

# SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

## I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: *therascreen*® EGFR RGQ PCR Kit

Device Procode: OWD

Applicant's Name and Address: QIAGEN Manchester Ltd  
Skelton House, Lloyd Street North  
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United Kingdom

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P120022

Date of FDA Notice of Approval: July 12, 2013

Expedited: Granted priority review status on January 15, 2012 because the device addresses an unmet medical need, as demonstrated by significant clinically meaningful advantage.

## II. INDICATIONS FOR USE

The *therascreen*® EGFR RGQ PCR Kit 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 (FFPE) non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used to select patients with NSCLC for whom GILOTRIF™ (afatinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated. Safety and efficacy of GILOTRIF™ (afatinib) have not been established in patients whose tumors have L861Q, G719X, S768I, exon 20 insertions, and T790M mutations, which are also detected by the *therascreen*® EGFR RGQ PCR Kit.

Specimens are processed using the QIAamp® DSP DNA FFPE Tissue Kit for manual sample preparation and the Rotor-Gene® Q MDx instrument for automated amplification and detection.

## III. CONTRAINDICATIONS

None.

#### IV. **WARNINGS AND PRECAUTIONS**

The warnings and precautions can be found in the QIAGEN *therascreen*® EGFR RGQ PCR Kit labeling.

#### V. **DEVICE DESCRIPTION**

The following components comprise the overall device:

- QIAGEN QIAamp® DSP DNA FFPE Tissue Kit
- QIAGEN *therascreen*® EGFR RGQ PCR Kit
- QIAGEN Rotor-Gene Q MDx Software version 2.1.0 build 9, and EGFR Assay Package version 1.1.2

#### **Specimen Preparation**

Formalin-fixed, paraffin-embedded (FFPE) blocks are sectioned onto glass slides. A stained slide is used to confirm that there is tumor present. Two non-stained tissue sections are scraped from the slide for DNA extraction. DNA is manually extracted and purified from two 5-10 µm glass-mounted sections of FFPE tissue taken from NSCLC patients using the QIAGEN QIAamp® DSP DNA FFPE Tissue Kit. The tumor tissue is deparaffinized with xylene and the xylene is extracted with ethanol. The sample is lysed under denaturing conditions with proteinase K for one hour. The sample is heated at 90°C to reverse formalin cross-linking of genomic DNA. The sample is passed through a silica-based membrane so that genomic DNA binds to the membrane and contaminants are removed. Purified genomic DNA is eluted from the membrane into 120-µl of elution buffer. Extracted DNA is stored at -20°C.

#### **PCR Amplification and Detection**

The QIAGEN *therascreen*® EGFR RGQ PCR Kit contains reagents for eight separate reactions; seven mutation-specific reaction mixes to amplify and detect mutations in codons 18, 19, 20 and 21 of the EGFR oncogene, and one Control Reaction mix that amplifies and detects a region of exon 2 in the EGFR oncogene. The seven mutation-specific reaction mixes are the exon 19 Deletions, L858R, T790M, L861Q, G719X, S768I and exon 20 Insertions reaction mixes.

Each reaction in the *therascreen*® EGFR RGQ PCR Kit makes use of an amplification refractory mutation system (ARMS) allele specific polymerase chain reactions (PCR) to selectively amplify mutated genomic DNA templates (mutation-positive) in a background of non-mutated genomic DNA (mutation-negative; wild-type) combined with a fluorophore-labeled Scorpion primer to detect any resultant amplification product. ARMS technology exploits the ability of *Taq* polymerase to distinguish between a match and a mismatch at the 3' end of a PCR primer. Scorpions are bi-functional molecules containing a PCR primer covalently linked to a probe. The probes incorporate both a fluorophore (carboxyfluorescein [FAM™]) and a quencher which quenches the fluorescence of the fluorophore. During PCR, when the probe binds to the ARMS

amplicon, the fluorophore and quencher become separated leading to a detectable increase in fluorescence.

Before testing with the mutation-specific reaction mixes, each DNA sample must be tested with the Control Reaction mix to determine whether the quality and quantity of DNA is sufficient and appropriate for the working range of the assay. The Control Reaction Ct value is used to assess the total amplifiable DNA in a sample and must fall within pre-specified ranges for each sample. The interpretation of the results obtained from the Control Reaction is as follows:

Control Ct value	Interpretation	Action
> 31.10	Quantity of amplifiable DNA is not sufficient for mutation analysis.	Additional samples should be extracted and tested
< 23.70	Quantity of amplifiable DNA is too high for mutation analysis.	Dilute with the sample diluent water supplied in the kit
$23.70 \leq \text{Control Ct} \leq 31.10$	Quantity of amplifiable DNA is suitable for mutation analysis	---

The run parameters used for assessing the DNA sample with the Control Reaction mix are the same run parameters for mutation analysis using the mutation assays. The run parameters are:

- Hold at 95°C for 15 minutes to activate the *Taq* polymerase;
- PCR for 40 cycles of 95°C for 30 seconds, to denature, and 60°C for 1 minute, to anneal/extend.

The PCR cycle at which the fluorescence from a particular reaction crosses the pre-defined threshold value is defined as the Ct value. The *therascreen*® EGFR RGQ PCR Kit detects the following mutations in exon 19 of the EGFR gene: 2235\_2249del15, 2235\_2252>AAT, 2237\_2255>T, 2236\_2250del15, 2238\_2255del18, 2238\_2248>GC, 2238\_2252>GCA, 2239\_2253del15, 2239\_2256del18, 2239\_2248TTAAGAGAAG>C, 2239\_2258>CA, 2240\_2257del18, 2240\_2254del15, 2239\_2251>C, as well as the L858R substitution mutation 2573 T>G in exon 21 of the EGFR gene. The *therascreen*® EGFR RGQ PCR Kit also detects the following mutations where safety and efficacy of GILOTRIF™ (afatinib) has not been established: T790M (2369C>T), L861Q (2582T>A), G719A (2156G>C), S768I (2303G>T), and exon 20 insertions (2319\_2320insCAC and 2310\_2311insGGT). The list of mutation is also included in “SUMMARY OF PRECLINICAL STUDIES” section below.

### **Test Controls**

Each test run must contain an Internal Control (IC, already included in the PCR reaction mixes), a Positive Control (PC), and a No Template Control (NTC, Negative Control) tested with each reaction mix. A test run is considered invalid if any NTC indicates that the test run has been contaminated (one or more Ct values below a set value for the green FAM channel or outside a set range for the yellow Hex channel), or if a PC Ct value lies outside a set range (green FAM channel). For EGFR Kit test runs to be accepted as valid,

the RGQ software requires Ct values for PC, and NTC, meet validity criteria specified in the EGFR Locked Templates as shown in table below.

#### Run Validity Criteria

Reaction Control	Reaction Mix	Channel	Ct Range*
PC	Control	FAM (green)	28.13 – 34.59
	T790M	FAM (green)	30.22 – 34.98
	Deletions	FAM (green)	28.90 – 34.90
	L858R	FAM (green)	29.97 – 34.81
	L861Q	FAM (green)	28.49 – 34.02
	G719X	FAM (green)	29.42 – 34.19
	S768I	FAM (green)	28.98 – 35.19
	Insertions	FAM (green)	27.92 – 34.09
NTC	All 8 reaction mixes	FAM (green)	No Amp (i.e. not $\leq 40.00$ )
		HEX (yellow)	29.85 – 35.84

\*Ranges are inclusive.

#### ***Internal Control:***

All eight reaction mixes contain an additional ARMS primer and a HEX-labeled Scorpions primer for the amplification and detection of a synthetic non EGFR related oligonucleotide template that is used as an Internal Control. The Scorpions primer is labeled with HEX to distinguish from the FAM-labeled Scorpions in the Control and mutation assays. In each reaction mix, the Internal Control Reaction is designed to be the weaker of the two reactions. This is achieved through the use of a very low concentration of Internal Control template. The Internal Control Reaction is designed to work independently of mutation-specific amplification, but can fail in the presence of strong amplification if it is “outcompeted” by the FAM reaction. A no mutation detected result with a failed Internal Control Reaction in any one of the seven mutation assays will be reported as an invalid result. The Internal Control is used to detect inhibitors or gross reaction failures.

#### ***Positive Control (PC):***

The Positive Control comprises a mixture of synthetic oligonucleotides representing the mutations detected by the *therascreen*® EGFR RGQ PCR Kit. Detection of the Positive Control confirms the proper functioning of each of the reaction mixes in the Kit. Of the multiplex reaction mixes (exon 19 Deletions, G719X, Insertions), one mutation from each is included in the Positive Control.

#### ***Negative Control (NTC):***

The *therascreen*® EGFR RGQ PCR Kit contains nuclease-free water to be used as a No Template Control (NTC) reaction. The NTC serves as a negative control and assesses potential contamination during assay setup.

#### **Instrument and Software**

The Rotor-Gene Q (RGQ) MDx instrument is a real-time PCR analyzer designed for thermocycling and real-time detection of amplified DNA. The RGQ MDx instrument controls and monitors PCR reactions and includes the software that determines mutation

status based upon PCR results. It incorporates a centrifugal rotor design for thermal cycling during PCR reactions where each tube spins in a chamber of moving air. Samples are heated and cooled in a low-mass-air oven according to a software determined cycle that initiates the different phases of the PCR cycle for a total of 40 cycles for each PCR run. In the RGQ MDx Instrument, samples are excited from the bottom of the chamber by a light emitting diode. Energy is transmitted through the thin walls at the base of the tube. Emitted fluorescence passes through the emission filters on the side of the chamber and is detected by a photomultiplier tube. Detection is performed as each tube aligns with the detection optics; tubes spin past the excitation/detection optics every 150 milliseconds. The fluorescence signals monitor the progress of the PCR reactions. The instrument is capable of supporting up to six optical channels (six excitation sources and six detection filters), however only two of these channels (the yellow HEX and green FAM channels) are used with the *therascreen*® EGFR RGQ PCR Kit.

The *therascreen*® EGFR Assay Package consists of two templates: the “*therascreen* EGFR Control Run Locked Template” (for DNA sample assessment) and the “*therascreen* EGFR Locked Template” (for detection of EGFR mutations). These templates contain the PCR run parameters, assess run validity and calculate the results. The same run parameters are used for both the DNA sample assessment with the Control Reaction Mix and for detection of EGFR mutations using the mutation assays.

The RGQ MDx instrument software supports real-time analysis procedures. The software determines Ct values, calculates  $\Delta$ Ct values, and compares these to the mutation-specific cut-off values incorporated into the software as described above. A system of Flags/Warnings is embedded within the software in order to inform the user of potential problems with the assay and to indicate non-valid test runs or non-valid samples within a valid test run (inappropriate level of DNA or Internal Control failure). No results are reported for invalid runs or for non-valid samples. The  $\Delta$ Ct values will be reported to the users; however, users do not have access to the raw data.

### **Interpretation of Results**

The Ct for the Control Reaction reflects the total amount of amplifiable EGFR template in the sample, while the Ct for each mutation-specific reaction reflects the amount of EGFR mutation within the sample. The difference in Ct values ( $\Delta$ Ct) between the Control Reaction and the mutation-specific reaction (mutation assay) indicates the proportion of mutation within the sample. The  $\Delta$ Ct value reduces and approaches to 0 as the proportion of mutant DNA in the samples increases, a  $\Delta$ Ct value may be negative owing to individual assay variability. The  $\Delta$ Ct value increases as the proportion of mutant DNA in the sample decreases. When the  $\Delta$ Ct value exceeds the  $\Delta$ Ct cut-off values for all seven mutation assays, the assay reports “No Mutation Detected”. For each sample, a calculation is performed by the RGQ software to determine the  $\Delta$ Ct value (FAM channel) for each of the 7 mutation-specific reactions:

$$\Delta\text{Ct} = [\text{Mutation Reaction Ct Value}] - [\text{Control Reaction Ct Value}]$$

Each sample will have seven possible  $\Delta$ Ct values (one per mutation). These values are compared to pre-established specifications (cut-off values) incorporated into the RGQ software to determine whether a sample is “Mutation Detected” or “No Mutation Detected”, and which mutation(s), if any, is present. When the mutation assay  $\Delta$ Ct value is less than or equal to the cut-off value for that reaction, the sample is EGFR mutation-positive.

The assay results will be displayed as “Mutation Detected” (MD), “No Mutation Detected” (NMD), “Invalid” or, if a run control fails, “Run Control Failed”. For samples with “Mutation Detected” results, specific mutations are reported, i.e., exon 19 Deletions (Del), L858R, T790M, L861Q, G719X, S768I, or exon 20 Insertions (Ins). It is possible for a mutation positive sample to have more than one out of the seven possible EGFR mutations reported. The multiplex assays, i.e., Del, G719X, and Ins, do not distinguish among the mutations they detect.

**Mutation Assay Cut-Offs ( $\Delta$ Ct)**

Mutation Assay	T790M	Del*	L858R	L861Q	G719X	S768I	Ins**
Cut-Off ( $\Delta$ Ct)	$\leq 7.40$	$\leq 8.00$	$\leq 8.90$	$\leq 8.90$	$\leq 8.90$	$\leq 8.90$	$\leq 8.00$

\* Exon 19 Deletions

\*\* Exon 20 Insertions

**VI. ALTERNATIVE PRACTICES AND PROCEDURES**

There are no other FDA-cleared or approved alternatives for EGFR mutation testing of formalin-fixed, paraffin-embedded (FFPE) NSCLC tissue for the selection of patients who are eligible for first-line treatment with GILOTRIF™ (afatinib).

**VII. MARKETING HISTORY**

The QIAGEN *therascreen*® EGFR RGQ PCR Kit has not been marketed in the United States or any foreign country.

**VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH**

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect EGFR test results and subsequently improper patient management decisions in non-small cell lung cancer (NSCLC) treatment.

For the specific adverse events related to GILOTRIF™ (afatinib) that occurred in the clinical studies, please see Section X below.

**IX. SUMMARY OF PRECLINICAL STUDIES**

**A. Laboratory Studies**

The specific performance characteristics of the QIAGEN *therascreen*® EGFR RGQ PCR Kit (henceforth referred to as EGFR Kit) were determined by studies using formalin-fixed, paraffin-embedded (FFPE) tissue specimens collected from NSCLC patients, and FFPE human cell lines (FFPE cell lines). The FFPE cell lines were generated using a lung carcinoma cell line (A549) to produce cell lines harboring the desired specific EGFR mutations. Bi-directional Sanger sequencing and massively parallel sequencing were used to select the specimens for the following studies, and to determine the percentage of mutation in NSCLC FFPE samples. The similarity between FFPE clinical specimens and FFPE cell lines was demonstrated by comparing assay amplification efficiencies (AE) between the two sample types, and by assessing the Limit of Detection (LoD) for the specific mutation assays. FFPE cell lines were sectioned and processed similar to FFPE clinical specimens. DNA was extracted and tested according the instructions for use.

