**G719X & S768I**	**Ex20Ins**	**L861Q& G719X**	**Other Mutations[1]**	**WT**	**Total**
G719X	9	0	0	0	0	1	1	11
S768I	0	4	0	0	0	0	0	4
G719X & S768I	0	0	2	0	0	0	0	2
Ex20Ins	*0*	*0*	*0*	*0*	*0*	*4*	*0*	*4*
L681Q & G719X	0	0	0	0	1	0	0	1
Other Mutations[1]	1	1	0	1	0	326	2	331
Wild type	0	0	0	0	0	3	14	17
Total	10	5	2	1	1	334	17	370

[1] Other mutations include any mutation result that does not contain G719X, S7681, Ex20ins, or L861Q.
Italicized text indicates change in the number of Ex20Ins from the original 12 after cut-off recalculation.

2. Analytical Sensitivity

a. Analytical Sensitivity - Limit of Blank (LoB)

To assess performance of the **cobas**® EGFR Mutation Test v2 in the absence of template and to ensure that a blank sample or a sample with wild-type DNA does not generate an analytical signal that might indicate a low concentration of mutation, samples with no template and NSCLC FFPET EGFR wild-type specimens were evaluated.

i. Limit of Blank (LoB) no template – DNA Specimen Diluent reagent was run as the sample with no template. None of the replicates tested across each sample panel and reagent lot yielded a "Mutation Detected" result. Ct values can be measured out to 55 cycles and results reported as "NaN" for "Not a Number", indicating no growth curve was observed and no Ct value was determined.

ii. Limit of Blank (LoB) FFPET Specimens – NSCLC FFPET EGFR wild-type specimens were tested. Specifically, 30 wild-type specimens were tested using 50 ng DNA per amplification. There were no detectable Ct values in the EGFR mutation channels in the presence of EGFR wild-type DNA isolated from NSCLC FFPET specimens. Using the analysis prescribed in the CLSI EP17-A2 guideline, the LoB was determined to be zero for all mutations.

iii. The study data were reanalyzed to assess the impact of the cut-off change for the Exon 20 insertion mutations. No change in the established LoB was identified.

b. <u>Analytical Sensitivity - Limit of Detection (LoD)</u>

Replicate **cobas**® EGFR Mutation Test v2 measurements were performed on dilution panel members that contained various amounts of genomic DNA and various percentages of the EGFR mutation, which bracketed the expected analytical sensitivity of the **cobas**® EGFR Mutation Test v2. Several studies were performed by testing dilution panels prepared from FFPET specimen blends.

Specimen FFPET blends – Multiple FFPET specimen DNA extracts representing each of the mutations detected by the test were blended with EGFR wild-type FFPET specimen extracts to generate samples targeting 10, 5.0, 2.5, and 1.25% mutation levels as determined by an NGS method, that was validated for detecting the EGFR mutations in exons 18, 19, 20, and 21. Serial dilutions of each specimen blend were prepared and eight (8) replicates of each panel member were run using each of three **cobas**® EGFR Mutation Test v2 kit lots (n=24/panel member). The sensitivity of each sample was determined by the lowest amount of DNA that produced an EGFR "Mutation Detected" rate of at least 95% for the targeted mutation. The study results are summarized in Table 6.

Table 6. Sensitivity of the cobas® EGFR Mutation Test v2 using FFPET Specimen Blends

Exon	EGFR Mutation	Mutation Sequence	Percent Mutation in the Panel Member to achieve ≥95% "Mutation Detected" Rate with 50 ng DNA input per reaction well (n=24 replicates)	COSMIC ID
18	G719X	2155 G>T	5.6	6253
		2155 G>A	3.2	6252
		2156 G>C	4.7	6239
		2156 G>C	2.5	6239
19	Exon 19 Deletion	2235_2249del15	1.4	6223
		2236_2250del15	2.5	6225
		2238_2252del15	2.4[c]	23571
		2239_2248>C	2.2	12382
		2240_2254del15	7.2	12369
		2240_2257del18	13.4[b]	12370
		2237_2253>TTGCT[a]	6.32	12416
		2237_2255>T[a]	4.08	12384
		2239_2256del18[a]	4.74	6255
		2238_2252del15[a]	5.45	23571
		2239_2257>GT[a]	6.02	Not Found
20	T790M	2369 C>T	2.4	6240
		2369 C>T	3.0	6240
	S768I	2303 G>T	2.4	6241
		2303 G>T	1.3	6241
	Exon 20 Insertion	2307_2308insGCCAGCGTG	1.7	12376
		2310_2311insGGT	1.3	12378
		2319_2320insCAC	6.81[e]	12377
21	L858R[d]	2573 T>G	4.0	6224
		2573 T>G	4.2	6224
		2573 T>G	4.3	6224
		2573 T>G	4.3	6224
		2573 T>G	5.3	6224
	L861Q	2582T>A	2.1	6213

[a] Only a single level targeting approximately 5% mutation was tested for these non-predominant exon 19 deletion mutations present in the EURTAC cohort. Specimen DNA blends were tested across 3 study sites. Data is included for completeness.

[b] Analytical sensitivity of the **cobas**® EGFR Mutation Test v2 for detecting this mutation is greater than 10% mutation level using the standard input of 50 ng per reaction well.

[c] Two independent specimens for the exon 19 deletion (2238_2252del15) were tested.

[d] Five independent specimens for the exon 21 L858R mutation were tested.

[e] Analytical sensitivity of the **cobas**® EGFR Mutation Test v2 for detecting this mutation is greater than 5% mutation level using a standard input of 50 ng per reaction well after reanalysis using the revised exon 20 insertion cut-off.

The studies support the claim that the **cobas**® EGFR Mutation Test v2 can detect 5% EGFR mutant alleles in a background of 95% wild-type alleles in formalin-fixed, paraffin-embedded tumor samples when using 50 ng DNA per amplification reaction (50 µL), with the exception of five mutations: the 2240_2257del18 exon 19 deletion mutation, which is detected at a sensitivity of >10%, and 2319_2320insCAC exon 20 insertion and three exon 19 deletions (2237_2253>TTGCT, 2240_2254del15, and 2239_2257>GT) determined from a prior approval which were detected at >6% upon reanalysis of the data after the exon 20 insertion cut-off was changed.

3. Analytical Sensitivity – Genomic DNA Input Range

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 of five DNA input concentrations of 50, 12.5, 3.1, 0.8 and 0.2 ng per amplification reaction were evaluated as part of the LoD - Specimen FFPET blends study. The study results supported the recommended DNA input of 50 ng per PCR reaction for the **cobas**® EGFR Mutation Test v2.

4. Analytical Sensitivity – Minimum Tumor Content

Twenty (20) NSCLC FFPET specimens (ten wild-type and ten EGFR mutants) mounted on slides with tumor content data were tested in single replicates for macro-dissected vs. neat conditions to determine the impact of tumor content on the performance of the **cobas**® EGFR Mutation Test v2. The mutations represented in the study consisted of five L861Q mutations; two Ex19Del and T790M dual mutations, one L858R and T790M dual mutation, and one T790M and G719X dual mutation. The percent tumor content was determined by pathologist assessment and spanned 8 - 95% tumor content by area (1 sample ≤ 10% and 9 samples > 10%) for mutant specimens and spanned 1-90% tumor content by area (3 samples ≤ 10% and 7 samples > 10%) for wild-type specimens. EGFR mutation status was determined by NGS. Each specimen pair consisted of adjacent sections and macro-dissection was performed as described in the package insert. Macro-dissection did not affect the detectability of EGFR mutations in FFPET specimens with <10% tumor content by the **cobas**® EGFR Mutation Test v2. However macro-dissection is still considered necessary for NSCLC FFPET sections with less than 10% tumor content prior to testing with the **cobas**® EGFR Mutation Test v2.

5. Analytical Specificity

a. Primer and Probe Specificity

Sequence information and alignment of the primers and probes with the EGFR gene was provided. A traditional Basic Local Alignment Search Tool (BLAST) search was performed for all of the oligonucleotides (primers and probes) as well as the target EGFR exons and amplicons using the human reference genome GRCh37. The traditional BLAST search was conducted using BLASTN 2.2.10 to assess short matches between the query (i.e., oligonucleotides and target sequences) and the database sequences. Based on two threshold parameters, T and S, sequences are reported as potential matches. A ThermoBLAST analysis (BLASTN 2.2.17, version 1.2.2.4.0) was also conducted to assess potential mismatches in hybridization, including stabilizing G-T mismatches. Based on the combined results of the BLAST searches, no potentially cross-reacting sequences other than the targeted sequences were identified.

b. Cross Reactivity

Cross-reactivity of the **cobas**® EGFR Mutation Test v2 to other EGFR exon 19 mutations were evaluated using the Phase II AURA2 clinical trial specimens and EGFR plasmids. While the **cobas**® EGFR Mutation Test v2 demonstrated cross-reactivity to the mutations listed in Table 7 below, analytical performance of the **cobas**® EGFR Mutation Test v2 in detecting these mutations has not been evaluated.