## **1. Correlation with Reference Method**

To demonstrate the accuracy of the EGFR Kit relative to bi-directional Sanger sequencing, accuracy studies were conducted with two sets of samples (a) procured clinical specimens and (b) clinical trial specimens from the Phase 3 study for afatinib, the 1200.32 clinical trial.

### **a. Procured Specimens**

The procured specimen study was a blinded study using procured FFPE clinical specimens from NSCLC patients. EGFR testing was performed on DNA samples extracted from 373 specimens with bi-directional Sanger sequencing results for exons 18, 19, 20 and 21 that had been blinded to the operators. A total of 366/373 samples (98.1%) produced valid EGFR Kit results and the bi-directional Sanger sequencing results were then unblinded. Analysis of concordance and discordant results were performed on this data set. Concordant results were obtained with 343/366 (93.7%) of procured samples, while discordant results were obtained with 23/366 (6.3%) of procured samples. Any discordant results and an equal number of concordant samples were subject to discordant analyses. The overall results are shown below.

## EGFR Kit Compared to Sanger Sequencing – Procured Specimens

		Mutation Call by Bi-Directional Sanger Sequencing										
		Del	Del & L858R	G719X	Ins	L858R	L861Q	T790M	T790M & L858R	S768I	WT	Total
EGFR Kit Call	Del	52	3	0	0	0	0	0	0	0	7	62
	Del & L868R	0	0	0	0	1	0	0	0	0	1	2
	G719X	0	0	4	0	0	0	0	0	0	1	5
	Ins	0	0	0	1	0	0	0	0	0	3	4
	L858R	0	0	0	0	58	0	0	0	0	7	65
	L861Q	0	0	0	0	0	2	0	0	0	0	2
	T790M	0	0	0	0	0	0	1	0	0	0	1
	T790M & L868R	0	0	0	0	0	0	0	1	0	0	1
	S768I	0	0	0	0	0	0	0	0	0	0	0
	WT	0	0	1	0	3	0	0	0	0	220	224
Total	52	3	5	1	62	2	1	1	0	239	366	

Procured samples with both Sanger and EGFR Kit valid results were analyzed to assess the overall percent agreement (OPA), positive percent agreement (PPA) and negative percent agreement (NPA) based on agreement between the two methods for overall mutation status, i.e. that the samples either have a positive mutation, regardless of which mutation it is, or have a no mutation detected result. Additional agreement analyses were also conducted for each of the 7 EGFR mutation assays in the EGFR Kit (data not shown). These percentages, together with the corresponding two-sided exact 95% confidence intervals (95% CI) were reported. The results demonstrate a PPA of 96.9%, a NPA of 92.1% and an OPA of 93.7%.

### EGFR Kit vs. Sanger Concordance in Procured Specimens

Measure of Agreement	Percent Agreement % (N)	95% CI*
<b>Positive Percent Agreement (PPA)</b>	96.9% (123/127)	92.1, 99.1
<b>Negative Percent Agreement (NPA)</b>	92.1% (220/239)	87.9, 95.1
<b>Overall Percent Agreement (OPA)</b>	93.7% (343/366)	90.7, 96.0

\*95% CI is calculated based on exact method.

For the 23 overall mutation status discordant results, 4 (17.4%) sample gave wild-type (i.e., no mutation detected) results by the EGFR Kit and gave mutation detected results by Sanger sequencing, while 19 (82.6%) samples gave mutation detected results by the EGFR Kit and gave wild-type results by Sanger sequencing.

**b. Phase 3 Clinical Trial Specimens**

This was a blinded study using FFPE clinical specimens from a subpopulation of patients in the 1200.32 clinical trial. The baseline clinical and demographic characteristics of the patients whose specimens were available for this retrospective testing were comparable to those of otherwise eligible patients whose specimens were not available for retesting. EGFR testing was performed on DNA samples extracted from 360 specimens with bi-directional Sanger sequencing results for exons 18, 19, 20 and 21 that had been blinded to the operators. Concordant results were obtained with 332/360 (92.2%) of samples, while discordant results were obtained with 28/360 (7.8%) of samples. Discordant results were not further evaluated or re-tested as the quantity of DNA remaining was very low. The overall results are shown below.

**EGFR Kit Compared to Sanger Sequencing – the 1200.32 Clinical Trial Specimens**

EGFR Kit Call	Sanger Mutation Status												
	Del	Del & T790M	G719X	Insertions	L858R	L861Q	T790M	T790M & L858R	S768I	L858R & S768I	G719X & S768I	WT	Total
Deletions	76	-	-	-	1	-	-	-	-	-	-	12	89
Del & T790M	2	1	-	-	-	-	-	-	-	-	-	0	3
G719X	-	-	1	-	-	-	-	-	-	-	-	1	2
Insertions	-	-	-	1	-	-	-	-	-	-	-	3	4
L858R	-	-	-	-	69	-	-	-	-	-	-	10	79
L861Q	-	-	-	-	-	1	-	-	-	-	-	1	2
T790M	-	-	-	-	-	-	-	-	-	-	-	0	0
T790M & L858R	-	-	-	-	-	-	-	1	-	-	-	0	1
S768I	-	-	-	-	-	-	-	-	1	-	-	0	1
L858R & S768I	-	-	-	-	-	-	-	-	-	2	-	0	2
G719X & S768I	-	-	-	-	-	-	-	-	-	-	1	0	1
WT	1	0	0	0	0	0	0	0	0	0	0	175	176
<b>Total</b>	<b>79</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>70</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>202</b>	<b>360</b>

Samples with both Sanger and EGFR Kit valid results were analyzed to assess the OPA, PPA and NPA based on agreement between the two methods for overall mutation status. These percentages, together with the corresponding two-sided exact 95% CI are summarized below. Additional agreement analyses were also conducted for each of the 7 mutation assays in the EGFR Kit (data not shown). These percentages, together with the corresponding two-sided exact 95% CI were reported. The results demonstrate a PPA of 99.4%, a NPA of 86.6% and an OPA of 92.2%.

**EGFR Kit vs. Sanger Concordance in the 1200.32 Clinical Trial Specimens**

Measure of Agreement	Percent Agreement % (N)	95% CI*
<b>Positive Percent Agreement (PPA)</b>	99.4% (157/158)	96.5, 100.0
<b>Negative Percent Agreement (NPA)</b>	86.6% (175/202)	81.2, 91.0
<b>Overall Percent Agreement (OPA)</b>	92.2% (332/360)	89.0, 94.8

\*95% CI is calculated based on exact method.

For the 28 overall mutation status discordant results, 1 (3.6%) sample with an EGFR mutation as determined by Sanger sequencing gave a wild-type (i.e., no mutation detected) result by the EGFR Kit, while 27 (96.4%) samples gave mutation detected results by the EGFR Kit and gave wild-type results by Sanger sequencing.

**2. Analytical Sensitivity**

**a. Analytical Sensitivity – Limit of Blank (LoB)**

To assess performance of the EGFR Kit 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 FFPE EGFR wild-type cell line DNA were evaluated. The results demonstrated no positive mutation calls with No Template Control (NTC) samples and FFPE wild-type samples

- i. **Limit of Blank (LoB) no template** – The No Template Control (NTC, nuclease-free water) as supplied in the EGFR Kit was run as the sample with no template. All NTC tested results in each mutation assay (n=14/14 per mutation assay) were valid and were “No Mutation Detected”.
- ii. **Limit of Blank (LoB) FFPE Specimens** – High input DNA from NSCLC FFPE EGFR wild-type cell line was tested (n=60). All valid results (n=59) were “No Mutation Detected”. One

replicate of the 60 tested was invalid according to the run criteria of the EGFR Kit and was excluded from analysis.

b. **Analytical Sensitivity – Limit of Detection (LoD)**

The LoD is the minimum percentage of mutant DNA that can be detected in a background of wild-type DNA when the total amplifiable DNA (within the input range) produced correct mutation calls at 95% for each mutation positive sample (C95). The DNA input working range for the *therascreen*® EGFR RGQ PCR Kit is based on the Control Reaction Ct value at the pre-specified range of 23.70 to 31.10 (also refer to section 2c below), which is used to indicate the amount of amplifiable DNA in a sample. The EGFR Kit does not use a specific concentration of DNA as determined by spectrophotometry.

Multiple studies were conducted sequentially to estimate and determine LoD for each EGFR mutation. The initial study estimated the LoD across a broad dilution series (eight replicates per dilution). Based on the results of the initial study, subsequent studies were conducted to determine the LoD across a narrow dilution series (24 replicates per dilution). For each EGFR mutation, percentages of correct calls were assessed across dilution levels. The LoD was determined at both the high DNA input (Control Ct ~25.50) and at low DNA input (Control Ct ~30.1) levels.

The NSCLC FFPE clinical specimens or FFPE cell lines were used in the LoD study. The mutant DNA, extracted from the FFPE clinical specimens or FFPE cell lines, was diluted in a background of wild-type DNA in order to create a series of samples containing different percentages of mutant DNA. At each dilution level (% mutation), 24 replicates were evaluated using multiple EGFR Kit lots. For each mutation, the rate of correct calls by % mutation, mean control Ct value, mean mutation Ct value, and mean  $\Delta$ Ct value were tabulated by % mutation. An example of such table for the exon 21 L858R mutation is shown below.

**Rate of Correct Mutation Calls by % Mutation for Exon 21 L858R Mutation**

L858R	% Mutation (Dilution)	Rate of Correct Calls	Mean Control Ct Value (Range)	Mean Mutation Ct Value (Range)	Mean ΔCt Value (Range)
Low DNA Input	17.06	24/24	30.29 (29.92-30.57)	35.14 (34.44-37.41)	4.85 (4.05-7.26)
	12.15	24/24	30.21 (29.59-30.74)	35.57 (34.95-37.49)	5.36 (4.46-7.19)
	8.69	24/24	30.24 (29.60-30.97)	36.10 (35.04-38.12)	5.86 (4.76-8.16)
	6.21	23/24	30.17 (29.90-30.60)	36.62 (35.13-39.29)	6.45 (4.53-9.34)
	4.46	20/24	30.21 (29.62-30.95)	37.39 (35.62-39.68)	7.19 (5.35-9.71)
	3.15	17/24	30.31 (29.78-30.70)	37.44 (36.14-38.43)	7.16 (5.75-8.65)
	2.25	12/24	30.13 (29.65-30.66)	38.15 (36.39-39.51)	8.02 (6.49-9.76)
High DNA Input	1.55	24/24	26.29 (25.97-26.71)	33.61 (32.97-34.75)	7.31 (6.42-8.38)
	1.10	21/24	25.62 (25.29-26.07)	33.89 (33.21-34.97)	8.27 (7.21-9.29)
	0.79	22/24	25.67 (25.31-26.03)	34.04 (33.18-34.74)	8.37 (7.15-9.30)
	0.56	19/24	26.00 (25.64-26.35)	34.58 (33.67-35.87)	8.59 (7.68-9.66)
	0.40	9/24	25.68 (25.40-26.03)	34.76 (34.09-35.44)	9.08 (8.35-9.90)
	0.29	8/24	25.70 (25.38-26.06)	34.83 (34.05-35.73)	9.13 (8.20-9.96)
	0.21	3/24	25.69 (25.37-26.04)	34.91 (34.00-35.69)	9.23 (8.39-9.89)

Utilizing the mutational call data, the logistic regression analysis was applied to each mutation individually using each of the low and high input DNA datasets. In these analysis models, the predicted probability of detection of 95% ( $p = 0.95$ ) on the y-axis would intercept the fitted curve to read out a mutation percentage on the x-axis [ $\log_2$  (% mutation dilution)]. Hence, the LoD value was determined as the % mutation dilution which gave an estimated probability of detection of 95% (C95). The LoD was determined for each EGFR mutation at either the low or high DNA input levels (data not shown). The final LoD claims listed in tables below indicate the percentage of mutation which gave a predicted probability of correct calls of 95% for each of the 21 mutations. The final LoD claims for EGFR mutations were fully supported by results from the reproducibility study conducted at low DNA input (refer to Repeatability and Reproducibility section below). Among the 21 EGFR mutations detected by the EGFR Kit, safety and efficacy of GILOTRIF™ (afatinib) have been established for the 15 mutations listed in the first table below, but have not been established for the 6 mutations listed in the second table below. Refer to GILOTRIF™ (afatinib) drug labeling for more details.

**Sensitivity of the *therascreen*® EGFR RGQ PCR Kit – Safety and Efficacy of GILOTRIF™ (afatinib) Established**

Exon	Mutation	Cosmic ID	Base Change	Sample Type <sup>^</sup>	Final LoD Claim (% Mutation)
19	Deletions	6220	2238_2255del18	CL	2.7
		6223*	2235_2249del15	CL+CS	6.4
		6225*	2236_2250del15	CL+CS	6.5 <sup>\$</sup>
		6254	2239_2253del15	CS	10.2 <sup>\$</sup>
		6255	2239_2256del18	CS	0.81 <sup>\$</sup>
		12369	2240_2254del15	CS	4.94
		12370	2240_2257del18	CS	8.1
		12382	2239_2248TTAAGAGAAG>C	CS	1.45 <sup>\$</sup>
		12383	2239_2251>C	CS	4.58
		12384	2237_2255>T	CS	7.54 <sup>\$</sup>
		12387	2239_2258>CA	CL	4.91
		12419	2238_2252>GCA	CL	16.87
		12422	2238_2248>GC	CL	3.24
13551	2235_2252>AAT	CL	4.24		
21	L858R	6224*	2573T>G	CL+CS	5.94

**Sensitivity of the *therascreen*® EGFR RGQ PCR Kit – Safety and Efficacy of GILOTRIF™ (afatinib) Not Established**

Exon	Mutation	Cosmic ID	Base Change	Sample Type <sup>^</sup>	Final LoD Claim (% Mutation)
20	T790M	6240*	2369C>T	CL+CS	17.5%
21	L861Q	6213	2582T>A	CL	9.24 <sup>\$</sup>
18	G719A	6239	2156G>C	CL	32.5 <sup>\$</sup>
20	S768I	6241	2303G>T	CL	7.66
20	Insertions	12377	2319_2320insCAC	CL	3.72 <sup>\$</sup>
		12378	2310_2311insGGT	CL	19.96 <sup>\$</sup>

<sup>^</sup> CS denotes FFPE clinical specimen; CL denotes FFPE cell line.