Table 7. Mutations Observed in the Phase II AURA2 Study Determined to Cross-React with the cobas® EGFR Mutation Test v2

Exon	Mutation Sequence	Amino Acid Change	COSMIC ID
19	2253_2276del24	S752_I759delSPKANKEI	13556
	2236_2256>ATC	E746_S752>I	133190
21	2572_2573CT>AG	L858R	13553

Plasmid constructs containing the non-predominant mutations for exons 18, 19, 20, and 21 were blended with wild-type genomic DNA to create 5% mutant sample with 50 ng DNA input per PCR. Results demonstrated that the **cobas**® EGFR Mutation Test v2 cross-reacts to the following mutations at a ≥ 86% hit rate. Independently, the EGFR exon 19 substitution mutation L747S was also tested at a genomic copy number equivalent to 50 ng/PCR input and confirmed to be cross-reactive.

c. Microorganisms and EGFR Homologs

Specificity of the **cobas**® EGFR Mutation Test v2 was evaluated by testing lung-related microorganisms, and plasmids of EGFR homologs, i.e., plasmids containing the sequences from each of the HER2, HER3, and HER4 genomic regions analogous to the sequences in EGFR exons 18, 19, 20, and 21 amplified by the **cobas**® EGFR Mutation Test v2.

i. EGFR Homolog Panels – Structurally related epidermal receptor tyrosine kinase protein analog sequences (EGFR/HER1, HER2, HER3 and HER4) were shown to not cross-react with the **cobas**® EGFR Mutation Test v2 when the potential cross-reactive sequence was added at a genomic copy number equivalent to 50 ng/PCR input to the isolated pooled EGFR mutation positive DNA stock prior to the amplification/detection procedure. A control condition without plasmid DNA was included. Results indicated that the observed mutations for all tested FFPET specimens matched the expected mutation as determined by sequencing, in the presence and absence of the added HER gene plasmid DNA.

ii. Testing of Lung-Related Microorganisms – *Streptococcus pneumoniae* and *Haemophilus influenzae* at 4 x 10^5 colony forming units (CFU) were found not to cross react or interfere with the **cobas**® EGFR Mutation Test v2 when added to specimens containing wild-type and mutant EGFR sequences during the tissue lysis step. Presence of *Pseudomonas aeruginosa* and *Aspergillus Niger* at approximately 100 CFU/mL in EGFR MMx1, EGFR MMx2, and EGFR MMx3 were found not to cross react or interfere with the performance of the **cobas**® EGFR Mutation Test v2.

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

To evaluate the potential interference of high necrotic tissue content in NSCLC FFPET specimens using the **cobas**® EGFR Mutation Test v2, 41 NSCLC FFPET specimens, including 18 mutant specimens (four with exon 19 deletion mutations, three with the L858R mutation, one with both the S768I and L858R mutations, two with the S768I and G719X mutations, two with the L861Q mutation, five with the G719X mutation, and one with the exon 20 insertion mutation) and 23 wild-type specimens, were evaluated. Percent necrosis, as identified by a pathologist, varied from 0-60% for mutant FFPET specimens and 5-85% for wild-type FFPET specimens.

Eighteen (18) mutant and 23 wild-type specimens with tumor content of 35-80% and 14-82% mutation were used in this study to assess the impact of the necrotic tissues. All observed results matched the expected results for all the specimens tested. Data supported that necrotic tissue content up to 85% in NSCLC FFPET specimens do not interfere with the call results for the **cobas**® EGFR Mutation Test v2.

An additional review of the data from the AURA2 study showed the percent necrosis present in specimens with the T790M mutation ranged from 0-70%, percent tumor ranged from 5-95% and the presence of necrosis did not appear to interfere with detection of the mutation. Up to 30% necrosis was observed in specimens that tested positive for Ex20Ins after the cut-off was revised in those specimens that were identified as positive.

7. Interference – Triglycerides or Hemoglobin

To evaluate the potential interference of triglycerides and hemoglobin on the performance of the **cobas**® EGFR Mutation Test v2, five conditions were tested for each of 13 NSCLC FFPET specimens across two studies:

- Hemoglobin (2 mg/mL)
- Buffer Control for Hemoglobin
- Triglycerides (37 mM)
- Buffer Control for Triglycerides
- Neat (No Substance)

Five 5-µm sections were obtained from each of the NSCLC EGFR FFPET specimens. Each section was deparaffinized and spiked with one of the five potential interfering materials in tissue pellet suspension prior to DNA extraction. The levels of triglycerides (37 mM) and hemoglobin (2 mg/mL) were equal to the levels recommended to be tested by CLSI guideline EP7-A2, Appendix D. Following deparaffinization and the spiking of potential interfering substances, genomic DNA was isolated from each of the spiked tissue specimens using the **cobas**® EGFR Mutation Test v2.

Seven mutant specimens with tumor content of approximately 13-41% mutation were used in this study to assess the impact of the interference at approximately 3-fold to 4-fold analytical sensitivity. All observed results matched the expected results at the levels of triglycerides and hemoglobin tested, indicating that triglycerides and hemoglobin do not interfere with the performance of the **cobas**® EGFR Mutation Test v2.

8. Interference – Drugs

To evaluate the potential interference of therapeutic drugs which may be present in NSCLC FFPET specimens that could be tested with the **cobas**® EGFR Mutation Test v2, 13 NSCLC FFPET specimens were tested with drugs (i.e., albuterol, ipratropium, fluticasone, ceftazidime, imipenem, cilastin, piperacillin, tazobactam, betadine, and lidocaine) and the solvents used to dissolve each drug. Five (5) µm sections were obtained from each NSCLC EGFR FFPET specimen. Each of the sections was deparaffinized and spiked with tested drugs or solvent in tissue pellet suspension prior to DNA extraction. The levels of potential interfering substance were equal to the levels recommended to be tested by CLSI guideline EP7-A2 or at 3x C_{max} value as recommended by the drug's package insert with the exception of betadine, which is a topical solution that was tested as 10 µL of a 10% (w/v) solution. Genomic DNA was isolated and tested from each of the spiked tissue specimens using the **cobas**® EGFR Mutation Test v2. Seven mutant specimens with tumor content of 15-40% mutation were used in this study to assess the impact of the interference. All observed results matched the expected

results for all conditions tested, indicating the tested drugs do not interfere with the performance of the **cobas**® EGFR Mutation Test v2.

9. Repeatability

Repeatability was demonstrated across two studies.

The first study included five NSCLC FFPET specimens with mutations for each of the four (one Ex20Ins mutation, one L858R and T790M dual mutation, and one S768I and G719X dual mutation) out of the seven mutations detected by the **cobas**® EGFR Mutation Test v2. Each sample was tested in duplicate by two operators using two different **cobas**® DNA Sample Preparation and **cobas**® EGFR Mutation Test kit reagent lot combinations across four days; the testing was split between two **cobas z** 480 analyzers. Six incorrect or invalid calls were observed in the study due to either contaminated replicates (n = 4) or the presence of an additional mutation at very low levels resulting in intermittent calls (n=2). Therefore, 154/160 calls were correct, demonstrating a repeatability rate of 96.25%.

A second study was conducted which included three EGFR mutant NSCLC FFPET specimens (one L861Q, one G719X, one exon 20 insertion), each representing a different mutation, and one EGFR wild-type FFPET specimen. The study was conducted as described above but was conducted across eight days due to several invalid runs over three days. The invalid runs were due to user error, internal control (IC) Ct value being above the IC Ct_{max} cut-off, and contamination of a negative control and a single sample replicate. Overall, 127/128 (99.2%) results were accurately identified by the assay after resolution of the invalid results.

10. Reproducibility

A reproducibility study was performed to assess the reproducibility of the **cobas**® EGFR Mutation Test v2 across three external testing sites with two operators per site, three reagent lots (two lots per site), and five non-consecutive testing days (per operator), with an 11-member panel of DNA samples extracted from FFPET sections of wild-type (WT) and mutant NSCLC specimens. The panel included specimens representing the G719X mutation, T790M mutation, S768I mutation, exon 20 insertion mutations, and L861Q mutation. Each mutation-positive specimen was represented at two concentrations, near the mutation's LoD and 2X LoD, prepared from genomic DNA (gDNA) blends (mutation positive with WT gDNA). Each sample at each concentration was run in duplicate. Of 91 runs, 90 (98.9%) were valid. A total of 1,980 tests were performed with 11 panel members tested in duplicate in 90 valid runs; all test results were valid. There were no Mutation Detected results in 180 valid tests of WT panel members, producing 100% agreement. Agreements were 100% for all mutant panel members. Results by overall agreement are presented in Table 8. The coefficient of variation (CV)

was <9.2% in all mutant panel members. For the external control, the overall CV was ≤1.3%. The CV was ≤0.6% between lots and ≤ 1.1% within-lot.

Table 8. Overall Agreement Estimates by Panel Member

Panel Member	Mutation	Number of Valid Tests	Agreement (N)	
			n	% (95% CI)[a]
1	Wild-Type	180	180	100 (98.0, 100.0)
2	G719X - LoD	180	180	100 (98.0, 100.0)
3	T790M - LoD	180	180	100 (98.0, 100.0)
4	S768I - LoD	180	180	100 (98.0, 100.0)
5	Ex20Ins – LoD[b]	180	166	92.2 (87.3, 95.7)
6	L861Q - LoD	180	180	100 (98.0, 100.0)
7	G719X - 2X LoD	180	180	100 (98.0, 100.0)
8	T790M - 2X LoD	180	180	100 (98.0, 100.0)
9	S768I - 2X LoD	180	180	100 (98.0, 100.0)
10	Ex20Ins - 2X LoD	180	180	100 (98.0, 100.0)
11	L861Q - 2X LoD	180	180	100 (98.0, 100.0)

Note: Results were in agreement when a mutant panel member had a valid result of MD for the target mutation or when a wild-type panel member had a valid result of NMD.
[a] 95% CI = 95% exact binomial confidence interval.
[b] The overall agreement was revised from 100% for Ex. 20 insertions at the LoD to 92.2% after the mutation cut-off was changed and the data re-analyzed.
CI = confidence interval; LoD = limit of detection; NMD = No Mutation Detected

The total CV % ranged from 2.2% to 9.1% across all panel members. Within each component, CV% ranged from 0.0% to 8.0% across all panel members. Within-run accounted for the major percentage of the variance (from 29.1% to 79.0%) for the mutation panel members. Percentage of total variance attributed to lot varied from 0.0% to 26.3%; attributed to site/instrument varied from 0.0% to 16.1%; attributed to day varied from 11.8% to 55.7%; and attributed to operator varied from 0.0% to 13.6% to across mutant panel members. The summary results are shown in Tables 9 and 10 below for the mutant positive panel members and for Ex20Ins after cut-off revision. Panel member 1 consisted of an EGFR wild-type specimen.

Table 9. Overall Mean, Standard Deviation, and %CV for CtR from Valid Results of Mutant Panel Members and Ct from Valid Results for Ex20Ins Panel Members

		Standard Deviation (SD) and Percent Coefficient of Variation (CV)											
		Lot		Site/Inst.		Operator		Day		Within-Run		Total	
Panel Member	Mean CtR (95% CI)	SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
2	8.41 (8.2, 8.6)	0.09	1.0	0.00	0.0	0.00	0.0	0.09	1.0	0.14	1.7	0.19	2.2
3	6.80 (6.6, 7.0)	0.00	0.0	0.03	0.4	0.07	1.0	0.15	2.2	0.14	2.0	0.22	3.2
4	3.21 (3.0, 3.4)	0.00	0.0	0.07	2.2	0.05	1.5	0.12	3.6	0.16	4.9	0.21	6.7
6	4.20 (4.0, 4.4)	0.10	2.4	0.06	1.4	0.04	0.9	0.09	2.1	0.12	2.9	0.19	4.6
7	7.46 (7.3, 7.6)	0.07	0.9	0.03	0.4	0.00	0.0	0.08	1.1	0.13	1.7	0.17	2.3

		Standard Deviation (SD) and Percent Coefficient of Variation (CV)											
		Lot		Site/Inst.		Operator		Day		Within-Run		Total	
Panel Member	Mean CtR (95% CI)	SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
8	5.78 (5.7, 5.9)	0.02	0.4	0.00	0.0	0.07	1.3	0.15	2.5	0.11	1.8	0.20	3.4
9	2.30 (2.1, 2.5)	0.00	0.0	0.06	2.7	0.00	0.0	0.07	3.1	0.19	8.0	0.21	9.1
11	3.39 (3.2, 3.6)	0.07	2.2	0.01	0.4	0.04	1.2	0.08	2.5	0.11	3.3	0.16	4.8
	Mean Ct (95% CI)												
5	32.41 (32, 32.9)	0.17	0.5	0.20	0.6	0.5	0.1	0.18	0.5	0.32	1.0	0.45	1.4
10	31.63 (31.3, 32)	0.16	0.5	0.06	0.2	0.09	0.3	0.19	0.6	0.26	0.8	0.37	1.2

Table 10. Total Precision, Standard Deviation and Percentage of Total Variance of CtR Attributed to Lot, Site/Instrument, Operator, Day, and Within-Run by Mutant Type Panel Members

Panel Member	N	Total SD	Percentage of Total Variance [CV(%)]					Total CV
			Lot	Site/Instrument	Operator	Day	Within-Run	
2	180	0.19	21.17 (1.02)	0.00 (0.00)	0.00 (0.00)	21.00 (1.01)	57.84 (1.68)	2.2
3	180	0.22	0.00 (0.00)	1.69 (0.42)	10.15 (1.02)	47.81 (2.22)	40.36 (2.04)	3.2
4	180	0.21	0.00 (0.00)	11.03 (2.22)	4.82 (1.47)	29.45 (3.63)	54.71 (4.95)	6.7
5	180	0.45	14.09 (0.53)	19.4 (0.62)	1.02 (0.14)	15.41 (0.55)	50.08 (0.99)	1.4
6	180	0.19	26.35 (2.38)	9.19 (1.40)	3.55 (0.87)	20.78 (2.11)	40.13 (2.93)	4.6
7	180	0.17	15.62 (0.90)	2.82 (0.38)	0.00 (0.00)	23.47 (1.10)	58.09 (1.74)	2.3
8	180	0.20	1.51 (0.42)	0.00 (0.00)	13.62 (1.26)	55.72 (2.54)	29.14 (1.84)	3.4
9	180	0.21	0.00 (0.00)	9.16 (2.74)	0.00 (0.00)	11.83 (3.11)	79.01 (8.05)	9.1
10	180	0.37	18.84 (0.51)	2.67 (0.19)	5.47 (0.27)	25.52 (0.59)	47.50 (0.81)	1.2
11	180	0.16	20.02 (2.16)	0.62 (0.38)	6.11 (1.19)	25.75 (2.45)	47.50 (3.33)	4.8

11. <u>Lot-to-Lot Reproducibility</u>

The **cobas**® EGFR Mutation Test v2 utilizes two separate kits: (1) The **cobas**® DNA Sample Preparation kit for isolation of DNA from NSCLC FFPET specimens, and (2) the **cobas**® EGFR Mutation Test v2 for the amplification and detection of the isolated DNA for EGFR mutation status. Over two studies eight NSCLC FFPET specimens were tested with nine combinations of 3 lots of the **cobas**® DNA Sample Preparation Kit and 3 lots of the **cobas**® EGFR Mutation Test v2 kit. The eight NSCLC FFPET specimens included two L858R and T790M dual mutant specimens; one S768I and G719X dual mutation specimen; one L861Q specimen; one exon 20 insertion specimen; one G719X specimen; one L861Q specimen; and two EGFR wild-type specimens. The percent tumor content in EGFR mutant specimens ranged from 14% to 22.7%. The observed results matched the expected results for eight of the nine lot combinations. One EGFR wild-type specimen replicate yielded a G719X result. There was insufficient eluate remaining in that replicate to perform repeat testing and confirm the result using NGS. NGS testing was performed on the remaining replicate which resulted in a NMD result. A root cause for the incorrect result was not identified; however, overall the results demonstrated that different lots of the two kits can be used interchangeably. The mean Ct and % CV values for each channel were summarized across lots and no trend in Ct values was observed.

12. Specimen Handling – Curl Versus Slide Equivalency

To evaluate the equivalence of using DNA extracted from 5-µm unmounted NSCLC FFPET sections (FFPET "curls") and DNA extracted from NSCLC FFPET sections mounted on slides (FFPET "slides"), specimens were tested across two studies. The specimens from both studies represented a total of 81 NSCLC FFPET specimens (45 wild-type and 36 mutant specimens). The mutant specimens included 13 exon 20 insertions; six G719X; four L861Q; one exon 19 deletion and S768I dual mutation; five T790M and L858R dual mutations; two T790M and exon 19 deletion dual mutation; one T790M and G719X dual mutation; three S768I and G719X dual mutations; and one S768I and L858R dual mutation.

Two sections were sliced from each NSCLC FFPET specimen; one section was mounted on a slide and the other "curl" section placed into a microfuge tube, and prepared according to directions. Both the slide and curl sections for each specimen were tested using one lot combination of the **cobas**® DNA Sample Preparation Kit and **cobas**® EGFR Mutation Test v2 kit.

Thirty-one (31) specimens were included in the first study and were comprised of 11 mutation-positive specimens and 20 wild-type specimens. The mutant-positive specimens consisted of five exon 20 insertions; one G719X; one exon 19 deletion and S768I dual mutation; one T790M and L858R dual mutation; two S768I and G719X dual mutation; and one S768I and L858R dual mutation specimen. Tumor content ranged from 25% to 80% for mutant specimens and from 1% to 85% for wild-type specimens. The results demonstrated 97% (30/31) agreement between unmounted FFPET curls and FFPET slides.

The second study included 50 NSCLC FFPET specimens (25 wild-type and 25 mutant specimens). The mutant specimens included five G719X specimens, one G719X and S768I specimen, eight exon 20 insertion specimens, one G719X and T790M specimen, four L861Q specimens, two exon 19 deletion and T790M specimens, and four L858R and T790M specimens. Two sections were sliced from each NSCLC FFPET specimen; one section was mounted on a slide and the other "curl" section placed into a microfuge tube, and prepared according to directions. Both the slide and curl sections for each specimen were tested using one lot combination of the **cobas**® DNA Sample Preparation Kit and **cobas**® EGFR Mutation Test v2 kit. Tumor content ranged from 8% to 95% for mutant specimens and from 1% to 90% for wild-type specimens. Two specimens demonstrated discordant results between the slides and curls with reporting the presence of an extra mutation in the slide specimen (one L768I and one Ex19Del). In both specimens, the discordant sample demonstrated an additional mutation than expected. Upon investigating the cause for the discordant results, one slide sample did not demonstrate the extra mutation result after the same eluate was

reamplified. For the second specimen, when the DNA eluates were reamplifed the slide demonstrated another mutation result in addition to the previous extra mutation. After DNA was isolated from fresh samples the expected results were observed. While contamination was deemed the reason for the discordances, the study demonstrated a 98% (49/50) agreement between the FFPET curls and FFPET slides specimens.
The results from the curl vs. slide equivalency study were reanalyzed after the exon 20 insertion mutation was changed using the software containing the new algorithm. Only one specimen was impacted in which one sample, a exon 20 insertion mutation curl, was affected where the result changed from mutation detected to no mutation detected. An investigation of the data showed that the Ct of the exon 20 insertion channel was 34.6, which is beyond the newly established cut-off of 33.1.

13. Specimen Handling – Macro-dissection

The accuracy of samples following macro-dissection was evaluated with AURA2 clinical trial specimens that had less than 10% tumor content. Refer to Section 4 on "Analytical Sensitivity - Minimum Tumor Content" above for more details.

14. Guard banding

The objective of the guard banding studies was to establish the robustness of the PCR conditions for the **cobas**® EGFR Mutation Test v2. Guard banding studies were performed on the **cobas**® EGFR Mutation Test v2 Thermal Cycling Profile and Proteinase K concentration (for DNA isolation procedure).