\* LoD was determined using both FFPE clinical specimens and FFPE cell line at low DNA input level for these 4 EGFR mutations, which covers 68.71% of the reported EGFR mutations.

\$ Final LoD % mutation claims are based on results from reproducibility study.

The final LoD claims for EGFR mutations were fully supported by results from the reproducibility study conducted at low DNA input (refer to Repeatability and Reproducibility section below). The LoD values determined at the low DNA input were higher than those determined at high DNA input; this difference is expected as there is more abundant mutation DNA template at the higher DNA input level. The data overall support the final LoD claims listed above at the percent mutation which gave a predicted probability of correct calls of 95%. Although the analytical sensitivity studies have been conducted, the *therascreen*®

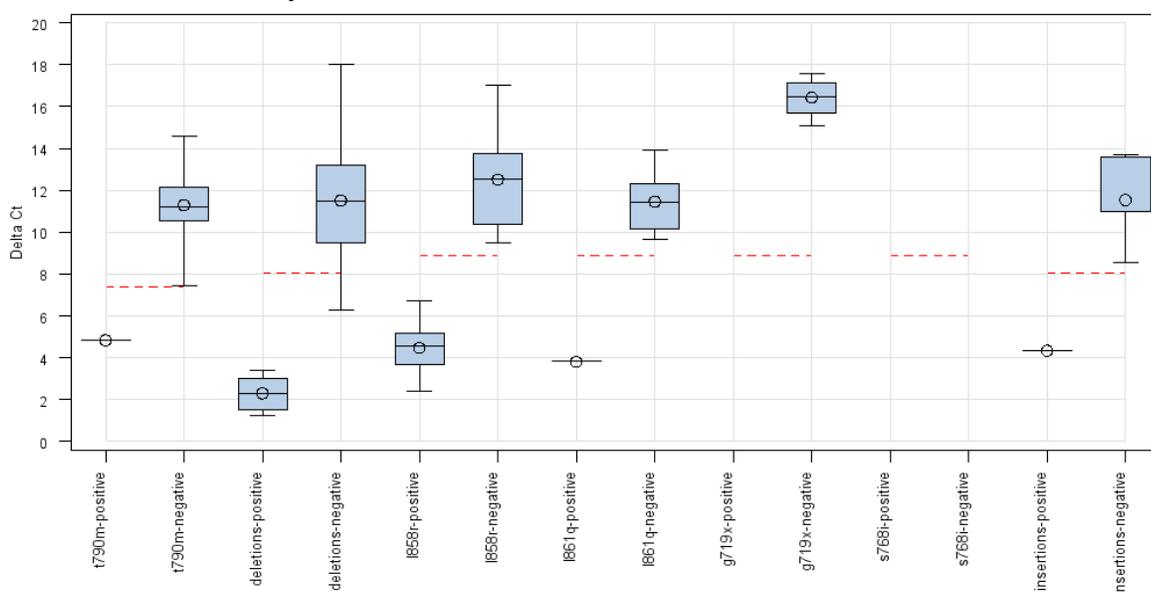
EGFR RGQ PCR Kit is for the qualitative detection of the EGFR mutation and is not intended to for quantitative measurements.

c. **Analytical Sensitivity – Control Ct Range, RFI Validation, and  $\Delta$ Ct Cut-offs**

- i. **Control Ct Ranges** – The objective of this study was to set an appropriate Control Reaction Ct range for use in assessing DNA sample validity. A set of 417 sectioned FFPE clinical specimen blocks were assayed and characterized using the EGFR Kit. Among these, 400 samples were wild-type and 17 samples were of mutation positive as determined by bi-directional Sanger sequencing. EGFR mutations representing five out of the seven mutation assays in the EGFR Kit were covered in this study. Samples for the remaining two mutation assays (i.e., G719X and S768I) were not available. Distribution of Control assay Ct values were tested for normality using graphical inspection and a standard test for normality. Data was not normally distributed therefore non-parametric methods were used. The nonparametric one-sided tolerance intervals used for each boundary were selected at 90% coverage with 99% confidence. The boundaries selected were further rounded following considerations in accordance with the user needs and risk management. The final Control Reaction Ct Working range selected for the EGFR Kit is determined to be 23.70 to 31.10 Ct.
- ii. **Correlation to Relative Fluorescence Increase (RFI)** – During development, the optimal analysis settings for the ‘Green’ (FAM) and ‘Yellow’ (HEX) Channels were determined to set thresholds, i.e., the mean fluorescence values which a curve must cross to be distinguished from background fluorescence as positive amplification. The thresholds at 0.075 for the ‘Green’ (FAM) channel and at 0.02 for the ‘Yellow’ (HEX) channel were chosen and applied during the analysis of 235 FFPE replicates, 35 NTC replicates and 35 Positive Control replicates. The analysis parameters were found to be suitable prior to assessing Ct values, and were configured in the locked templates contained within assay packages (i.e., operators would be unable to alter).
- iii.  **$\Delta$ Ct Cut-offs** – A risk-based approach was taken with regard to false positive rates when setting the assay cut-offs, and estimated LoB values were used as one component in developing cut-off values. The raw data plots and/or box plots of  $\Delta$ Ct were used as a visual aid based on distribution and separation of  $\Delta$ Ct values. The red-dashed line shows the cut-offs, above that are the mutation negative samples, and below that the mutation-positive samples.

There is no boxplot for S768I mutation-negative samples as no amplification was observed with wild-type samples in this assay, therefore no  $\Delta Ct$  value could be calculated. There are no boxplots displayed for G719X and S768I, as there were no mutation positive samples available for G719X and S768I due to the rarity of the samples. For the T790M assay, a cut-off of 7.4 was chosen to enable greater sensitivity of the assay whilst still ensuring no false positive or negative calls would result. For the exon 19 Deletions assay, a cut-off of 8.0 was selected to enable greater sensitivity of the assay with a false positive rate of 0.7%. Refer to section V above on Interpretation of Results for the cut-off values selected.

### Cut-off $\Delta Ct$ Values by Mutation



### 3. Analytical Sensitivity – Effect of DNA Input on $\Delta Ct$

The DNA input level is defined as the total quantity of amplifiable EGFR DNA in a sample and is determined by the Ct values from the Control Reaction. When samples at different total DNA levels contain the same proportion of mutant DNA, it is expected that the measured  $\Delta Ct$  values will remain consistent. The objective of the study was to demonstrate that the performance of the EGFR Kit is consistent over the total DNA input (Control Ct) range of the assay.

Nineteen (19) of the mutations detected by the EGFR Kit were tested, including all 7 EGFR mutation assays. DNA extracted from 19 FFPE cell lines was used to prepare pools of DNA at the lower end of the Control Reaction working range. For each point within the working range sufficient material was prepared to carry out 6 replicate tests. The target Ct were set for dilution 1 (100% or undiluted) for each mutation of Ct at ~24.70. For some mutations it was not possible to achieve a Ct of 24.70; thus, the lowest possible Ct was used. This pool of DNA was used to generate 6 equally spaced dilution levels across and

beyond the working range, resulting a dilution at approximately 1:3 ratio (i.e., each subsequent dilution level contained approximately 3 fold less DNA). The final dilution point was outside of the lowest DNA input level of the working range, Ct ~32-33 Ct (0.41%).

For each of the EGFR mutations tested, the mean control Ct, mutant Ct and  $\Delta$ Ct values for each mutation assay at each dilution were displayed in tables below, as a function of the Control Ct ranges for the dilutions. Overall, the  $\Delta$ Ct values measured at different total DNA input levels were consistent across the working range of the EGFR Kit, and passed the pre-specified acceptance criteria for the study.

**Mean Control Ct Values across the Control Ct Range – FFPE cell lines**

Sample Mutation	Relative DNA Input Level					
	0.41%	1.23%	3.70%	11.11%	33.33%	100.00%
WT	33.34	31.38	29.76	28.22	26.09	24.74
T790M	32.98	31.05	29.34	27.62	26.06	24.39
Del6210	33.53	31.28	29.64	27.90	26.48	24.65
Del6218	32.82	31.24	29.42	27.78	26.32	24.68
Del6220	33.84	31.81	30.19	28.38	26.59	24.97
Del6223	33.93	31.14	29.45	27.82	26.19	24.42
Del6225	32.83	31.03	29.51	27.80	26.18	24.48
Del12367	33.50	31.40	29.64	28.05	26.41	24.79
Del12387	33.31	31.47	29.70	28.01	26.41	24.73
Del12422	33.42	31.48	29.91	28.17	26.35	24.78
Del12419	33.42	31.19	29.35	27.78	26.17	24.58
Del12728	33.19	31.32	29.69	28.04	26.45	24.78
Del13551	32.86	30.98	29.51	27.89	26.22	24.62
L858R	32.93	31.34	29.61	27.85	26.26	24.64
L861Q	33.25	31.67	30.00	28.42	26.56	24.99
G719A	33.26	31.63	29.97	28.32	26.88	25.00
S768I	33.13	31.33	29.79	27.96	26.43	24.74
Ins12377	33.68	31.63	29.64	27.87	26.26	24.72
Ins12378	33.47	31.23	29.64	27.83	26.31	24.58

**Mean Mutant Ct Values across the Control Ct Range – FFPE cell lines**

Sample Mutation	Relative DNA Input Level					
	0.41%	1.23%	3.70%	11.11%	33.33%	100.00%
<b>T790M</b>	35.12	32.87	31.31	29.60	27.97	26.31
<b>Del6210</b>	36.18	34.29	32.50	30.91	29.19	27.60
<b>Del6218</b>	37.75	35.34	33.62	31.77	30.22	28.56
<b>Del6220</b>	33.00	30.98	29.39	27.52	25.90	24.22
<b>Del6223</b>	36.00	32.87	31.24	29.63	27.96	26.42
<b>Del6225</b>	34.54	32.70	31.23	29.53	27.91	26.27
<b>Del12367</b>	34.35	32.30	30.56	28.98	27.34	25.70
<b>Del12387</b>	35.09	33.20	31.46	29.82	28.19	26.63
<b>Del12422</b>	36.10	34.05	32.29	30.62	28.95	27.39
<b>Del12419</b>	37.92	36.12	34.32	32.71	31.12	29.68
<b>Del12728</b>	33.56	31.84	30.18	28.62	26.88	25.23
<b>Del13551</b>	33.32	31.68	30.24	28.43	26.76	25.24
<b>L858R</b>	32.97	31.40	29.76	28.07	26.47	24.98
<b>L861Q</b>	34.45	33.09	31.43	29.64	28.00	26.45
<b>G719A</b>	36.22	34.19	32.25	30.58	28.96	27.34
<b>S768I</b>	36.23	34.17	32.42	30.70	29.14	27.60
<b>Ins12377</b>	35.75	33.75	31.63	30.19	28.62	26.93
<b>Ins12378</b>	33.71	31.71	29.91	28.21	26.55	24.76

**Mean  $\Delta$ Ct Values across the Control Ct Range – FFPE cell lines**

Sample Mutation	Relative DNA Input Level					
	0.41%	1.23%	3.70%	11.11%	33.33%	100.00%
T790M	2.14	1.82	1.97	1.98	1.91	1.92
Del6210	2.64	2.96	2.86	3.01	2.71	2.95
Del6218	4.93	4.10	4.20	4.00	3.90	3.89
Del6220	-0.84	-0.84	-0.80	-0.87	-0.69	-0.75
Del6223	2.08	1.73	1.79	1.81	1.77	2.00
Del6225	1.71	1.67	1.72	1.74	1.73	1.79
Del12367	0.85	0.90	0.92	0.93	0.94	0.90
Del12387	1.78	1.72	1.75	1.81	1.78	1.89
Del12422	2.68	2.56	2.39	2.45	2.60	2.61
Del12419	4.60	4.93	4.98	4.93	4.96	5.11
Del12728	0.37	0.52	0.49	0.57	0.43	0.45
Del13551	0.45	0.70	0.73	0.53	0.53	0.62
L858R	0.04	0.06	0.15	0.23	0.21	0.34
L861Q	1.19	1.42	1.43	1.22	1.44	1.46
G719A	2.96	2.56	2.28	2.26	2.08	2.34
S768I	3.10	2.84	2.63	2.74	2.71	2.85
Ins12377	2.07	2.12	1.98	2.33	2.35	2.21
Ins12378	0.23	0.48	0.28	0.37	0.24	0.21

The dilution levels were actually different for Ins12376 than the other mutation samples, and these are reported in a separate table below. Dilution points are labeled as relative percent DNA input level, with the undiluted FFPE cell line specified as 100%.

**Mean Control, Mutant and ΔCt Values for Insertions 12376 across the Control Ct Range – FFPE cell lines**

Sample Mutation		Relative DNA Input Level					
		1.80%	4.01%	8.62%	20.04%	45.04%	100.00%
Ins12376	Control Ct	31.69	30.48	29.25	27.96	26.84	25.65
Ins12376	Mutant Ct	34.86	33.62	32.06	30.95	29.78	28.75
Ins12376	Delta Ct	3.16	3.13	2.81	3.00	2.94	3.10

**4. Linearity**

a. **Amplification Efficiency as a Function of DNA Input**

The linearity and amplification efficiency of PCR for each mutation assay, relative to the Control Reaction, across the working range of the EGFR Kit was investigated. Amplification efficiency was calculated using linear regression with assay Ct as the response variable and log<sub>2</sub> relative DNA input level as the explanatory variable for 19 EGFR mutations detected by the EGFR Kit including all the 7 EGFR mutation assays, and the Control Reaction.

$$\text{Amplification Efficiency} = [2^{**(-1 / \text{slope})}] - 1$$

(\*\* indicates to the power of)

EGFR mutations were tested targeting the lower end of the Control Reaction working range (~25Ct, high DNA input) and were serially diluted with ATE buffer, effectively diluting the input DNA and mutant DNA equally. The final dilution point was outside of the lowest DNA input level of the working range, Ct ~32-33Ct. The largest difference in the amplification efficiencies (AE) between the Control Reaction and a mutant reaction was observed for the exon 19 deletion DEL6218 (mean difference in efficiencies approximately 12.2%). The amplification efficiency of the control compared to the mutation assay indicates that the ΔCt, and thus mutation call, is consistent across the working range of the assay. A summary of the data is shown below.

### Assay Linearity as a Function of DNA Input

FFPE Cell Line	Assay Ct	Intercept	Intercept Standard Error	Slope Estimate	Slope Standard Error	Slope Two-Sided 95% CI	Amplification Efficiency(AE)	Difference in AE
DEL12367	Control Ct	31.868	0.058	-1.083	0.015	-1.114,-1.052	0.896	.
	Mutant Ct	32.756	0.068	-1.076	0.018	-1.112,-1.04	0.904	-0.008
DEL12387	Control Ct	31.828	0.047	-1.077	0.012	-1.102,-1.053	0.903	.
	Mutant Ct	33.579	0.056	-1.062	0.015	-1.092,-1.033	0.920	-0.017
DEL12419	Control Ct	31.686	0.073	-1.097	0.019	-1.135,-1.058	0.882	.
	Mutant Ct	36.430	0.071	-1.040	0.018	-1.077,-1.002	0.948	-0.066
DEL12422	Control Ct	31.935	0.050	-1.088	0.013	-1.114,-1.061	0.891	.
	Mutant Ct	34.490	0.127	-1.091	0.033	-1.159,-1.023	0.888	0.003
DEL12728	Control Ct	31.731	0.036	-1.052	0.009	-1.071,-1.033	0.933	.
	Mutant Ct	32.190	0.069	-1.047	0.018	-1.084,-1.01	0.939	-0.006
DEL13551	Control Ct	31.441	0.040	-1.029	0.010	-1.05,-1.008	0.961	.
	Mutant Ct	32.028	0.051	-1.026	0.013	-1.053,-0.999	0.965	-0.003

**Assay Linearity as a Function of DNA Input (continued)**

FFPE Cell Line	Assay Ct	Intercept	Intercept Standard Error	Slope Estimate	Slope Standard Error	Slope Two-Sided 95% CI	Amplification Efficiency(AE)	Difference in AE
DEL6210	Control Ct	31.850	0.080	-1.093	0.021	-1.135,-1.051	0.885	.
	Mutant Ct	34.666	0.088	-1.077	0.023	-1.124,-1.031	0.903	-0.018
DEL6218	Control Ct	31.470	0.031	-1.030	0.008	-1.046,-1.014	0.960	.
	Mutant Ct	35.931	0.105	-1.139	0.027	-1.194,-1.083	0.838	0.122
DEL6220	Control Ct	32.283	0.054	-1.114	0.014	-1.143,-1.085	0.863	.
	Mutant Ct	31.446	0.057	-1.099	0.015	-1.129,-1.068	0.879	-0.016
DEL6223	Control Ct	31.917	0.098	-1.153	0.026	-1.206,-1.101	0.824	.
	Mutant Ct	33.791	0.154	-1.158	0.040	-1.24,-1.076	0.820	0.004
DEL6225	Control Ct	31.436	0.034	-1.045	0.009	-1.063,-1.027	0.941	.
	Mutant Ct	33.133	0.070	-1.034	0.018	-1.071,-0.997	0.955	-0.014
G719A	Control Ct	31.941	0.047	-1.031	0.012	-1.056,-1.007	0.958	.
	Mutant Ct	34.571	0.077	-1.113	0.020	-1.154,-1.072	0.864	0.094
INS12376	Control Ct	32.541	0.043	-1.043	0.010	-1.064,-1.022	0.944	.
	Mutant Ct	35.631	0.100	-1.060	0.024	-1.108,-1.013	0.923	0.021
INS12377	Control Ct	31.996	0.074	-1.130	0.019	-1.169,-1.09	0.847	.
	Mutant Ct	34.090	0.073	-1.098	0.019	-1.138,-1.059	0.879	-0.033
INS12378	Control Ct	31.791	0.061	-1.100	0.016	-1.133,-1.067	0.878	.
	Mutant Ct	32.132	0.036	-1.117	0.010	-1.137,-1.097	0.860	0.018
L858R	Control Ct	31.593	0.026	-1.053	0.007	-1.067,-1.039	0.931	.
	Mutant Ct	31.667	0.023	-1.017	0.006	-1.029,-1.005	0.977	-0.046
L861Q	Control Ct	31.963	0.060	-1.049	0.016	-1.081,-1.017	0.936	.
	Mutant Ct	33.267	0.064	-1.028	0.017	-1.062,-0.994	0.962	-0.026

### Assay Linearity as a Function of DNA Input (continued)

FFPE Cell Line	Assay Ct	Intercept	Intercept Standard Error	Slope Estimate	Slope Standard Error	Slope Two-Sided 95% CI	Amplification Efficiency(AE)	Difference in AE
S768I	Control Ct	31.721	0.037	-1.054	0.010	-1.073,-1.034	0.931	.
	Mutant Ct	34.609	0.088	-1.081	0.023	-1.129,-1.034	0.898	0.032
T790M	Control Ct	31.455	0.050	-1.075	0.013	-1.102,-1.049	0.905	.
	Mutant Ct	33.450	0.080	-1.090	0.021	-1.133,-1.048	0.889	0.017
WT	Control Ct	31.840	0.049	-1.089	0.013	-1.115,-1.063	0.890	.

#### b. Amplification Efficiency as a Function % Mutation

The objective of this study was to evaluate the linearity of each mutant assay across the working range of the assay, when the total amount of DNA is held constant but the percentage of mutant DNA is varied. To maintain an equivalent Control Ct across the dilution series, EGFR mutation positive FFPE cell line DNA was diluted with the wild-type FFPE cell line DNA. Dilution series at both the high DNA input (Control Ct ~26) and low DNA input (Control Ct ~29-30) were tested. The dilution factor used was dependent on the  $\Delta$ Ct value generated by the undiluted FFPE cell line. Different cell lines generated different  $\Delta$ Ct values. Combined with different cut-offs for each mutation assay, each cell line required a slightly different dilution series to ensure that the final dilution point was beyond the assay cut-off.

For each EGFR mutation test, pools of DNA sufficient for 6 replicates at each dilution level were prepared. The Ct and  $\Delta$ Ct data for each mutation at each dilution level were calculated. The Control Ct values corresponding to either ~26Ct or ~29-30 Ct were consistent over the dilution series of each mutation. A linear regression model was fitted to estimate the difference in mean  $\Delta$ Ct between the two DNA input levels. A plot of the  $\Delta$ Ct values was generated showing the data for both high and low DNA input levels on the same plot. The slope and 95% confidence intervals (95% CI) were reported. The study results are summarized in table below, which showed that dilution of mutations in a background of a constant amount of total DNA resulted in amplification efficiencies (“ $\Delta$ Ct Amplification Efficiency” column) that are mostly comparable ( $\pm$  10%) to the mutation amplification efficiency determined in the above linearity study (“Mutation Amplification Efficiency” column). Amplification efficiencies differing close to or greater than 10% are noted for Del6220, Del6223, G719A, Ins12377, and L861Q mutations.

## Assay Linearity as a Function of % Mutation

FFPE Cell Line	R-Square	Root Mean Square Error	Intercept Estimate	Slope Estimate	Difference In Estimated Mean $\Delta$ Ct Between DNA Input Levels	Difference In Estimated Mean $\Delta$ Ct Two-Sided 95% CI	$\Delta$ Ct Amplification Efficiency	Control Amplification Efficiency	Mutation Amplification Efficiency
Del12367	0.975	0.373	0.064	1.013	0.018	-0.192, 0.227	0.983	0.896	0.904
Del12387	0.970	0.376	-0.096	1.019	0.177	-0.040, 0.393	0.974	0.903	0.920
Del12419	0.945	0.277	-0.454	1.100	-0.162	-0.323, -0.001	0.878	0.882	0.948
Del12422	0.876	0.626	-0.009	1.066	-0.478	-0.844, -0.112	0.916	0.891	0.888
Del12728	0.945	0.650	-0.596	0.976	1.134	0.756, 1.512	1.035	0.933	0.939
Del13551	0.979	0.373	-0.394	1.027	0.473	0.265, 0.681	0.963	0.961	0.965
Del6210	0.888	0.571	-0.222	1.057	0.007	-0.325, 0.339	0.926	0.885	0.903
Del6218	0.914	0.418	-0.786	1.117	0.325	0.082, 0.568	0.860	0.960	0.838
Del6220	0.989	0.309	-0.351	1.015	0.341	0.160, 0.521	0.980	0.863	0.879
Del6223	0.966	0.368	0.855	1.008	-0.954	-1.195, -0.713	0.989	0.824	0.820
Del6225	0.975	0.336	-0.287	0.992	0.119	-0.074, 0.312	1.011	0.941	0.955
G719A	0.947	0.520	-0.083	1.033	0.310	0.021, 0.599	0.957	0.958	0.864
Ins12376	0.931	0.383	0.306	0.955	-0.081	-0.305, 0.143	1.066	0.944	0.923
Ins12377	0.957	0.353	0.078	1.016	-0.174	-0.381, 0.033	0.978	0.847	0.879
Ins12378	0.987	0.312	-0.079	1.122	-0.243	-0.420, -0.065	0.855	0.878	0.860
L858R	0.982	0.395	-0.053	1.067	0.435	0.213, 0.656	0.915	0.931	0.977
L861Q	0.943	0.634	-0.365	1.122	-0.104	-0.460, 0.251	0.855	0.936	0.962
S768I	0.950	0.458	0.459	1.048	-0.619	-0.917, -0.322	0.938	0.931	0.898
T790M	0.949	0.487	-0.050	1.146	-0.308	-0.579, -0.037	0.831	0.905	0.889

## 5. Analytical Specificity

### a. Primer and Probe Specificity

The primers and probes have been designed to avoid any known EGFR polymorphisms. A specificity analysis was conducted using the Basic Local Alignment Search Tool (BLAST) to ensure that the primers used in the EGFR Kit would amplify only human EGFR sequences and not sequences from other species or non-EGFR human sequences (e.g., pseudogenes). No non-specific amplification is predicted from non-EGFR genes. In addition, alignments of pairs of oligonucleotides (primers, probes, and templates) used in the EGFR Kit were performed to ensure there is no unexpected binding that could lead to non-specific amplification. There was no significant homology between the various reagents.

### b. Cross Reactivity to Other EGFR Mutations

Cross-reactivity of the *therascreen*® EGFR RGQ PCR Kit to other EGFR mutations was observed in the Phase 3 clinical trial specimens, FFPE cell lines and EGFR plasmids. The *therascreen*® EGFR RGQ PCR Kit gave “Mutation Detected” results for the following EGFR mutations in the

specific sample types indicated in the table below. Analytical performance of the *therascreen*® EGFR RGQ PCR Kit in detecting these mutations has not been evaluated for its intended use.

**Mutations Determined to Cross-React with the *therascreen*® EGFR RGQ PCR Kit**

<b>Exon 19 – Mutation Assay Del</b>		
<b>Mutation</b>	<b>COSMIC ID*</b>	<b>Sample Type</b>
2237_2251del15	12678	FFPE Clinical Trial Specimen <sup>^</sup>
2239_2247del9	6218	FFPE Cell Line
2236_2253del18	12728	FFPE Cell Line
2237_2254del18	12367	FFPE Cell Line
2240_2251del12	6210	FFPE Cell Line
<b>Exon 18 – Mutation Assay G719X</b>		
G719S	6252	FFPE Clinical Trial Specimen <sup>#</sup>
G719C	6253	Plasmid
<b>Exon 20 – Mutation Assay Ins</b>		
Insertion	12376	FFPE Cell Line

\*Catalogue of Somatic Mutations in Cancer (COSMIC), <http://www.sanger.ac.uk/genetics/CGP/cosmic>  
<sup>^</sup>The exon 19 deletion 12678 was observed in non-randomized study population of the 1200.32 clinical trial.  
<sup>#</sup>The exon 18 G719S mutation was observed in randomized study population of the 1200.32 clinical trial.

c. **Cross-Reactivity / Exclusivity**

The *therascreen*® EGFR RGQ PCR Kit is comprised of 8 separate reaction mixes: one single Control Reaction that detects a non-polymorphic region of the EGFR gene and seven mutation assays that detect EGFR mutations. There is no reaction that specifically measures the wild-type EGFR sequence at exons 18, 19, 20 or 21. The EGFR Kit “No Mutation Detected” result is determined from the absence of any positive mutation results.

The objective of this study is to demonstrate the amount of non-specific amplification, or cross reactivity that occurs in each reaction with excess amounts of EGFR wild-type DNA to ensure no false positive results occur. Similarly, non-specific amplification of EGFR mutations does not result in erroneous mutation calls in the presence of excess amounts of mutant DNA. Since the DNA input for this assay is based on the Control Ct range (23.70 – 31.10), the highest concentration of DNA input is based on having a Control Ct value of approximately 25. FFPE cell lines were used for this evaluation.

- i. **Non-Specific Amplification/Cross Reactivity: Wild-type EGFR DNA** – To address the amount of non-specific amplification of wild-type EGFR DNA by reaction mixes designed to amplify

specific mutations, 60 replicates of wild-type FFPE cell line DNA at approximately the highest concentration of amplifiable DNA input level were evaluated using the EGFR Kit. The Control Ct values were approximately 25 Ct. These results demonstrated that the lowest  $\Delta$ Ct values exceeded the established cut-offs indicating that non-specific amplification was not observed. The lowest  $\Delta$ Ct values observed for each reaction are shown below.

**Lowest Mean  $\Delta$ Ct Observed for Wild-type Samples in Mutant Reactions**

Assay	Cut-off	Lowest $\Delta$ Ct Observed
T790M	$\leq 7.4$	12.77
Deletions	$\leq 8.0$	13.25
L858R	$\leq 8.9$	*
L861Q	$\leq 8.9$	13.34
G719X	$\leq 8.9$	*
S768I	$\leq 8.9$	*
Insertions	$\leq 8.0$	*

\*denotes no  $\Delta$ Ct value.

- ii. **Non-Specific Amplification/Exclusivity: Mutation positive EGFR DNA** – The exclusivity of the EGFR Kit is intended to discriminate between mutation negative and mutation positive status. Mutant samples with a high concentration of input DNA were tested against all reaction mixes by preparing DNA samples from FFPE cell lines (19 EGFR mutations detectable by the EGFR Kit) such that the Control Reaction Ct value corresponded to approximately 25. Sixty (60) replicates of each mutation sample were evaluated. The percentage of mutation in the sample was governed by the percentage of mutant in the cell line DNA. The minimum  $\Delta$ Ct for each mutant is presented in the table below and demonstrates that there is no impact due to the cross reactivity between mutant reactions as the minimum  $\Delta$ Ct values were all higher than the respective assay cut-off values for all non-matching reaction mixes and mutant DNA samples.