Six FFPET NSCLC specimens consisting of one T790M and G719X dual mutation; one S768I and G719X dual mutation; one L861Q mutation, one G719X mutation; one exon 20 insertion mutation; and one wild-type specimen were used. At least ten 5-µm sections were obtained from each of the specimens and processed to isolate DNA using a single lot of the **cobas**® DNA Specimen Preparation Kit according to the **cobas**® EGFR Mutation Test v2 Instructions for Use. After processing, all replicates of each specimen were combined to make a pool of extracted DNA. Three replicates of each specimen pool were tested for each condition using a single reagent lot of the **cobas**® EGFR Mutation Test v2.

a. Thermal Cycling Profile

The thermal cycling profile was guard banded by varying both the denaturation and annealing temperatures by ± 1°C. All replicates of each specimen pool produced their expected results. For each specimen tested, the average Ct for each guard band condition was within 1 Ct of the average Ct of the control condition. The Ct difference from control condition for all specimens combined ranged from -0.19 to 0.52. The results showed that the

cobas® EGFR Mutation Test v2 is able to tolerate variations of ± 1°C of the thermal cycling profile in denaturation and annealing temperatures.

b. Proteinase K (PK)

During sample preparation, the NSCLC FFPET specimen is lysed by incubation at an elevated temperature with a proteinase and chaotropic lysis/binding buffer that release nucleic acids and protect the released genomic DNA from DNases. For each specimen processed by the **cobas**® DNA Sample Preparation kit, 70 µL of PK and 180 µL of DNA Tissue Lysis Buffer are added. The mixture is first incubated at 56°C for 60 minutes and then at 90°C for 60 minutes.

PK was guard banded by varying the PK volume (±20%), the first incubation temperature (±2°C), and the first incubation time (±25%) using the **cobas**® DNA Sample Preparation kit. Following the **cobas**® EGFR Mutation Test v2 Instructions for Use, three operators each processed one replicate of three NSCLC FFPET specimens for nine PK conditions. A single reagent lot was used in this study and a total of three runs were performed. All replicates of each specimen produced their expected results. For each PK condition tested, the average Ct for each guard band condition was within 1 Ct of the average Ct of the control condition. The Ct difference from control condition for all specimens combined ranged from -0.26 to 0.32. The results showed that the **cobas**® EGFR Mutation Test v2 is able to tolerate differences of ±20% for PK volume, ±2°C for the first incubation temperature, and ±25% for the first incubation time.

15. Stability Studies

a. Clinical Specimen, Slide-Mounted and Slide Curl

To demonstrate the stability of sections from NSCLC FFPET clinical specimens when mounted on a slide ("slide") or when not mounted on a slide ("curl"), three NSCLC FFPET specimens, representing three EGFR mutations and one EGFR wild-type specimens were evaluated. The three EGFR mutations were represented by two specimens and consisted of one T790M and L858R dual mutation (17.48% and 16.38% mutation) and one L861Q mutation (14% mutation) specimens. Ten 5-µm sections were obtained from each of the three NSCLC FFPET specimens, mounted or not mounted on slides (one section per slide). The slides and curls were stored at 2-8°C and 32°C and tested after 0 and 61 days. At each time point, two slides and two curls for each specimen and storage temperature were processed using one lot of the reagent. Results from the three specimens matched the expected results (based on sequencing results) at both time points. These results indicated that sections stored as slides or curls are stable at least 61 days at 2-8°C and 32°C storage for testing with the **cobas**® EGFR Mutation Test v2.

b. Extracted DNA From FFPET Specimens

Stability of DNA extracted from NSCLC FFPET specimens was evaluated using one EGFR wild-type specimen, one T790M and L858R dual mutant specimen (31.73% and 28.69% mutation), and one L861Q mutant specimen (16.16% mutation). DNA extracts obtained from each NSCLC FFPET specimen was tested as follows:

1. after storage at -20°C for 15, 31 or 61 days;
2. after storage at 2 to 8°C for 0, 15, 31, or 61 days;
3. after storage at 32°C for 2, 4, or 8 days;
4. after one, two, or three freeze-thaw cycles consisting of storage at -20°C, thawing, re- freezing and sampling at the specified -20°C time points.

After storage at -20°C, 2 to 8°C, or 32°C, results from the specimens matched the expected results (based on sequencing results) at each of the time points, indicating that DNA extracted from NSCLC FFPET specimens using the **cobas**® DNA Specimen Preparation Kit is stable for at least 61 days when stored at 2-8°C or -20°C. The results also indicated that the extracted DNA is stable for at least 8 days when stored at 32°C, and up to three freeze-thaw cycles when stored at -20°C. No trend in Ct values was detected over these testing conditions.

c. Working (Activated) Master Mix

To evaluate the stability of **cobas**® EGFR Mutation Test v2 Working (activated) Master Mixes stored at 2-8°C and 32°C for up to 125 minutes, three NSCLC FFPET specimens (two EGFR mutation-positive and one EGFR wild-type), were tested using 1 lot of reagent. The three NSCLC FFPET specimens were the same as those used in the extracted DNA stability study. Working (activated) Master Mixes were prepared by adding magnesium acetate to each EGFR Master Mix, and then stored at 2-8°C for 0, 35, 65, and 125 minutes, and at 32°C for 0, 35, 65, and 125 minutes. Ten 5-µm sections were obtained from each of the NSCLC FFPET specimens. Each of the sections was processed to isolate DNA using the **cobas**® DNA Sample Preparation Kit. DNA obtained from a single specimen was combined, resulting in a pool of extracted DNA for each specimen. After the indicated storage time, duplicate samples of DNA extracts from each of the NSCLC specimens were combined with the Working Master Mixes and amplified and detected using the **cobas**® EGFR Mutation Test v2. All valid specimen results matched the expected results at each of the time points, indicating that **cobas**® EGFR Mutation Test v2 working (activated) Master Mixes are stable for at least two hours when stored at 2-8°C or 32°C

d. Extracted DNA Plus Working (Activated) Master Mix

To evaluate the stability of the combination of extracted DNA from NSCLC

FFPET specimens and **cobas**® EGFR Mutation Test v2 Working (activated) Master Mixes, three NSCLC FFPET specimens were tested using 1 lot of reagent. The three NSCLC FFPET specimens were the same as those used in the extracted DNA stability study. DNA was extracted from each of the NSCLC FFPET specimens using the **cobas**® DNA Specimen Preparation Kit, combined with Working (activated) Master Mixes, and stored at 2-8°C for 0, 35, 65, and 125 minutes, and at 32°C for 0, 35, 65, and 125 minutes. After the indicated storage time, the combined DNA extract/Working Master Mixes were amplified and detected using the **cobas**® EGFR Mutation Test v2. Results from the specimens matched the expected result (based on sequencing results) at each of the time points after storage at 2-8°C or 32°C. No trend in Ct values was detected over these testing conditions. These results indicated that DNA extracted from NSCLC FFPET specimens using the **cobas**® DNA Specimen Preparation Kit combined with Working (activated) the **cobas**® EGFR Mutation Test v2 Working Master Mixes is stable for up to 125 minutes when stored at 2-8°C or 32°C prior to the start of amplification.

e. Open Vial, cobas® EGFR Mutation Test v2 Kit Reagents

To determine the open vial stability of the reagents in the **cobas**® EGFR Mutation Test v2 kit, two kits were used to test three NSCLC FFPET specimens. The three NSCLC FFPET specimens were the same as those used in the extracted DNA stability study. One kit was tested on Days 0, 15, 21, and 31 and the second kit was tested on Days 0, 45, 61, and 91. The study was conducted to demonstrate the open vial stability of the **cobas**® EGFR Mutation Test v2 kit reagents up to 30 days and 90 days with up to 4 uses per kit. Ten 5-µm sections were obtained from each NSCLC FFPET specimen. All results from the six specimens matched the expected result when the **cobas**® EGFR Mutation Test v2 kit reagents were used 4 times over 91 days when stored at 2-8°C between uses. No trend in Ct values was detected over these open-vial storage periods. The results indicate that the open vial stability of the **cobas**® EGFR Mutation Test v2 kit reagents is at least 91 days.

f. cobas® EGFR Mutation Test v2

Stability of the **cobas**® EGFR Mutation Test v2 kit and its components were assessed at various time points after storage at 2-8°C (real-time) in upright and inverted orientations using three lots of the kit reagents. The test samples were the EGFR Mutant Control (EGFR MC) and the DNA Specimen Diluent (DNA SD), which serve as a positive control and negative control, respectively, in the **cobas**® EGFR Mutation Test v2. Eight replicates of the EGFR MC and DNA SD were tested at each storage condition. Stability was evaluated by performing functional testing at 4 weeks, 8 weeks, 3 months, 6 months, 9 months, 12 months, 13 months, 18 months, 19 months, 24 months, and 25 months. For a given storage condition at any time point to pass, the eight replicates of the EGFR MC and DNA SD must be within the pre-

specified ranges of Ct, which are identical to the Ct values for assessing validity of a run and assigning mutation status. To date, testing has been completed and met the acceptance criteria through 18 months storage at 2-8°C for all three lots of the kit reagents. Current real-time stability data support stability of the **cobas**® EGFR Mutation Test v2 at 2-8°C for 18 months. Real-time stability studies are ongoing to support the 24 months expiry.

g. cobas® DNA Sample Preparation Kit

Stability of the **cobas**® DNA Sample Preparation Kit, including open-vial stability, was demonstrated in P110020 approval of the **cobas**® 4800 BRAF V600 Mutation Test.

h. Shipping

Shipping stability was established under the **cobas**® EGFR Mutation Test (v1) and is not expected to be different for the **cobas**® EGFR Mutation Test v2 kit.

16. Antimicrobial Effectiveness Testing (AET)

To assess the effectiveness of the preservatives in the **cobas**® EGFR Mutation Test v2 kit components, a total of five microorganisms (*Staphylococcus aureus, Candida albicans, Aspergillus niger, Pseudomonas aeruginosa and Escherichia coli*) were individually spiked into each of the kit components on Day 0, and the colony forming units (CFU) were counted in log10 on Day 14 and Day 28. There is no impact of microbial contamination on the functional performance of the **cobas**® EGFR Mutation Test v2 when stored for up to 28 days at 25ºC.