## Cross-Reactivity between Mutation Assays Using FFPE Cell Line DNA

		Cut-Off	Mutant Reaction						
			Deletions	G719X	Insertions	L858R	L861Q	S768I	
Mutant DNA	DEL12367	8.0	0.31	*	*	*	*	*	12.07
	DEL12387	8.0	1.02	*	*	*	*	*	11.89
	DEL12419	8.0	4.19	*	*	13.57	*	13.96	11.67
	DEL12422	8.0	2.03	*	*	*	*	*	12.89
	DEL12728	8.0	0.31	*	*	*	*	*	13.03
	DEL13551	8.0	-0.16	*	*	*	*	*	12.09
	DEL6210	8.0	2.16	*	*	*	13.87	*	10.93
	DEL6218	8.0	2.83	*	*	12.79	10.2	*	10.9
	DEL6220	8.0	-1.01	*	*	11.46	*	*	11.37
	DEL6223	8.0	1.27	*	*	14.34	*	*	12.7
	DEL6225	8.0	0.24	*	*	*	*	*	12.52
	G719A	8.9	11.91	1.62	*	*	13	*	12.47
	INS12376	8.0	13.21	*	2.09	*	*	*	11.82
	INS12377	8.0	12.27	*	1.5	*	13.11	*	11.93
	INS12378	8.0	13.71	*	-0.61	*	*	*	11.09
	L858R	8.9	13.68	*	14.2	-0.83	14.39	*	12.46
	L861Q	8.9	10.36	*	*	*	0.88	*	13.27
	S768I	8.9	13.81	*	13.8	10.93	13.48	2.46	11.49
	T790M	7.4	14.43	*	14.34	*	14.39	*	1.59

Minimum  $\Delta$ Cts from matched reactions are shaded. Blank cells indicate no cross reactivity  
 \* indicates no  $\Delta$ Ct value

### 6. Interference – Effects of Necrotic Tissue

To evaluate the potential interference of necrotic tissue content in NSCLC FFPE specimens on the performance of the EGFR Kit, FFPE clinical specimens from the 1200.32 clinical trial with both EGFR Kit test results and Sager sequencing results were analyzed. A total of 66 EGFR mutant specimens (i.e., 30 exon 19 deletion, 32 L858R, 1 G719X, 1 exon 20 insertion, 1 L861Q, and 1 S768I specimens) and 81 wild-type specimens were evaluated. Percent necrosis, as identified by a pathologist, varied from 0-50% for both mutant and wild-type FFPE specimens.

For both mutant and wild-type FFPE specimens, all except 2 samples have EGFR Kit results that matched the expected Sanger sequencing results. The two mismatched results were from one wild-type and one mutant samples with less than 10% necrotic content; thus, it is unlikely that necrosis was the reason for the discordant result. The overall percent correct calls were 98.5% (65/66) and 98.8% (80/81) for mutant and wild-type FFPE specimen, respectively. The results support the use of the EGFR Kit with NSCLC FFPE specimen with necrotic tissue content up to 50%.

### 7. Interference – Exogenous Substance

To evaluate the impact of interfering substance on performance of the EGFR Kit, potentially interfering substances present in the DNA extraction process, were tested at 10x concentration in mutant and wild-type samples with a target Ct value of approximately 27.5. The impact of each substance on the  $\Delta$ Ct

values and mutation status of the samples was assessed. The substances tested were (1) paraffin wax, (2) xylene, (3) ethanol, and (4) Proteinase K. The difference between the  $\Delta$ Ct of samples with interferent was compared to samples without interferent according to statistical methods outlined in CLSI guidance document EP7-A2. For mutant samples, of the 672 replicates tested (7 mutation assays x 8 substances x 3 levels of interfering substance x 4 replicates), there were no invalid or indeterminate results and no false mutation negative results. For wild-type samples, 0 of the 96 replicates tested were invalid or indeterminate and there were no false mutation detected results. The results demonstrated that these substances did not interfere with the *therascreen*® EGFR RGQ PCR Kit call results.

#### **8. Repeatability and Reproducibility**

The repeatability and reproducibility of the EGFR Kit was investigated by testing DNA extracted from NSCLC FFPE clinical specimens or FFPE cell lines, representing all 7 mutation assays in the EGFR Kit. NSCLC wild-type FFPE clinical specimens were also included in the study. Reproducibility was conducted across three sites (i.e., United Kingdom, Germany, and USA). At each site, samples were tested in duplicate (for within-run repeatability assessment), on 2 different RGQ instruments, using 2 operators and 2 EGFR Kit lots (3 lots across 3 sites) over a total of 16 days. Reproducibility for each individual mutation was conducted over non-consecutive days at each site. This resulted in a total of 32 data points for each test sample at each site, and a total of 96 data points for each EGFR mutation per mutation level. One lot of QIAGEN QIAamp® DSP DNA FFPE Tissue Kit was used to extract DNA from FFPE samples. Samples were prepared to have low DNA input levels where a control Ct value of approximately 30.10 was targeted. A sample pool for each mutation was created from a single specimen, and the wild-type pool comprised 5 different specimens combined.

For each sample, the proportions of correct mutation calls and the lower one-sided exact 95% confidence intervals (95% CI) were reported in table below. There were no “Mutation Detected” results in 84 valid tests of wild-type sample, producing 100% correct calls. The percentage of correct calls ranged from 96% -100% for mutant samples tested at 1-3x LoD across sites.

### Assay Reproducibility – Proportion of Correct Calls for EGFR Mutation Tested

Exon	Mutation	COSMIC ID	% Mutation Tested	% Mutation Tested Relative to Final LoD Claim	Number of Valid Results (N)	Correct Calls (N)	% Correct Calls	% Correct Call Lower One-Sided 95% CI
	Wild-type	NA			84	84	100.00	96.50
19	Deletions	6220*	5.69%	2-3x LoD	96	96	100.00	96.93
		6223	15.99%	2-3x LoD	95	95	100.00	96.90
		6225	7.06%	1-2x LoD	95	91	95.79	90.62
		6254	10.02%	LoD	92	92	100.00	96.80
		6255	0.81%	LoD	96	94	97.92	93.59
		12369	9.29%	1-2x LoD	95	95	100.00	96.90
		12370	8.06%	LoD	63^	62	98.41	92.69
		12382	1.45%	LoD	95	92	96.84	92.04
		12383	8.43%	1-2x LoD	93	93	100.00	96.83
		12384	7.54%	LoD	92	92	100.00	96.80
		12387*	9.53%	1-2x LoD	95	95	100.00	96.90
		12419*	28.75%	1-2x LoD	83	83	100.00	96.46
		12422	7.85%	2-3x LoD	94	94	100.00	96.86
13551*	11.12%	2-3x LoD	95	95	100.00	96.90		
21	L858R	6224	5.77%	LoD	92	92	100.00	96.80
20	T790M <sup>#</sup>	6240	34.02%	1-2xLoD	94	94	100.00	96.86
21	L861Q <sup>#</sup>	6213	9.24%	LoD	84	83	98.81	94.48
18	G719A <sup>#</sup>	6239	32.50%	LoD	78	77	98.72	94.06
20	S768I <sup>#</sup>	6241*	11.57%	1-2x LoD	82	82	100.00	96.41
20	Insertions <sup>#</sup>	12377*	10.45%	2-3x LoD	93	93	100.00	96.83
		12378*	19.96%	LoD	92	92	100.00	96.80

^Control Ct for deletion 12370 at LoD dropped out of the working range and no data could be generated at one site (n=32). The missing data from this site for this mutation was not retested due to lack of sample availability.

\*Reproducibility for these mutations was conducted using FPPE cell lines.

**#Safety and efficacy of GILOTRIF™ (afatinib) has not been established for patients with these EGFR mutations. Refer to GILOTRIF™ (afatinib) drug labeling for more details.**

A variance component analysis was used to estimate the standard deviation and 95% confidence intervals for within-run, between-run, between-day, between-lot and between-site variability. These estimates were reported along with %CV and the number of observations for the mean of  $\Delta C_t$ , Control Ct, and Mutant Ct values. Results by variance components and total variance are presented below for  $\Delta C_t$ . The column “N” included the number of data points that generated a  $\Delta C_t$  value. Across all variance components, the total coefficient of variation (CV) was  $\leq 14.11\%$  in all EGFR mutations tested. Across all mutant panel members, the %CV was in general  $< 6\%$  for between-lots, between lots, between days, and between runs. The %CV for within-run (repeatability) ranged from 5.99% to 13.49%.

**Overall Mean, Standard Deviation (SD), and Coefficient of Variation (% CV) for  $\Delta$ Ct – Between Lot, Between Site, and Total Variance**

Exon	Mutation	COSMIC ID	LoD Level Tested	N	Mean	Between Lot (SD)	Between Lot (%CV)	Between Site (SD)	Between Site (%CV)	Total (SD)	Total (%CV)
19	Deletions	6220	2-3x LoD	96	4.57	0.00	0.00	0.07	1.64	0.62	13.56
		6223	2-3x LoD	95	3.59	0.00	0.00	0.20	5.46	0.39	10.95
		6225	1-2x LoD	94	6.06	0.35	5.79	0.00	0.00	0.86	14.11
		6254	LoD	92	4.91	0.00	0.00	0.05	1.03	0.47	9.48
		6255	LoD	96	6.11	0.00	0.00	0.00	0.00	0.68	11.20
		12369	1-2x LoD	95	4.83	0.10	2.02	0.17	3.60	0.51	10.55
		12370	LoD	62	6.15	0.00	0.00	0.00	0.00	0.64	10.48
		12382	LoD	94	6.25	0.00	0.00	0.04	0.66	0.79	12.61
		12383	1-2x LoD	93	4.71	0.00	0.00	0.09	1.85	0.40	8.58
		12384	LoD	92	4.31	0.00	0.00	0.00	0.00	0.46	10.62
		12387	1-2x LoD	95	4.81	0.00	0.00	0.02	0.33	0.58	12.13
		12419	1-2x LoD	83	5.64	0.00	0.00	0.17	3.07	0.52	9.16
		12422	2-3x LoD	94	4.07	0.00	0.00	0.10	2.49	0.33	8.00
13551	2-3x LoD	95	4.46	0.03	0.59	0.12	2.74	0.60	13.39		
21	L858R	6224	LoD	92	4.77	0.00	0.00	0.19	4.00	0.55	11.59
20	T790M <sup>#</sup>	6240	1-2xLoD	94	4.74	0.00	0.00	0.25	5.22	0.49	10.34
21	L861Q <sup>#</sup>	6213	LoD	84	6.04	0.00	0.00	0.11	1.79	0.76	12.56
18	G719A <sup>#</sup>	6239	LoD	78	5.73	0.00	0.00	0.11	1.86	0.63	11.06
20	S768I <sup>#</sup>	6241	1-2x LoD	82	5.30	0.00	0.00	0.12	2.28	0.55	10.31
20	Insertions <sup>#</sup>	12377	2-3x LoD	93	4.14	0.00	0.00	0.00	0.00	0.42	10.05
		12378	LoD	92	4.9	0.00	0.00	0.00	0.00	0.57	11.56

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**Overall Mean, Standard Deviation (SD), and Coefficient of Variation (% CV) for ΔCt – Within Run, Between Run, and Between Day**

Exon	Mutation	COSMIC ID	LoD Level Tested	N	Mean	Within Run (SD)	Within Run (%CV)	Between Run (SD)	Between Run (%CV)	Between Day (SD)	Between Day (%CV)
19	Deletions	6220	2-3x LoD	96	4.57	0.62	13.49	0.00	0.00	0.00	0.00
		6223	2-3x LoD	95	3.59	0.35	9.73	0.00	0.00	0.08	2.37
		6225	1-2x LoD	94	6.06	0.78	12.91	0.00	0.00	0.21	3.47
		6254	LoD	92	4.91	0.45	9.11	0.1	1.95	0.08	1.6
		6255	LoD	96	6.11	0.68	11.20	0.00	0.00	0.00	0.00
		12369	1-2x LoD	95	4.83	0.48	9.90	0.00	0.00	0.00	0.00
		12370	LoD	62	6.15	0.5	8.10	0.41	6.71	0.00	0.00
		12382	LoD	94	6.25	0.79	12.60	0.00	0.00	0.00	0.00
		12383	1-2x LoD	93	4.71	0.40	8.39	0.04	0.95	0.00	0.00
		12384	LoD	92	4.31	0.36	8.43	0.23	5.40	0.17	3.97
		12387	1-2x LoD	95	4.81	0.57	11.91	0.11	2.3	0.00	0.00
		12419	1-2x LoD	83	5.64	0.4	7.05	0.32	5.63	0.00	0.00
		12422	2-3x LoD	94	4.07	0.28	6.98	0.14	3.37	0.00	0.00
13551	2-3x LoD	95	4.46	0.55	12.42	0.20	4.52	0.00	0.00		
21	L858R	6224	LoD	92	4.77	0.41	8.65	0.34	7.04	0.02	0.47
20	T790M <sup>#</sup>	6240	1-2xLoD	94	4.74	0.28	5.99	0.29	6.08	0.20	4.21
21	L861Q <sup>#</sup>	6213	LoD	84	6.04	0.66	10.95	0.36	5.89	0.08	1.32
18	G719A <sup>#</sup>	6239	LoD	78	5.73	0.41	7.17	0.48	8.33	0.00	0.00
20	S768I <sup>#</sup>	6241	1-2x LoD	82	5.30	0.48	9.02	0.20	3.72	0.15	2.82
20	Insertions <sup>#</sup>	12377	2-3x LoD	93	4.14	0.42	10.05	0.00	0.00	0.00	0.00
		12378	LoD	92	4.90	0.48	9.83	0.30	6.10	0.05	1.08

<sup>#</sup>Safety and efficacy of GILOTRIF™ (afatinib) has not been established for patients with these EGFR mutations. Refer to GILOTRIF™ (afatinib) drug labeling for more details.

**9. Lot-to-Lot Reproducibility**

This study addressed the potential for lot-to-lot variability to impact the mutation detection by the EGFR Kit. A maximum of three FFPE clinical specimens or FFPE cell lines representing each of the seven mutation assays of the EGFR Kit were used in this study, along with 21 wild-type samples. The EGFR Kit test system utilizes two separate kits: (1) The QIAamp® DSP DNA FFPE Tissue Kit for isolation of DNA from NSCLC FFPE specimens, and (2) the *therascreen*® EGFR RGQ PCR Kit for the amplification and detection of the isolated DNA for its EGFR mutation status. FFPE mutant and wild-type specimens were tested with 3 lots of the QIAamp® DSP DNA FFPE Tissue Kit and 3 lots of the *therascreen*® EGFR RGQ PCR Kit.

Sections from each FFPE clinical specimen or FFPE cell line were extracted in triplicate, and extracted DNA was tested with the Control assay and the corresponding mutation assay. The samples were tested in duplicate giving 18 results for each sample per EGFR Kit. This produced a maximum of 54 data points in total per mutation assay. The exceptions to this were S768I, which only had one representative sample and gave 18 data points, and the Insertions, which had two representative samples and gave a total of 36 data points. Only valid results were included in the analysis. The test results were analyzed for

each mutation assay and also by the kit lots. The overall percentage of correct calls across lots for EGFR mutation assay was 97.8% (317/324) and that for wild-type samples was 100% (379/379). The Control Ct, Mutant Ct and  $\Delta$ Ct values for each specimen were summarized across lots and no trend in Ct or  $\Delta$ Ct values were observed.

#### **10. Specimen Handling – Reproducibility**

The objective of this study was to assess sample handling variability as part of the EGFR Kit test system process. The reproducibility of the QIAamp® DSP DNA FFPE Tissue Kit was examined using sections taken from three FFPE specimen blocks, one containing an exon 19 deletion mutation (2235-2249 del15), one containing the exon 21 L858R mutation (2573T>G), and one that is wild-type. From the mutant FFPE clinical specimens, 48 sequential FFPE slide sections were randomized and divided into four batches where one batch of slide sections was used per site and one batch was kept as a contingency batch. For the wild-type FFPE clinical specimen, 36 sequential FFPE slide sections were randomized and divided into three batches where one batch of slide sections was used per site.

For each specimen, extractions were carried out in duplicate at each test site and tested on three non-consecutive days over a period of six days across three sites, yielding a total of 18 data points per specimen. At each site, two operators conducted the testing using one lot of the QIAamp® DSP DNA FFPE Tissue Kit (one lot per site, 3 lots total) in combination with the same lot of the EGFR Kit reagents across sites. One RGQ instrument was used to conduct the testing at Site 1 and two RGQ instruments were used at Sites 2 and 3. The acceptance criterion was that each clinical specimen should give the correct mutation call at least 17 out of 18 times. All mutant and wild-type specimen results were valid and yielded the expected call result (correct call =100%, 18/18 for each specimen), supporting the reproducibility and repeatability for the *therascreen*® EGFR RGQ PCR Kit at the pre-analytical step of DNA isolation

#### **11. Guard banding**

The objective of the guard banding studies was to establish the robustness of the EGFR Kit. The following studies were conducted to assess proteinase K digestion step for the QIAamp® DSP DNA FFPE Tissue Kit and the RGQ PCR cycling parameters for the *therascreen*® EGFR RGQ PCR Kit.

##### **a. Proteinase K**

This study was designed to determine the effect on the mutation Ct and  $\Delta$ Ct when varying the 56°C and 90°C incubation times and temperatures during the extraction of FFPE samples. FFPE sample extractions were performed using the QIAamp® DSP DNA FFPE Tissue Kit according to the Instruction For Use (IFU) provided with the kit. According to the IFU, the extraction process has two incubation steps: 1) Incubate at 56°C  $\pm$  3°C for  $\geq$ 1 hour, and 2) Incubate at 90°C  $\pm$  5°C for 1 hour  $\pm$ 5 minutes.

A total of 8 samples representing the Control and the seven mutation assays were tested in singlet at 26 experimental conditions, including variation of length of time and the temperature of the two incubation steps using a central composite design. FFPE clinical specimens were used for the exon 19 deletion assay and the exon 21 L858R assay, while FFPE cell lines were used for the other 5 mutation assays.

A total of 187 mutation detected calls of repeated sample testing at varying cycling tolerances were reported, with 5 of the 187 as false positive calls. The conditions which resulted in these false positive calls were all  $\pm 5^{\circ}\text{C}$  away from the standard conditions for both incubation steps. However, the data generated shows that varying the incubation temperatures by  $\pm 3^{\circ}\text{C}$  can be tolerated with a maximum shift of 0.40  $\Delta\text{Ct}$  observed across all the variables, from the standard condition. The results indicate that varying the incubation temperatures by  $\pm 5^{\circ}\text{C}$  cannot be tolerated; however, varying the time by  $\pm 10$  minutes can be tolerated. Thus, the EGFR Kit IFU recommendations are incubation at  $56^{\circ}\text{C} \pm 3^{\circ}\text{C}$  for 1 hour  $\pm 5$  minutes, followed by incubation at  $90^{\circ}\text{C} \pm 3^{\circ}\text{C}$  for 1 hour  $\pm 5$  minutes.

b. **Thermal Cycling Profile**

The study objective was to determine effect of mutation calling by varying the thermal cycling profile of the RGQ instrument, using FFPE clinical samples. Three FFPE clinical specimens, one containing an exon 19 deletion mutation (Del6223 or 2235-2249 del15), one containing an L858R mutation (2573T>G), and one that is wild-type, were processed to isolate DNA on the same day using the QIAamp® DSP DNA FFPE Tissue Kit. The standard cycling condition for the *therascreen*® EGFR RGQ PCR Kit is as follows:

- Activation:  $95^{\circ}\text{C}$  for 15 minutes
- Denaturation:  $95^{\circ}\text{C}$  for 30 seconds
- Annealing:  $60^{\circ}\text{C}$  for 1 minute

The thermal cycling profile was guard banded by varying  $\pm 2^{\circ}\text{C}$ , in  $1^{\circ}\text{C}$  steps, during activation, denaturation and annealing steps. A full 5 by 5 factorial design was used to test a total of 25 PCR conditions using 5 RGQ instruments over 4 days. Each extraction sample was tested in triplicate using the control assay to derive a mean Ct, and further normalized to a target Control Ct  $\sim 30$ . Three replicates of each specimen pool were then tested for each condition using a single reagent lot of the EGFR Kit. For each condition with temperatures within  $\pm 1^{\circ}\text{C}$  from the standard condition, the mutation positive  $\Delta\text{Ct}$  should be  $\pm 1.5$  Ct from the standard condition. All observed mutation calls matched the expected results when the cycling temperature shifts by  $\pm 1^{\circ}\text{C}$ . Across all variables, a maximum  $\Delta\text{Ct}$  shift of 0.74 was observed for the exon 19 deletion assay and a maximum  $\Delta\text{Ct}$  shift of 0.46 was observed for the exon 21 L858R mutation assay. All

wild-type samples showed amplification in the Control assay, and no positive mutation calls were made. The results showed that the *therascreen*® EGFR RGQ PCR Kit is able to tolerate variations of  $\pm 1^\circ\text{C}$  of the thermal cycling profile activation, denaturation and annealing temperatures.

c. **PCR Amplification Mix**

The study objective was to determine effect of mutation calling and tolerance of the EGFR Kit to volumetric variations of each PCR components in the PCR amplification mixture. Three FFPE clinical specimens, one containing an exon 19 deletion mutation (Del 6223, also known as 2235-2249 del15), one containing an L858R mutation (2573T>G), and one that is wild-type. The same DNA samples prepared for the thermal cycling guard banding study above were utilized, with the target Control Ct  $\sim 30$ . The volume of each PCR component (PCR mix, *Taq* polymerase, Master mix and DNA sample) was varied incrementally up to  $\pm 20\%$  while keeping all other components at the standard volumes as stated in the IFU. A total of 22 test conditions were analyzed, including 2 test conditions combining extreme variations in pipetting most likely to result in a false positive or false negative result. Each DNA sample type (Del 6223, L858R, wild-type) was investigated on separate runs, and tested a total of 3 times to achieve 3 replicates per test condition.

Across all test conditions and samples tested, all mutation calls were correct with one exception with the wild-type sample, which might be due to low copy number contamination. Study acceptance criteria was that differences in mean  $\Delta\text{Ct}$  values between the different PCR component levels should be  $\leq 1.34$  for the Deletions assay and  $\leq 1.56$  for the L858R assay. For the Deletions assay, all test conditions passed the acceptance criteria, with exception to one test condition (*Taq* at  $0.4\ \mu\text{L}$ ). This exhibited an estimated difference in mean  $\Delta\text{Ct}$  of  $-1.475$  which is  $0.135\ \Delta\text{Ct}$  above the acceptance criterion. This indicates that the Deletions assay cannot tolerate a *Taq* volume error of  $-20\%$  from the standard condition ( $0.5\ \mu\text{L}$ ). For the L858R assay all test conditions passed the acceptance criteria. This indicates that the volume of each variable can be varied up to  $\pm 20\%$ . As one test condition generated a result that did not meet the study acceptance criterion, a volume increment of  $\pm 10\%$  will be recommended within the IFU. The study results confirm that the EGFR Kit is able to tolerate volume differences of  $\pm 10\%$  from the standard condition across all variables individually (i.e., PCR mix, *Taq* polymerase, Master mix and DNA sample).

## **12. Cross-Contamination**

Studies were performed to demonstrate the absence of cross-contamination between test samples. Two FFPE cell lines were used for this study: one contains wild-type cells only, and the other contains cells harboring the exon 21

L858R mutation. The exon 21 L858R mutation was selected for this study because it is the most common EGFR mutation. The study was aimed to mimic the most likely situation where a high level L858R mutation could cross contaminate other samples within the assay or run. The key selection criterion for the mutation sample was that it had high mutation content. All sections had high mutation content as illustrated with the low  $\Delta$ Cts of the slides (min -0.23, max 0.89).

The study was conducted over five consecutive days, where DNA extraction was conducted to challenge the procedure by extracting L858R mutant DNA followed by extraction of wild-type DNA using one lot of reagents and one RGQ instrument. Two sections were used per DNA extraction where 87.1% of the extractions were performed on serial sections. The study consisted of ten test runs designed to investigate the potential for contamination both within and between runs. Results were summarized by Ct and  $\Delta$ Ct. From the valid L858R and wild-type samples used, the results showed the expected mutation call when tested with the EGFR Kit, that wild-type was “No Mutation Detected” and L858R was ‘L858R detected’. The analysis showed that there were no false positive calls from the 330 valid wild-type replicates. The results of this study indicate no detectable cross contamination for both within-runs and between-runs.

### 13. Stability Studies

#### a. Clinical Specimen, Slide-Mounted

To assess the stability of slides prepared from NSCLC FFPE tissue samples to determine limits of suitability for the *therascreen*® EGFR RGQ PCR Kit, slide sections were prepared from representative NSCLC FFPE specimens, one wild-type, one exon 19 deletion (Del 6223 or 2235-2249 del15) and one exon 21 L858R mutant, mounted onto glass slides, and stored in the dark at room temperature for up to 4 weeks. The acceptance criteria were that, at each time point (i.e., week 1, 2, and 4), DNA extracted from FFPE slides would be considered stable when a change of less than 1  $\Delta$ Ct relative to the corresponding baseline time point zero (i.e., T0) was observed. For each time point tested, DNA was extracted from slides, pooled and tested in 5 replicates across 3 runs.

The wild-type FFPE slides gave “No Mutation Detected” results for all replicates at all four time points. The mutant FFPE slides gave “Mutation Detected” results for all replicates at all four time points. All slides met the stability criteria across all time points. No trend in Ct values was detected over these testing conditions. These results indicate that slide sections are stable up to 4 weeks for storage at room temperature in the dark prior to testing with the *therascreen*® EGFR RGQ PCR Kit.

b. **Extracted DNA From FFPE Specimens**

Stability of the DNA extracted from FFPE clinical specimens was demonstrated in P110030 approval of the *therascreen*® KRAS RGQ PCR Kit.

c. **Reagents and Shipping**

Three NSCLC FFPE clinical specimens, one wild-type, one exon 19 deletion (Del 6223 or 2235-2249 del15, % mutation at 2.5x LoD) and one exon 21 L858R mutant (% mutation at 7.3x LoD) were used to conduct the EGFR Kit reagent and shipping stability study. DNA was extracted from mutant specimens and adjusted to contain a low DNA input using DNA extracted from wild-type FFPE clinical specimens. The wild-type specimen was used at a high DNA input level to allow assessment of any nonspecific amplification across the course of the study, and to monitor any stability related effect.

The stability study conducted for the *therascreen*® EGFR RGQ PCR Kit included:

- Real-time storage conditions for closed bottle and open bottle (simulating multiple uses by the operators) conditions.
- Open bottle light sensitivity condition (i.e., reaction mix + *Taq* polymerase = master mix), because fluorescently-labeled Scorpions in the reaction mix reagents are light sensitive.
- Transport conditions (extreme temperatures during storage and shipping, including inversion of reagents).
- Stress (freeze/thaw) conditions. The stress test conditions are defined as -15°C to -30°C (frozen condition) and 15°C to 25°C (thaw condition, bench-top).

**Overview of Stability Studies Conditions and Temperatures**

Study*	Storage condition		Cycling condition	
	definition	temperature	definition	temperature
Closed bottle	Real time	-15°C to -30°C	n.a.	n.a.
Open bottle	Real time	-15°C to -30°C	n.a.	n.a.
Open bottle (light sensitivity)	Ambient temperature	Ambient temperature	n.a.	n.a.
Transport simulation study EGFR Kit	Real time	-15°C to -30°C	Extreme cold	On dry ice
			Interim storage	-15°C to -30°C
			Thawing*	-15°C to 25°C
			Freezing	-15°C to -30°C
Stress test	Real time	-15°C to -30°C	Thawing	-15°C to 25°C
			Freezing	-15°C to -30°C

\* Thawing at ambient temperature; 50% of the bottles will be inverted.

Three independent lots of the EGFR Kit, manufactured according to standard procedures, were used for this study, with the exception of the transport stability study, which used one EGFR Kit lot. For each specimen, testing was conducted in duplicate with the exception of the open bottle (multi-use) study, for which each specimen was run only once. Real-time stability testing was conducted up to 3+ months and is planned to continue to 25 months.

For stability studies of real-time closed bottle, open bottle, and open bottle light sensitivity conditions, the acceptance criteria for each time point are as follows.

- (1) correct call is made (i.e., relevant mutation should be detected for the mutant specimens and “No Mutation Detected” call for the wild-type specimen).
- (2) For all clinical specimens, the linear regression lines and corresponding 95% confidence intervals fall within the 23.7 Ct to 31.1 Ct range for the Control Ct.
- (3) For the mutant clinical specimens, the linear regression lines and corresponding 95% confidence intervals fall within the 23.7 Ct to 40.0 Ct range for the Mutant Ct and are below the  $\Delta$ Ct cut-offs (8.00  $\Delta$ Ct for Deletions and 8.90  $\Delta$ Ct for L858R).
- (4) For the Positive Control, the linear regression lines and corresponding 95% confidence intervals fall within the acceptable Positive Control Ct ranges for all reaction mixes.
- (5) For the Internal Control, the linear regression lines and corresponding 95% confidence intervals fall within the acceptable Ct range of 29.85 Ct to 35.84 Ct.

For stability studies of transport and stress conditions, the acceptance criteria for each time point are as follows.