B. Animal Studies

None.

C. Additional Studies

None.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The AURA2 study (D5160C00002) was a global phase II, open-label, single-arm study conducted by AstraZeneca Pharmaceuticals LP to assess the safety and efficacy of osimertinib as a second or ≥ third-line therapy in patients with locally advanced or metastatic NSCLC (Stage IIIB-IV), who had progressed following prior therapy with an approved EGFR TKI agent and whose tumor specimens demonstrated a T790M positive result. The AURA2 study began with the first patient dosed on June 13, 2014 and completed, with the last patient dosed on October 27, 2014. The AURA2 study was one

of two efficacy studies submitted to support the accelerated approval of osimertinib under NDA 208065.

In the AURA2 study, patients' EGFR mutation status was determined at one of three central laboratories using a clinical trial assay (CTA) which was an investigational use only (IUO) version of the **cobas**® EGFR Mutation Test (v1), which identified mutations masked in the previously approved version of the kit, in order to identify those patients with a T790M mutation. All specimens were later retested using the **cobas**® EGFR Mutation Test v2. To establish the clinical validity of the **cobas**® EGFR Mutation Test v2, objective response rate (ORR) according to RECIST 1.1 by blinded independent central review (BICR) was estimated for all patients enrolled for enrollment who were determined to be T790M positive by both versions of the **cobas**® EGFR Mutation Test. A summary of the clinical study is presented below. Retrospective testing with the **cobas**® EGFR Mutation Test v2 was conducted under protocol COB-EGFR-341 for bridging to the CTA, which was approved under IDE G140034 on April 3, 2014. The study was completed with the last sample tested on March 25, 2015.

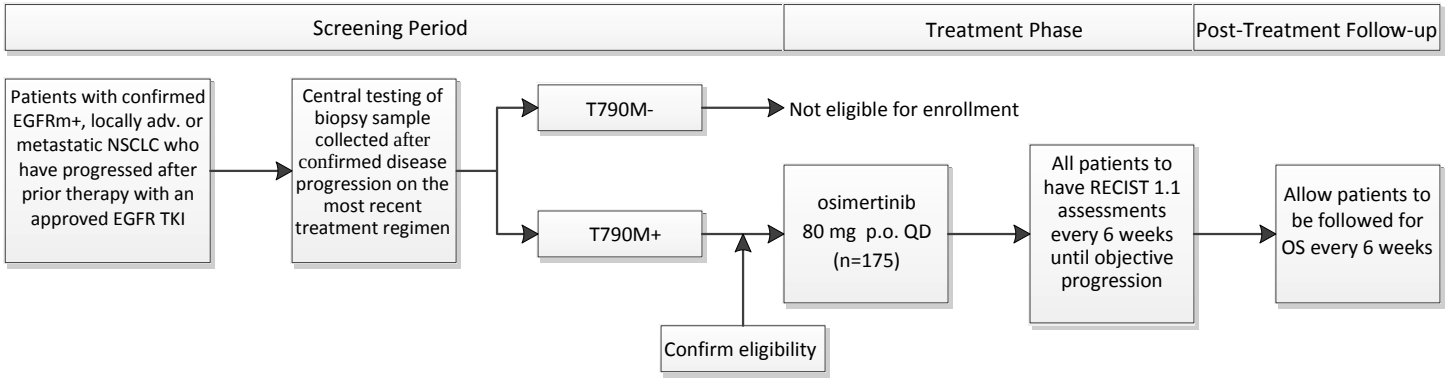
A. Study Design

The AURA2 study was a global phase II, open-label, single-arm study, assessing the safety and efficacy of osimertinib as a second or greater-line therapy for treatment in patients with locally advanced or metastatic NSCLC (Stage IIIB-IV), who had progressed following prior therapy with an approved EGFR TKI agent and whose tumor specimens demonstrated a T790M positive result. The study was conducted at 44 centers in 8 countries. A biopsy was required for central testing of EGFR T790M mutation status following confirmed disease progression on the most recent treatment regimen. The EGFR T790M mutation status of the patient's tumor was prospectively determined using the **cobas**® EGFR Mutation Test (v1) by one of three designated central laboratories, located in the US, Belgium, and Singapore. The study consisted of two cohorts:

1. Second-line therapy cohort: patients whose disease had progressed following first line therapy with an EGFR TKI agent but who had not received further treatment.
2. ≥ third-line therapy cohort: patients whose disease had progressed following treatment with both an EGFR TKI and a platinum-based doublet chemotherapy (patients may have also received additional lines of treatment).

A graphical representation of the study is shown in Figure 1 below:

Figure 1. Study Flow Chart.



[a] A total of approximately 175 patients were planned to be dosed with osimertinib. Patient enrollment consisted of 2 cohorts: 1) approximately 50 patients planned with EGFR T790M mutation whose disease had progressed following first-line therapy with 1 EGFR TKI agent but who had not received further treatment, and 2) approximately 125 patients planned with EGFR T790M mutation-positive NSCLC whose disease had progressed following treatment with both EGFR TKI and a platinum-based doublet chemotherapy (patients may have also received additional lines of treatment).

[b] Patients were considered enrolled at the time osimertinib treatment was started. Patients continued to receive osimertinib treatment until objective disease progression (according to RECIST 1.1) or for as long they were receiving clinical benefit in the opinion of the investigator. Patients who discontinued study treatment for reasons other than disease progression had to continue tumor assessents per the protocol schedule until progression.

1. Clinical Inclusion and Exclusion Criteria

   Patients enrolled in the AURA2 study continued on treatment with osimertinib until RECIST 1.1-defined progression or until a treatment discontinuation criterion was met. There was no maximum duration of treatment as patients could continue to receive osimertinib beyond RECIST 1.1-defined progression as long as they continued to show clinical benefit, as judged by the investigator. Prospective patients were required to meet all inclusion and exclusion criteria listed below.

   Inclusion Criteria

   1. Provision of signed and dated, written informed consent prior to any study-specific procedures, sampling and analyses. If a patient declined to participate in any voluntary exploratory research and/or genetic component of the study, there was no penalty or loss of benefit to the patient and he or she was not to be excluded from other aspects of the study.
   2. Male or female, aged at least 18 years. Patients from Japan aged at least 20 years.
   3. Histological or cytological confirmation diagnosis of NSCLC.
   4. Locally advanced or metastatic NSCLC, not amenable to curative surgery or radiotherapy.

5. Radiological documentation of disease progression: following first line EGFR TKI treatment but who had not received further treatment OR following prior therapy with an EGFR TKI and a platinum-based doublet chemotherapy. Patients may have also received additional lines of treatment. All patients had to have documented radiological progression on the last treatment administered prior to enrolling in the study.
6. Confirmation that the tumor harbored an EGFR mutation known to be associated with EGFR TKI sensitivity (including G719X, Ex. 19del, L858R, L861Q).
7. Patients had to have central confirmation of tumor EGFR T790M mutation positive status from a biopsy sample taken after confirmation of disease progression on the most recent treatment regimen.
8. World Health Organization (WHO) performance status 0 to1 with no deterioration over the previous 2 weeks and a minimum life expectancy of 12 weeks.
9. At least 1 lesion, not previously irradiated and not chosen for biopsy during the study screening period, that could be accurately measured at baseline as ≥10 mm in the longest diameter (except lymph nodes which had to have short axis ≥15 mm) with computerized tomography (CT) or magnetic resonance imaging that was suitable for accurate repeated measurements.
10. Females were to be using adequate contraceptive measures, were not to be breastfeeding and had to have a negative pregnancy test prior to the start of dosing if of childbearing potential, or had to have evidence of non-childbearing potential by fulfilling one of the following criteria at screening:
    - Post-menopausal defined as aged more than 50 years and amenorrheic for at least 12 months following cessation of all exogenous hormonal treatments.
    - Women under 50 years old were considered post-menopausal if they had been amenorrheic for 12 months or more following cessation of exogenous hormonal treatments and with luteinizing hormone and follicle-stimulating hormone levels in the post-menopausal range for the institution.
    - Documentation of irreversible surgical sterilization by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation.
11. Male patients were to be willing to use barrier contraception (i.e., condoms)
12. For inclusion in the optional genetics research, study patients had to provide informed consent for genetic research.

<u>Exclusion criteria</u>

1. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study center).
2. Treatment with any of the following:
    - Treatment with an EGFR TKI (e.g., erlotinib, gefitinib or afatinib) within 8 days or approximately 5 half-lives, whichever was the longer, of the first dose of study treatment. (If sufficient washout time had not occurred due to the schedule or PK properties, an alternative appropriate washout time

based on known duration and time to reversibility of drug-related AEs could be agreed upon by AstraZeneca and the investigator.)

- Any cytotoxic chemotherapy, investigational agent or other anti-cancer drugs from a previous treatment regimen or clinical study within 14 days of the first dose of study treatment.
- Prior treatment with osimertinib or a third generation EGFR TKI (e.g., CO-1686).
- Major surgery (excluding placement of vascular access) within 4 weeks of the first dose of study treatment.
- Radiotherapy treatment to more than 30% of the bone marrow or with a wide field of radiation within 4 weeks of the first dose of study treatment.
- Patients currently receiving (or unable to stop use at least 1 week prior to receiving the first dose of study treatment) medications or herbal supplements known to be potent inhibitors or inducers of cytochrome P450 3A4 (CYP3A4) (see Appendix F of the CSP).

3. Any unresolved toxicities from prior therapy greater than CTCAE grade 1 at the time of starting study treatment with the exception of alopecia and grade 2, prior platinum therapy-related neuropath.
4. Spinal cord compression or brain metastases unless asymptomatic, stable and not requiring steroids for at least 4 weeks prior to the start of study treatment.
5. Any evidence of severe or uncontrolled systemic diseases, including uncontrolled hypertension and active bleeding diatheses which, in the investigator's opinion, made it undesirable for the patient to participate in the study or which would jeopardize compliance with the protocol, or active infection including hepatitis B, hepatitis C and human immunodeficiency virus. Screening for chronic conditions was not required.
6. Refractory nausea and vomiting, chronic gastrointestinal diseases, inability to swallow the formulated product or previous significant bowel resection that would preclude adequate absorption of osimertinib.
7. Any of specified cardiac criteria related to QTc, arrhythmia, or abnormalities in in rhythm, conduction or morphology of resting ECG (e.g., complete left bundle-branch block, second- or third-degree heart block, partial response (PR) interval).
8. Past medical history of interstitial lung disease (ILD), drug-induced ILD, radiation pneumonitis that required steroid treatment, or any evidence of clinically active ILD.
9. Inadequate bone marrow reserve or organ function, as demonstrated by specified laboratory values regarding: absolute neutrophil and platelet count, and levels of hemoglobin, ALT, AST, total bilirubin, and creatinine.
10. History of hypersensitivity to active or inactive excipients of osimertinib or drugs with a similar chemical structure or class to osimertinib.
11. Women who were breastfeeding.
12. Judgment by the investigator that the patient should not participate in the study if the patient was unlikely to comply with study procedures, restrictions and requirements.

2. Follow-up Schedule

All patients were followed every six weeks from the date of first dose for RESIST 1.1 assessments until objective progression and every six weeks for overall survival assessment. Adverse events were assessed at treatment and all follow-up visits.

3. Clinical Endpoints

The AURA2 study's primary efficacy endpoint variable was the objective response rate (ORR) according to RECIST 1.1 by blinded independent central review (BICR) using the Full Analysis Set (FAS). FAS was defined as all T790M+ patients by the **cobas**® EGFR Test (v1) who received at least 1 dose of osimertinib. The ORR was defined as the number (%) of patients with at least 1 visit response of complete response (CR) or partial response (PR) that was confirmed at least 4 weeks later (i.e., a best objective response [BOR] of CR or PR).

Secondary objectives were to:

- Further assess the efficacy of osimertinib in terms of duration of response (DoR), disease control rate (DCR), tumour shrinkage, progression-free survival (PFS) and OS.
- Assess the safety and tolerability profile of osimertinib.
- Investigate the effect of AZD9291 on QT interval corrected for heart rate (QTc) interval after oral dosing to NSCLC patients.
- Assess the impact of osimertinib on patients' disease-related symptoms and health-related quality of life (HRQoL).
- Characterize the pharmacokinetics of osimertinib and its metabolites (AZ5104 and AZ7550).

4. Bridging Study:

A total of 472 patients who had progressed following an EGFR TKI were screened for enrollment into the AURA2 study. Fifty-five of the 472 patients screened did not meet eligibility criteria for pathology assessment, and 383 had successful pathology assessment and were eligible for **cobas**® EGFR Mutation Test (v1) testing. Of these, 233 test results were T790M mutation positive (60.8%), 140 test results were T790M negative (36.6%), and 10 test results were invalid (2.6% invalid rate). Of the 233 T790M mutation positive patients, 210 received osimertinib. All specimens with successful pathology assessment (n = 383) were tested with the **cobas**® EGFR Mutation Test v2. Of these, 231 test results were T790M mutation positive (60.3%), 139 test results were T790M negative (36.3%), and 13 test results were invalid (3.4% invalid rate). Of 383 specimens tested with NGS, 261 were T790M mutation positive (68.2%), 118 were T790M negative (30.8%), and 4 were invalid (1.0%).

Agreement was determined between the two **cobas**® EGFR Mutation Test versions which is shown in Table 11. Additionally a three-way comparison

table (Table 12) shows the results between the two **cobas**® EGFR Mutation Test versions and the NGS reference method.

Table 11. cobas® EGFR Mutation Test v1 vs. v2 Using AURA2 Specimens

		cobas® EGFR Mutation Test v1 (CTA)			
		Exon T790M Deletion			
		MD	NMD	Invalid	Total
cobas® EGFR Mutation Test v2 Result	MD	225	5	1	231
	NMD	8	131	0	139
	Invalid	0	4	9	13
	Total	233	140	10	383
Without Invalid Results	PPA (95% CI)	225/233 = 96.6% (93.4%, 98.3%)			
	NPA (95% CI)	131/136 = 96.3% (91.7%, 98.4%)			
	OPA (95% CI)	(225+131)/369 = 96.5% (94.1%, 97.9%)			
With Invalid Result	PPA (95% CI)	225/242 = 93% (89%, 95.6%)			
	NPA (95% CI)	131/150 = 87.3% (81.1%, 91.7%)			
	OPA (95% CI)	(225+131)/383 = 93% (89.9%, 95.1%)			

Note: Estimates with invalid results assume that the results invalid by both methods are discordant with the reference method (worst case scenario)

Table 12. Three-way Summary of Results by cobas® EGFR Test v1, v2, and NGS

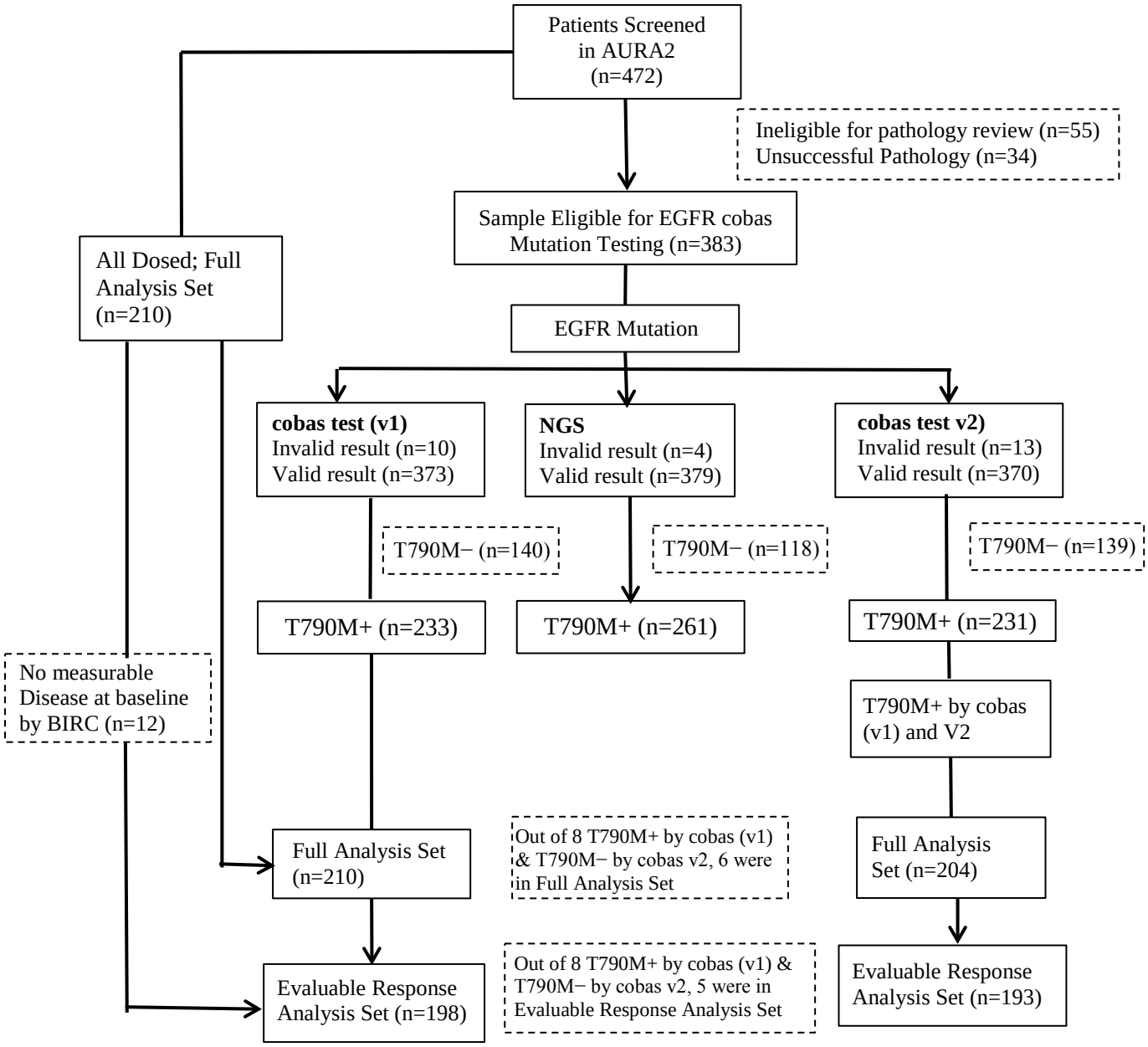
cobas® EGFR Mutation Test Result (v1)[1]	**cobas**® EGFR Mutation Test Result (v2)[1]	NGS Result[1]			
		Mutation Detected	No Mutation Detected	Invalid	Total
		N = 261	N = 118	N = 4	N = 383
MD (n=233)	MD (n=225)	222	1	2	225
	NMD (n=8)	7	1	0	8
	Invalid (n=0)	0	0	0	0
NMD (n=140)	MD (n=5)	3	2	0	5
	NMD (n=131)	23	108	0	131
	Invalid (n=4)	1	3	0	4
Invalid (n=10)	MD (n=1)	1	0	0	1
	NMD (n=0)	0	0	0	0
	Invalid (n=9)	4	3	2	9

[1]Mutation Detected indicates the presence of EGFR T790M, as identified by the testing method. No Mutation Detected indicates the absence of the EGFR T790M as identified by the testing method.
NGS = Next generation sequencing.