- (1) correct call is made (i.e., relevant mutation should be detected for the mutant specimens and “No Mutation Detected” call for the wild-type specimen).
- (2) For all clinical specimens, 95% confidence intervals of the mean for each of inverted and upright tubes at each of the post-cycle time points and pre-cycle baseline fall within the 23.7 Ct to 31.1 Ct range for the Control Ct values, the 23.7 Ct to 40.0 Ct range for the mutant Ct values, and are below the  $\Delta$ Ct cut-offs (8.00  $\Delta$ Ct for Deletions and 8.90  $\Delta$ Ct for L858R).
- (3) For the Positive Control, all Ct values fall within the acceptable ranges for each of the eight reaction mixes.
- (4) For the Internal Control, the 95% confidence interval of the means, for all eight reaction mixes analyzed together, at the post-cycle time points and pre-cycle baseline fall within the 29.85 Ct to 35.84 Ct range.

Real-time closed bottle stability data support 3 month expiry for the *therascreen*® EGFR RGQ PCR Kit when stored at -15°C to -30°C. The open bottle stability study results support 90 day in-use stability. The open bottle light sensitivity study results demonstrate 3 hour stability of the master mixes when exposed to light. Current data also demonstrate EGFR Kit stability after 12 freeze/thaw cycles for 49 days, and after simulated transport to the customer, and then stored at the customer site, for upright and inverted bottles for 50 days.

**B. Animal Studies**

None.

**C. Additional Studies**

None.

## **X. SUMMARY OF PRIMARY CLINICAL STUDY**

The Phase 3 study for afatinib (also known as the 1200.32 clinical trial or the LUX-Lung 3), and the bridging study between the Clinical Trial Assay (CTA) and the *therascreen*® EGFR RGQ PCR Kit were the clinical basis of the PMA approval decision. The 1200.32 clinical trial was an international, multi-center, open label, randomized Phase 3 trial of afatinib versus chemotherapy as first-line treatment for patients with stage IIIB or IV adenocarcinoma of the non-small cell lung cancer (NSCLC) harboring an EGFR mutation (ClinicalTrials.gov number NCT00949650).

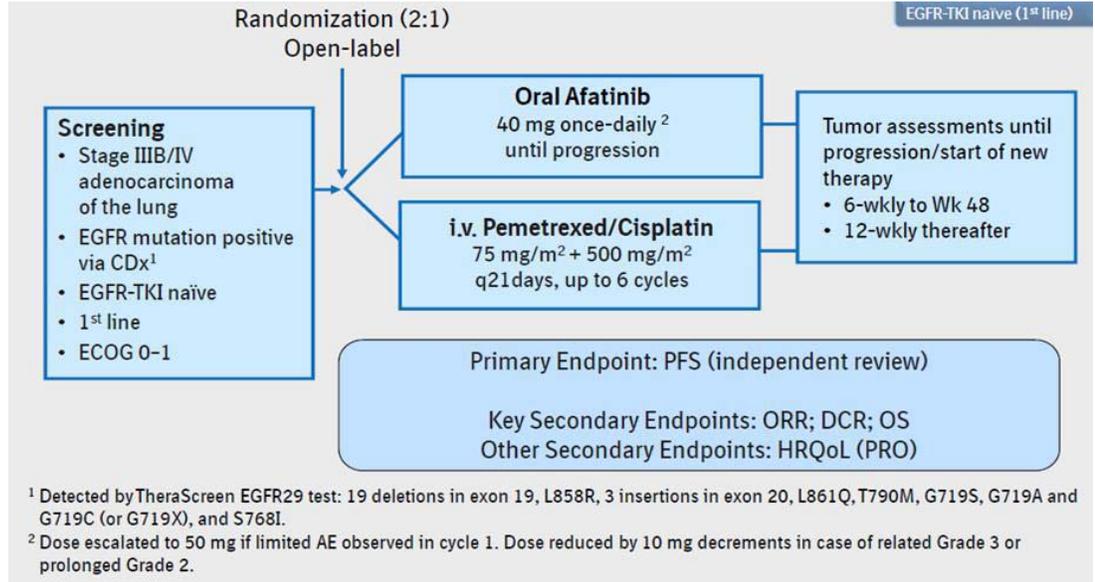
The 1200.32 clinical trial was sponsored by Boehringer Ingelheim, and was conducted at 133 sites across 25 countries in Asia, Australia, Europe, North America, and South America. The study dates were from August 17, 2009 to February 9, 2012 (cut-off date for primary analysis of PFS). The trial objective was to compare the efficacy and safety of afatinib monotherapy with pemetrexed /cisplatin chemotherapy (chemotherapy) as first-line treatment for EGFR mutation positive NSCLC patients. The primary endpoint was: progression-free survival (PFS) as assessed by central independent review according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1.

The eligibility of patients for enrollment in the 1200.32 clinical trial was determined by testing the mutation status of NSCLC patients' EGFR status using the Clinical Trial Assay (CTA) from QIAGEN. EGFR mutation screening was performed at 3 testing sites. Retrospective testing of tissue specimens from patients screened for the 1200.32 clinical trial was performed using the *therascreen*® EGFR RGQ PCR Kit. A bridging study was conducted to assess the concordance of the *therascreen*® EGFR RGQ PCR Kit with the CTA used to select patients for the 1200.32 clinical trial. To establish the clinical utility of the *therascreen*® EGFR RGQ PCR Kit, clinical outcomes (i.e., progression-free survival or PFS) for all patients enrolled in the 1200.32 clinical trial (i.e., CTA positive) were compared to the outcomes of patients whose specimens were mutation-positive upon retrospective testing with the *therascreen*® EGFR RGQ PCR Kit. A summary of the clinical study is presented below.

### **A. Study Design**

The 1200.32 clinical trial was a randomized, open-label, active-controlled, parallel-group Phase 3 trial to compare the efficacy and safety of afatinib monotherapy with chemotherapy as first-line treatment in treatment-naïve patients with Stage IIIB (with cytologically proven pleural effusion or pericardial effusion) or IV adenocarcinoma of the lung harboring an EGFR mutation. Stratification was conducted according to three EGFR mutation categories (L858R, Del 19 – the common mutations and 'Other' that comprised all other detected mutations) and race (Asian vs. Non-Asian). Randomized patients were assigned 2:1 to receive afatinib or chemotherapy (pemetrexed / cisplatin) as summarized in the figure below. Among the 345 patients randomized in the 1200.32 clinical trial; 230 were allocated to receive afatinib, and 115 to receive chemotherapy.

## The 1200.32 Clinical Trial – Study Design



The demographic and other baseline characteristics were balanced between the treatment arms. As expected for EGFR mutation positive population, the trial included substantial proportions of females (64.9%), East Asians (71.9%), and never smokers (68.4%). All but one patient were either fully functional at baseline (ECOG PS 0, 38.6%) or had a mild decrease in function performance status (ECOG PS 1, 61.2%). The majority of patients had NSCLC Stage IV at screening (89.3%); the predominant tumor histology was adenocarcinoma (98.0%).

The primary objectives of the 1200.32 clinical trial were to compare the efficacy and safety of afatinib monotherapy with pemetrexed / cisplatin chemotherapy as first-line treatment in treatment-naïve patients with Stage IIIB (with cytologically proven pleural effusion or pericardial effusion) or IV adenocarcinoma of the lung harboring an EGFR mutation. The pre-specified primary analysis was the analysis of PFS based on central independent review. It was planned after 217 patients developed progressive disease (based on central independent review) or died, whichever is earlier. The cut-off date was estimated based on the observed discrepancy in the number of PFS events by investigator assessment and by central independent review during the trial. An analysis of overall survival (OS) was also planned at the time of primary PFS analysis, and at a time when more complete information was available on OS (expected around December 2013). The stratified log-rank test (stratification factors at randomization: EGFR mutation category [L858R vs. Del 19 vs. Other]; race [Asian vs. Non-Asian]) was used. Safety was assessed based on the incidence and severity of adverse events (AEs) according to the common terminology criteria for adverse events (CTCAE) version 3.0 and changes in safety laboratory parameters.

A data monitoring committee (DMC) was responsible for assessing the safety and efficacy data to ensure the overall safety of the patients treated in this trial. This

DMC was an independent multidisciplinary group and comprised 3 voting members, including 1 independent statistician and 2 independent oncologists. These experts had expertise in the management of patients with NSCLC and in the conduct and monitoring of randomized clinical trials.

### **1. Two-Step Patient Consent and Screening Process**

Due to low frequency of EGFR mutation in the unselected NSCLC population (<20%), a high number of patients had to be screened for the trial in order to identify the required number of eligible EGFR mutation positive patients.

Anticipating high screen failure rate due to EGFR wild-type status in the majority of the patients and in order to minimize the trial burden to patients and site personnel (e.g., potentially unnecessary screening assessments and collection of baseline clinical data etc), patient consent and screening process were performed in a step-wise manner:

- Step 1 (Screening visit 1): patients signed the consent form 1 allowing mutation testing from the tumor sample using the CTA. Minimal demographic data (e.g., gender, age) were collected for all patients undergoing Screening visit 1. In case of positive mutation test results, the patients proceeded to step 2. If ineligible based on the CTA test results, the patients would be discontinued with no further data collected from them.
- Step 2 (Screening Visit 2): EGFR mutation positive patients would be consented for the main study with all pertaining procedures as described in the Informed consent 2. The data for these patients would be collected as long as they participated in the study and in line with the standard processes. Because EGFR mutation negative patients would not be eligible to proceed to Screening visit 2 where more comprehensive medical data would have been collected, the information on the baseline characteristics of CTA EGFR mutation negative patients is very limited.

### **2. Tumor Specimens and Testing**

Specimens for EGFR testing were collected at the first screening visit, and formalin fixed and paraffin embedded (FFPE). Specimens were submitted in the form of sections mounted on glass slides. DNA was extracted using DNA extraction kit in the CTA. The genetic analysis of DNA extracted from biopsy specimens to determine tumor EGFR mutation status was conducted by three central laboratories sub-contracted by QIAGEN Manchester Ltd (Manchester, UK). These laboratories were HistoGeneX in Belgium, Clariant Inc in the USA (Clariant) and Mitsubishi Chemical Medience Corporation in Japan (Mitsubishi).

### **3. Clinical Inclusion and Exclusion Criteria For Patient Enrollment**

#### *Inclusion Criteria*

1. Pathologically confirmed diagnosis of Stage IIIB (with cytologically proven pleural effusion or pericardial effusion) or Stage IV adenocarcinoma of the

lung. Patients with mixed histology were eligible if adenocarcinoma was the predominant histology.

2. EGFR mutation detected by central laboratory analysis of tumor biopsy material.
3. Measurable disease according to RECIST version 1.1.
4. Eastern Cooperative Oncology Group (ECOG) scores of 0 or 1.
5. Age  $\geq$ 18 years.
6. Life expectancy of at least three months.
7. Written informed consent that was consistent with ICH-GCP Guidelines.

#### *Exclusion Criteria*

1. Prior chemotherapy for relapsed or metastatic NSCLC. Neo-adjuvant or adjuvant chemotherapy was permitted if at least 12 months had elapsed between the end of chemotherapy and randomization.
2. Prior treatment with EGFR-targeting small molecules or antibodies.
3. Radiotherapy or surgery (other than biopsy) within four weeks prior to randomization.
4. Active brain metastases (defined as stable for  $<$ 4 weeks and/or symptomatic and/or requiring treatment with anticonvulsants or steroids and/or leptomeningeal disease).
5. Any other current malignancy or malignancy diagnosed within the past five years (other than non-melanomatous skin cancer and in situ cervical cancer).
6. Known pre-existing interstitial lung disease.

#### **4. Follow-up Schedule**

Patients were monitored according to the procedures specified in the protocol and summarized below.

##### *Patients were followed for efficacy:*

After permanent discontinuation of study medication, the patient was to be evaluated in an end-of-trial (EOT) visit. All patients were to have the first follow-up (FU) visit 21 days ( $\pm$ 7 days) after the EOT visit. Patients without progression who had not started subsequent anti-cancer treatment at the first FU visit were to have further FU visits every 21 days ( $\pm$ 7 days) until progression or start of subsequent anti-cancer treatment. The last FU visit of a patient was the FU visit when progressive disease was first documented or when subsequent anti-cancer treatment had begun. The patient was then considered to have completed the trial.

##### *Patients were followed for safety:*

Adverse events (AE) were recorded as they were encountered during the study, and the first Follow-up visit. Adverse events that occur between Follow-up 1 (21 days after End of Treatment) and the final follow-up visit were only to be reported if they are considered related to trial medication or procedures by the investigator. AE severity was graded according to CTCAE version 3.0. The maximum CTCAE Grade was assigned to occurrences and records, and the CTCAE Grade was displayed in AE listings.

## **5. Clinical Endpoints**

The primary objectives of the 1200.32 clinical trial were to compare the efficacy and safety of afatinib monotherapy with pemetrexed / cisplatin chemotherapy as first-line treatment in EGFR tyrosine kinase inhibitor (TKI)-naïve patients with Stage IIIB or IV adenocarcinoma of the lung harboring an EGFR mutation. The primary endpoint was progression-free survival (PFS), based upon the evaluation of tumor imaging according to the modified RECIST version 1.1 criteria, and the clinical information provided for each patient as reviewed by independent radiologists and an independent oncologist. The primary analysis of PFS considered all data collected until the cut-off date (09 February 2012), i.e., the estimated date of the 217th PFS event as determined by central independent review. Patients without a PFS event prior to the cut-off date were censored at the date of the last evaluable tumor imaging. Patients who were randomized but never received any study medication were censored at the date of randomization unless they died before the second scheduled assessment. Further censoring rules had been specified in the clinical trial protocol and statistical analysis plan.

## **6. Bridging Study**

The aim of the bridging study was to determine the concordance between EGFR mutation results using the Clinical Trial Assay (CTA) generated at the time of accrual into the 1200.32 clinical trial and the EGFR results generated using the *therascreen*® EGFR RGQ PCR Kit (refer to as EGFR Kit below). The clinical specimens used for the bridging study were all available retained DNA samples from patients randomized into the 1200.32 clinical trial and an equal number of specimens from patients designated as mutation negative by CTA and not eligible for treatment. If the original DNA sample was not available, then FFPE sections would be used.

The study was conducted at HistoGeneX in Belgium during August and September 2012. Patient identifiers were blinded and samples were randomized. Thus, operators were blinded to the identity of individual patients and their previous CTA results. Samples without proper informed consent for mutation testing were excluded from the bridging study.