B. Accountability of the PMA Cohort

The disposition of patients/specimens and test results are described in Figure 2. Briefly, of the 472 patients screened, 383 patients were eligible for testing with the **cobas**® EGFR Mutation Test (v1). Among the 383 patients, 233 T790M+ NSCLC patients were recruited for AURA2 to use Tagrisso® (osimertinib). The Full Analysis Set (FAS) was defined as all T790M+ patients by the **cobas**® EGFR Mutation Test (v1) who received at least 1 dose of osimertinib (n = 210 patients).

Figure 2. Patients/Specimens and Test Results Disposition



AURA2 = AstraZeneca, Inc. Study D5160C00002; cobas test (v1) = cobas EGFR Mutation Test (v1); cobas v2 = cobas EGFR Mutation Test v2; BICR = blinded independent central review; EGFR = epidermal growth factor receptor; NGS = next generation sequencing.

C. Study Population Demographics and Baseline Parameters

The median age of the FAS was 64 years, included nearly twice as many females as males, and the majority of patients had metastatic disease. Justification for the acceptance of foreign data was included in the NDA 208065 submission. A summary

of patient demographic information and disease characteristics are shown in Tables 13 and 14, respectively.

Table 13. Patient Demographics (AURA2)

Demographic Characteristics		**Second-line**	**≥ Third-line**	**Total**
	N	68	142	210
Age (years)	Mean	64.0	62.4	62.9
	SD	11.76	10.48	10.91
	Median	64.5	63.5	64.0
	Min	36	35	35
	Max	88	84	88
Age group (years) n (%)	<50	5 (7.4)	15 (10.6)	20 (9.5)
	≥50 to <65	29 (42.6)	59 (41.5)	88 (41.9)
	≥65 to <75	20 (29.4)	49 (34.5)	69 (32.9)
	≥75	14 (20.6)	19 (13.4)	33 (15.7)
Sex n (%)	Male	24 (35.3)	40 (28.2)	64 (30.5)
	Female	44 (64.7)	102 (71.8)	146 (69.5)
Race n (%)[a]	White	26 (38.2)	46 (32.4)	72 (34.3)
	Black or African American	0	3 (2.1)	3 (1.4)
	Asian	39 (57.4)	93 (65.5)	132 (62.9)
	Native Hawaiian or other Pacific Islander	1 (1.5)	0	1 (0.5)
	Other	2 (2.9)	0	2 (1.0)
Ethnic group, n (%)[b, c]	Hispanic or Latino	2 (3.1)	3 (2.2)	5 (2.5)
	Asian (other than Chinese and Japanese)	17 (26.2)	18 (12.9)	35 (17.2)
	Chinese	12 (18.5)	39 (28.1)	51 (25.0)
	Japanese	10 (15.4)	36 (25.9)	46 (22.5)
	Other	24 (36.9)	43 (30.9)	67 (32.8)

[a] The category of "Other" is as collected on the eCRF; any race data missing on eCRFs was not reported as a category in summaries of RACE data.

[b] Caucasian ethnicity is not presented as it was not offered as a category in the eCRF.

[c] Six patients from the United States did not report an "ethnic population" for ethnicity summaries reported in this table (n=204/210); all 6 patients reported themselves as "non-Hispanic or Latino" and all also reported race as "white" in the eCRF.

Abbreviation: eCRF, electronic case report form. Source: CSR.

Table 14. Disease Characteristics (AURA2)

	Second-line (N= 68)	**≥ Third-line (N = 142)**	**Total (N = 210)**
EGFR mutations by **cobas**® central test[a]			
T790M	68 (100)	140 (98.6)	208 (99.0)
Ex19Del	45 (66.2)	92 (64.8)	137 (65.2)
L858R	20 (29.4)	47 (33.1)	67 (31.9)
G719X	2 (2.9)	2 (1.4)	4 (1.9)
S768I	1 (1.5)	2 (1.4)	3 (1.4)

	Second-line (N= 68)	≥ Third-line (N = 142)	Total (N = 210)
Ex20Ins	0	1 (0.7)	1 (0.5)
T790M only	1 (1.5)	0	1 (1.5)
Overall disease classification			
Metastatic[b]	64 (94.1)	134 (94.4)	198 (94.3)
Locally advanced only[c]	4 (5.9)	8 (5.6)	12 (5.7)
WHO performance status			
0 (normal activity)	29 (42.6)	54 (38.0)	83 (39.5)
1 (restricted activity)	39 (57.4)	88 (62.0)	127 (60.5)
Baseline target lesion size, (mm)			
N	62	136	198
Mean	52.6	63.3	59.9
SD	37.35	41.56	40.50
Median	44.4	55.7	50.5
Minimum	10	12	10
Maximum	208	218	218
Baseline target lesion size category (mm), n (%)			
<40	26 (38.2)	40 (28.2)	66 (31.4)
40 to 79	23 (33.8)	67 (47.2)	90 (42.9)
80 to 119	10 (14.7)	17 (12.0)	27 (12.9)
≥120	3 (4.4)	12 (8.5)	15 (7.1)
Brain metastases[d]	23 (33.8)	65 (45.8)	88 (41.9)
Visceral metastases[e]	53 (77.9)	115 (81.0)	168 (80.0)

[a] EGFR mutation identified by the central **cobas**® EGFR Mutation Test v1 (by biopsy taken after confirmation of disease progression on the most recent treatment regimen).

[b] Metastatic disease (patient had any metastatic site of disease).

[c] Locally advanced (patient had only locally advanced sites of disease).

[d] Brain metastases (patients with metastatic site of brain and/or those that reported radiotherapy in anatomical locations unequivocally in the brain and/or those that reported surgical excision of tumor from anatomical locations unequivocally in the brain).

[e] Visceral metastases (patients in whom the metastatic or locally advanced site was "Brain" or "Hepatic", those where the metastatic site was "Lymph nodes" and/or those that had specified 'other sites' such as stomach, spleen, peritoneum, ascites, renal or adrenal).

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; WHO, World Health Organization

Prevalence information of different EGFR mutations as well as T790M co-mutations identified from the AURA2 study by the **cobas**® EGFR Mutation Test v2 are shown in the Table 15.

Table 15. Prevalence of Different EGFR Mutations and Co-Mutations with T790M by cobas® EGFR Mutation Test v2 in AURA 2

		Co-Mutation with T790M	
Mutation	**N (%)**	**Co-Mutation**	**N (%)**
G719X	8 (2.27%)		

		Co-Mutation with T790M	
Mutation	**N (%)**	**Co-Mutation**	**N (%)**
Ex19Del	56 (15.86%)		
Ex19Del; S768I; T790M	1 (0.28%)	Ex. 19del	147 (63.64%)
Ex19Del; T790M	144 (40.79%)		
Ex19Del; T790M; Ex20Ins	*2 (0.57%)*		
L858R	54 (15.30%)		
L858R; G719X	1 (0.28%)		
L858R; T790M	74 (20.96%)	L858R	77 (33.33%)
L858R; T790M; Ex20Ins	*2 (0.57%)*		
L858R; T790M; S768I	1 (0.28%)		
Ex. 19del; L858R; T790M	1 (0.28%)	Ex. 19del and L858R	1 (0.43%)
S768I	2 (0.57%)		
S768I; G719X	1 (0.28%)		
S768I; T790M; G719X	1 (0.28%)	S768I/G719X/L861Q	4 (1.73%)
T790M; G719X	2 (0.57%)		
T790M; L861Q; G719X	1 (0.28%)		
T790M	2 (0.57%)	T790M only	2 (0.87%)
Total	353		231

Italicized text indicates change in the number of Ex20Ins from the original 12 after cut-off recalculation.

D. Safety and Effectiveness Results

1. Safety Results

   The safety with respect to treatment with Tagrisso® (osimertinib) is not addressed in details in this SSED for the **cobas**® EGFR Mutation Test v2. However adverse events related to Tagrisso® (osimertinib) are summarized below. Please see section 6 of the Tagrisso® (osimertinib) drug label for additional information. At the initial data cut-off date (DCO) of January 9, 2015 the majority of patients [87.1% (183/210) of patients] continued to receive treatment. At the time of the 90-day safety update (May 1, 2015), no patient had been exposed to osimertinib for longer than 12 months. Adverse reactions (ARs) were reported in 95.2% (200/210) of patients in the study and a total of 79.0% (166/210) of patients had ARs considered by the investigator to be possibly causally related to osimertinib, with the majority of AEs being CTCAE grade 1 or 2. Less than 20% were reported to be grade 3 or above. Patients who experienced a CTCAE grade 3 and/or unacceptable toxicity of any grade that was not attributable to their disease or disease-related processes under investigation and where the investigator considered the AR to be specifically associated with the study treatment, their dosing was to be interrupted. For patients whose AR did not resolve within the three weeks specified by the study protocol or who exhibited corneal ulcerations were permanently withdrawn from the study. In a later update, interstitial lung disease (ILD) was added as a reason for permanent withdrawal.

The most frequent treatment-emergent ARs on osimertinib were diarrhea, rash, dry skin, and nail toxicity. The most frequent fatal ARs were pneumonitis/ILD and pneumonia, which led to the deaths of four patients while on study. Pneumonia, pulmonary embolism, pneumonitis and abdominal pain were the most common non-fatal serious adverse event occurring in patients. Refer to the drug label for more information.

2. Effectiveness Results

An open label, single arm Phase II study, AURA2, was performed to investigate the efficacy of osimertinib by assessment of objective response rate (ORR) by Blinded Independent Central Review (BICR) in patients with a confirmed diagnosis of EGFR mutation positive metastatic NSCLC who had progressed following prior therapy with an approved EGFR TKI agent and whose tumors are positive of the EGFR T790M resistance mutation. The study was submitted to FDA's Center for Drug Evaluation and Research (CDER) support accelerated approval of osimertinib in a second line or greater setting.

A bridging study was performed to establish effectiveness of the **cobas**® EGFR Mutation Test v2 through retrospective testing of all patient specimens which were enrolled into the trial with an IUO version of the test approved under P120019 (**cobas**® EGFR Mutation Test v1).

Of the 472 patients screened for the AURA2 study, 383 patients were eligible for testing with the **cobas**® EGFR Mutation Test v1. Of those eligible, 233 T790M+ patients were recruited into the AURA2 study, and 210 patients were enrolled and received osimertinib. Table 16 presents the ORR by BICR and investigator assessment in AURA2. Of 198 patients who received at least one dose of osimertinib and had measureable disease confirmed by BICR [Evaluable Response Analysis Set (ERAS)], 127 were confirmed responders by BICR with ORR as 64.1% (95% CI: 57.0%,% 70.8%).

Of 210 patients who received at least 1 dose of osimertinib (FAS), 128 were confirmed responders by BICR with ORR as 61.0% (95% CI: 54.0%, 67.6%) and 135 by investigator assessment with ORR as 64.3% (95% CI: 57.4%, 70.8%).

All 383 patients eligible for AURA2 trial, were retested by the **cobas**® EGFR Mutation Test v2. Of 233 T790M positive patients recruited into the AURA 2 trial, 225 were T790M+ by the **cobas**® EGFR Mutation Test and 204 were in the FAS. Of 204 patients who received at least one dose of Tagrisso®, 193 ERAS had measureable disease confirmed by BICR (ERAS). Of 193 patients, 126 were confirmed responders by BICR with ORR as 65.3% (95% CI: 58.1% to 72.0%).

Of 204 patients who received osimertinib (FAS), 126 were confirmed responders by BICR with ORR as 62.3% (95% CI: 55.2% to 68.9%) and 133 by investigator assessment with ORR as 65.2% (95% CI: 58.2% to 71.7%). These data are also included in Table 16.

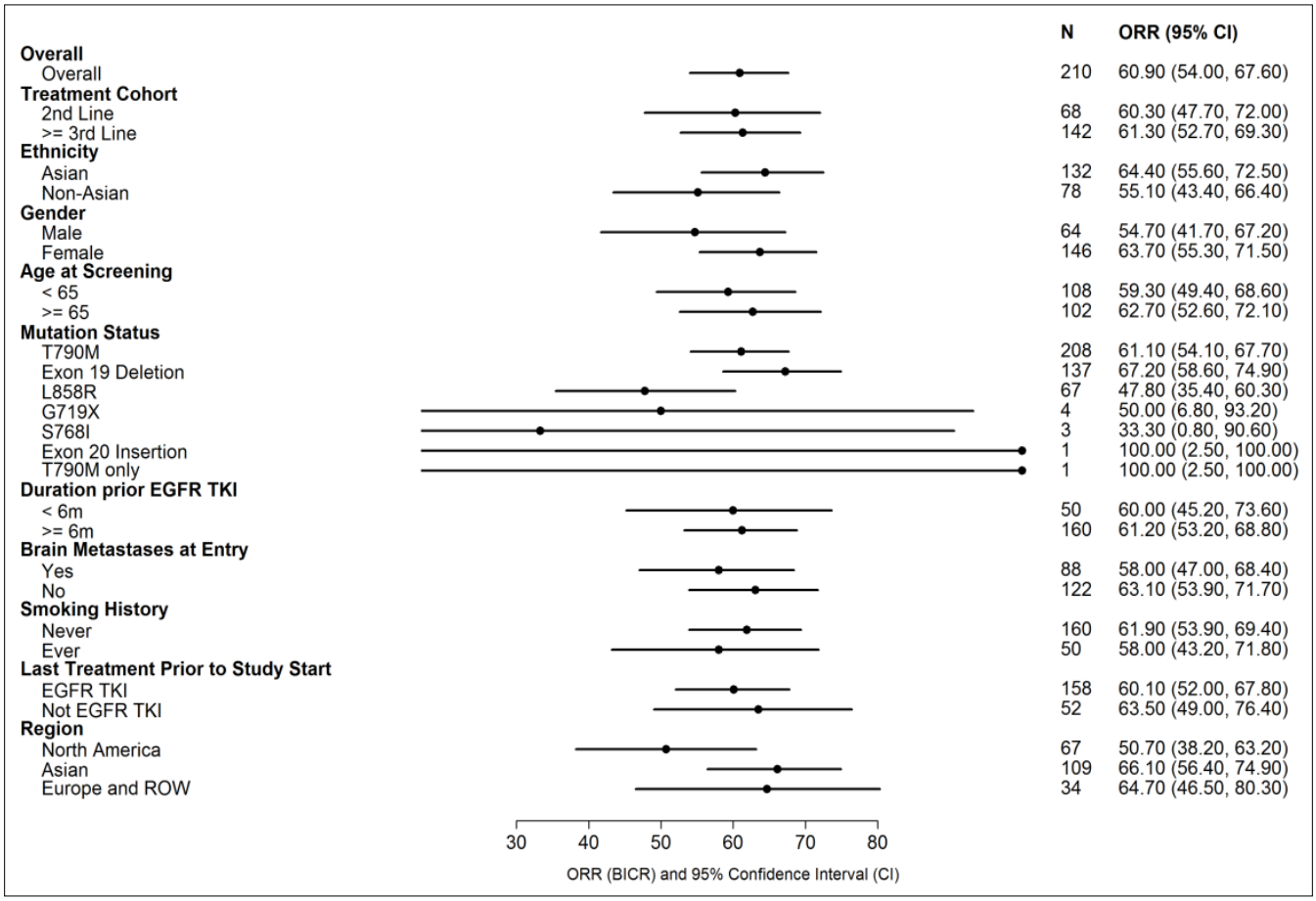
Table 16. Clinical Benefit of T790M Mutation Positive Patients Tested with the cobas® EGFR Mutation Test v2 in the AURA2 Trial

		AURA 2			cobas® EGFR Mutation Test v2 T790M Positive		
Analysis Set	**Assessed by**	**N**	**Number of Confirmed Responders**	**ORR (95% CI)**	**N**	**Number of Confirmed Responders**	**ORR (95% CI)**
FAS	BICR	210	128	61.0% (54.0%, 67.6%)	204	127	62.3% (55.2%, 68.9%)
	Investigator		135	64.3% (57.4%, 70.8%)		133	65.2% (58.2%, 71.1%)

3. Subgroup Analysis

The results of the AURA2 study are based on the **cobas**® EGFR Mutation Test v1 results. A subgroup analysis was performed which demonstrates the effectiveness of osimertinib in patients whose tumors are positive for the EGFR T790M resistance mutation and is depicted in the Figure 3.

Figure 3. Subgroup analyses per BICR assessment for AURA2



E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENATION AND FDA'S POST-PANEL ACTION

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

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of the **cobas**® EGFR Mutation Test v2 was demonstrated in retrospective analyses of patients enrolled in the Phase II AURA2 study for osimertinib. Analytical performance studies with the **cobas**® EGFR Mutation Test v2, when used according to the directions provided, demonstrate the ability to detect the T790M mutation with an analytical sensitivity of 3% mutation in DNA extracted from FFPET tissue of patients with advanced non-small cell lung cancer (NSCLC).

The safety and effectiveness of Tagrisso® (osimertinib) has not been established in patients whose tumors have G719X, exon 19 deletions, S768I, exon 20 insertions, L858R, or L861Q mutations which are also detected by the **cobas**® EGFR Mutation Test v2.

B. Safety Conclusions

As a diagnostic test, the **cobas**® EGFR Mutation Test v2 involves testing on formalin-fixed, paraffin embedded human NSCLC cancer tissue sections. The risks of the **cobas**® EGFR Mutation Test are associated with the potential mismanagement of patients resulting from false results of the test. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect EGFR test results, and consequently improper patient management decisions in NSCLC treatment. A patient with a false positive result may undergo treatment with osimertinib with inappropriate expectation of therapeutic benefit and experience side effects. A patient with a false negative result may be treated without osimertinib and not experience the potential therapeutic benefit.

C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in the AURA2 study, which were used to support PMA approval as described above. The clinical benefit of the **cobas**® EGFR Mutation Test v2 was demonstrated in a retrospective analysis of efficacy and safety data obtained from an open-label, single arm study in which Tagrisso® (osimertinib) demonstrated a robust objective response rate of 62.3% (95% CI, 55.2%, 68.9%) in the full analysis set based on blinded independent central review (BICR) and the 65.3% (95% CI, 58.1%, 72.0%) in the subset of patient who had measurable disease confirmed by BICR.

The risks of the **cobas**® EGFR Mutation Test v2 are associated with the potential mismanagement of patients resulting from erroneous test results. The device is a key part of diagnostic evaluation for non-small cell lung cancer in decisions regarding treatment with erlotinib and osimertinib. There is currently no FDA approved test for the selection of candidate metastatic NSCLC patients for treatment with osimertinib.

In conclusion, given the available information above, the data support the use of the **cobas**® EGFR Mutation Test v2 as an aid in selecting NSCLC patients for osimertinib treatment based on a **cobas**® EGFR Mutation Test v2 "Mutation Detected" result for the EGFR T790M mutation, and the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the Phase II AURA2 clinical study support the utility of the **cobas**® EGFR Mutation Test v2 as an aid in selecting patients with advanced NSCLC for whom Tagrisso® (osimertinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated. Tagrisso® (osimertinib) demonstrated an objective response rate that appears to be robust and of a magnitude to reasonably predict clinical benefit for osimertinib in patients identified with the **cobas**® EGFR Mutation Test v2.

XIII. CDRH DECISION

CDRH issued an approval order on November 13, 2015.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Limitations in the device labeling. Refer to the drug label for Tagrisso® (osimertinib) for additional information related to use of the drug.

Post-approval Requirements and Restrictions: See approval order.