The disposition of samples from the clinical trial through to bridging study results is illustrated in “Accountability of PMA Cohort” section below. A total of 1269 specimens were presented to the test sites for screening in 1200.32 clinical trial. CTA results were obtained for 1171 specimens. Among the 1171 specimens with CTA results, 630 specimens were available for re-testing by the EGFR Kit, and a total of 526 valid EGFR Kit results were obtained (273 were CTA positive and 253 were CTA negative). Among the 345 patients randomized, valid EGFR Kit results were obtained for the 273 samples where 266 samples were EGFR Kit positive and 7 samples were EGFR Kit negative. Among 719 patient samples with mutation not detected CTA results, valid EGFR Kit results were obtained for the 253 samples where 247 samples were EGFR Kit negative and 6 samples were EGFR Kit positive.

a. **Bridging Study Sample Selection**

Validity of the bridging study rests on whether there is selection bias for the bridging study samples. Imbalances between the EGFR Kit evaluable set (N=526) and the un-evaluable set (N=645) were investigated using logistic regression. Due to the limited data collection for screening failure patients at the Screening visit 1, only the covariates of race (Asian, Non-Asian), gender, age and smoking history were investigated. Given the relatively low number of discordant patients (N=13), the logistic regression using two possible results was performed. Note a total of 525 patients was analyzed as one patient did not have race recorded.

A stepwise logistic regression was performed using a 10% significance level of entry, and no covariates were identified to have effects on EGFR Kit concordance with CTA. Table below presents the baseline demographics for the two sets of patients which were generally comparable with the exception of race where a higher proportion of Asian patients were present in the EGFR Kit evaluable set (64%) than in the un-evaluable set (42%). For the evaluable data set, more mutations were detected from Asian patients and therefore a higher proportion was evaluated within the bridging study as compared to the un-evaluable set. There were no major concerns with respect to covariate imbalance.

Additional comparative analysis was conducted to ensure that bridging study sample is representative of EGFR Kit intended use population, and that estimated PPA and NPA are not subject to selection bias or spectrum bias. CTA negative patients in bridging study (n=253) were compared with CTA negative patients in clinical trial screening sample who are not included in the bridging study (n=467). Similarly, CTA positive patients in bridging study (n=273) were compared with CTA positive patients in clinical trial screening sample who are not included in the bridging study (n=178). Baseline characteristics for which data were available were mostly comparable.

## Demographic Data for EGFR Kit Evaluable vs. Un-evaluable Sets

	MRS evaluable	MRS un-evaluable
Total	526 (100.0)	645 (100.0)
Gender [N (%)]		
Male	250 ( 47.5)	335 ( 51.9)
Female	276 ( 52.5)	310 ( 48.1)
Age [years]		
N	526	645
Mean	60.6	60.4
SD	10.0	10.8
Min	28	29
Median	61.0	61.0
Max	86	88
Age group 1 [N (%)]		
<65 years	323 ( 61.4)	402 ( 62.3)
>=65 years	203 ( 38.6)	243 ( 37.7)
Age group 2 [N (%)]		
<75 years	492 ( 93.5)	597 ( 92.6)
>=75 years	34 ( 6.5)	48 ( 7.4)
Race [N (%)]		
Amer.Ind./Alaska Nat	6 ( 1.1)	6 ( 0.9)
Asian	337 ( 64.1)	271 ( 42.0)
Indian	1 ( 0.2)	1 ( 0.2)
Southeast Asian	117 ( 22.2)	119 ( 18.4)
Far east Asian - Japanese	110 ( 20.9)	63 ( 9.8)
Far east Asian - Korean	27 ( 5.1)	28 ( 4.3)
Far east Asian - China/Taiwan	81 ( 15.4)	58 ( 9.0)
Asian - Other	1 ( 0.2)	2 ( 0.3)
Black/African Amer.	1 ( 0.2)	13 ( 2.0)
Hawaiian/Pacif. Isle	0 ( 0.0)	0 ( 0.0)
White	181 ( 34.4)	354 ( 54.9)
Missing	1 ( 0.2)	1 ( 0.2)
	MRS evaluable	MRS un-evaluable
Race group [N (%)]		
Caucasian	181 ( 34.4)	354 ( 54.9)
Eastern Asian	335 ( 63.7)	268 ( 41.6)
Other	7 ( 1.3)	19 ( 2.9)
Other Asian	2 ( 0.4)	3 ( 0.5)
Missing	1 ( 0.2)	1 ( 0.2)
Region [N (%)]		
Europe	103 ( 19.6)	264 ( 40.9)
North America	27 ( 5.1)	38 ( 5.9)
Asia	330 ( 62.7)	261 ( 40.5)
Other	66 ( 12.5)	82 ( 12.7)
Smoking status [N (%)]		
Never smoked	248 ( 47.1)	287 ( 44.5)
Ex-smoker	228 ( 43.3)	245 ( 38.0)
Currently smokes	50 ( 9.5)	107 ( 16.6)
Missing	0 ( 0.0)	6 ( 0.9)
Smoking history [N (%)]		
<15 pack years + stopped >1 year before diagnosis	24 ( 4.6)	8 ( 1.2)
Never smoked	248 ( 47.1)	287 ( 44.5)
Other current or ex-smokers	254 ( 48.3)	344 ( 53.3)
Missing	0 ( 0.0)	6 ( 0.9)
Alcohol status [N (%)]		
Non drinker	329 ( 62.5)	386 ( 59.8)
Drinks but not interfere	186 ( 35.4)	243 ( 37.7)
Drinks could interfere	0 ( 0.0)	3 ( 0.5)
Missing	11 ( 2.1)	13 ( 2.0)

b. **CTA vs. EGFR Kit – Agreement Analysis Based on CTA Results**

A total of 630 samples from the 1200.32 clinical trial were considered for the bridging study. Valid EGFR Kit results were obtained for 526 patients from 397 DNA samples and 129 FFPE sections. Among the 526 samples with EGFR Kit valid results, 254 patients were tested CTA negative and 272 patients were tested CTA positive. Some samples did not return valid results with the EGFR Kit (n=104) due to insufficient DNA sample volume (~50%), insufficient DNA concentration as assessed by the Control Assay (~ 40%), or invalid sample results (~ 10%).

The mutation call results (N), positive percent agreement defined as  $\Pr(\text{EGFR Kit}=\text{MD} \mid \text{CTA}=\text{MD})$  and negative percent agreement defined as  $\Pr(\text{EGFR Kit}=\text{NMD} \mid \text{CTA}=\text{NMD})$  based on the CTA-tested results for the bridging study samples are presented below.

**Bridging Study Agreement Analysis – CTA vs. EGFR Kit Based on CTA Results**

Measure of Agreement	Percent Agreement (N)	95% CI*
Positive Percent Agreement	97.8% (266/272)	95.3, 99.0
Negative Percent Agreement	97.2% (247/254)	94.4, 98.7

\*The 95% CI is calculated based on score method.

c. **CTA vs. EGFR Kit – Agreement Analysis Based on EGFR Kit Results**

The bridging study was enriched with EGFR mutation positive population ( $272/526 = 51.7\%$ ) as compared to the EGFR mutation positive population in the 1200.32 clinical trial ( $451/1171 = 38.5\%$ ). Thus, agreement analysis between CTA and EGFR Kit conditional on EGFR Kit results should be adjusted for differential sampling. The positive percent agreement between CTA and EGFR Kit conditional on EGFR Kit results is defined as  $\Pr(\text{CTA}=\text{MD} \mid \text{EGFR Kit}=\text{MD})$  and negative percent agreement between CTA and EGFR Kit conditional on EGFR Kit results is defined as  $\Pr(\text{CTA}=\text{NMD} \mid \text{EGFR Kit}=\text{NMD})$ . The value of  $\phi$  used to adjust for differential sampling was:

$$\phi = (344 + 107)/(344 + 1 + 107 + 719) = 451/1171 = 38.5\%.$$

The 95% confidence intervals (CI) for percent agreements were calculated using a non-parametric bootstrap. As indicated in table below, percent positive agreement, PPA or  $\Pr(\text{CTA}+ \mid \text{EGFR Kit}+)$ , was estimated as 96.3% with 95% confidence interval (93.3%, 98.7%). Percent negative agreement, NPA or  $\Pr(\text{CTA}- \mid \text{EGFR Kit}-)$  was estimated as 98.4% with 95% confidence interval (97.1%, 99.5%). The PPA of 96.3% and NPA of 98.4% were used to estimate drug efficacy in EGFR Kit mutation detected

population as described in “Safety and Effectiveness Results” section D-2-b below.

**Bridging Study Agreement Analysis – CTA vs. EGFR Kit Based on EGFR Kit Results, Adjusted for Enrichment**

Measure of Agreement	Percent Agreement*	95% CI*
Positive Percent Agreement (PPA)	96.3%	93.3, 98.7
Negative Percent Agreement (NPA)	98.4%	97.1, 99.5

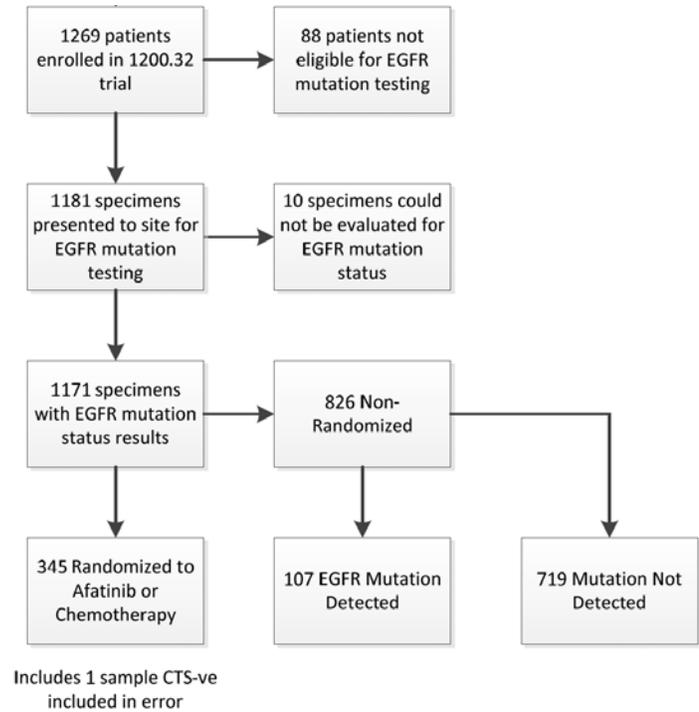
\*The percent agreement point estimate is adjusted for enrichment and the 95% CI is estimated using bootstrap.

**B. Accountability of PMA Cohort**

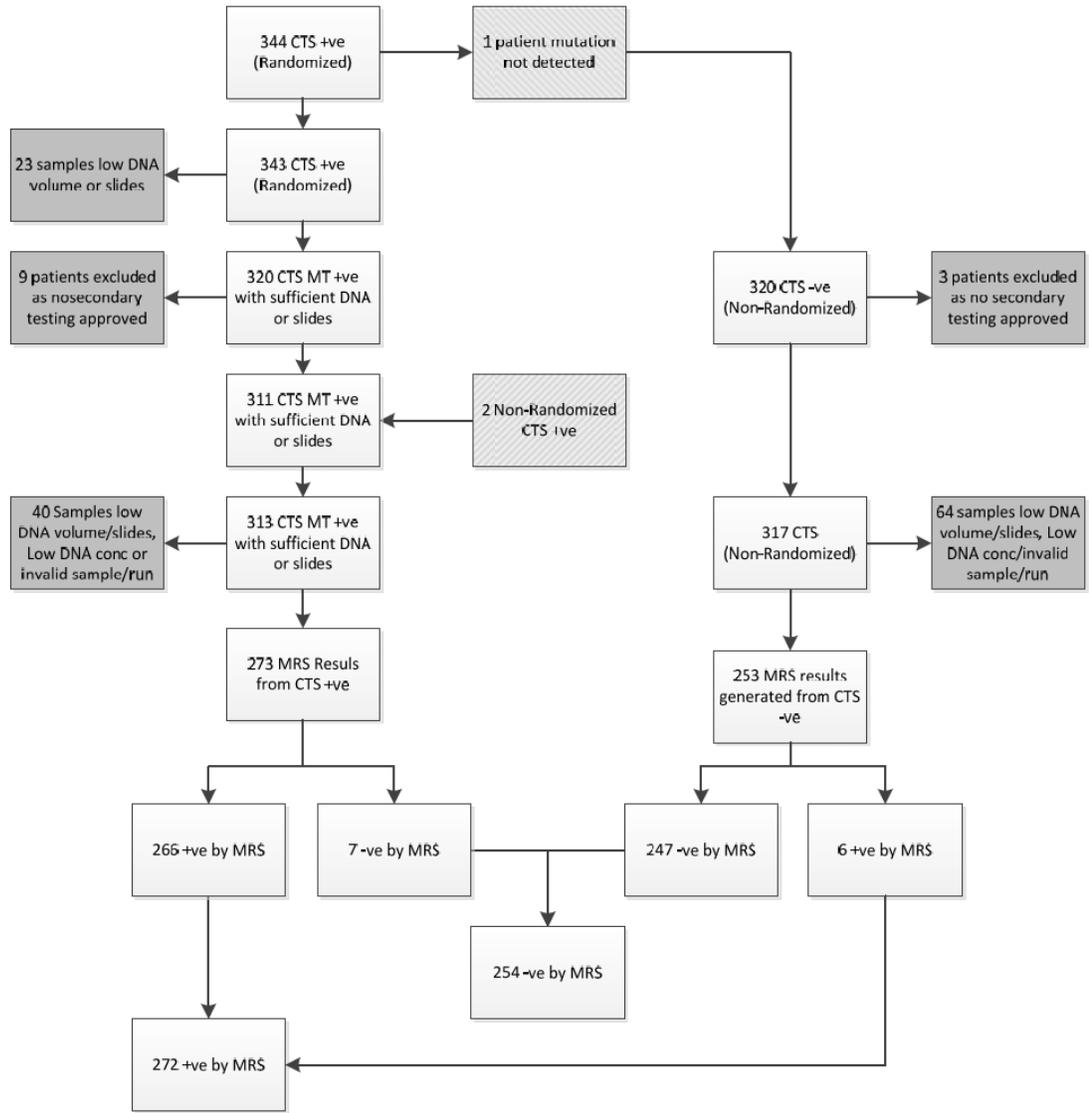
A total of 1269 specimens were presented to the test sites for screening in the 1200.32 clinical trial. CTA results were obtained for 1171 specimens. A total of 345 specimens were from EGFR mutation positive patients that were eligible for the 1200.32 clinical trial and these patients were randomized for treatment. Figures below outline the specimen accountability and sample disposition to the bridging study.

**Specimen Accountability for the 1200.32 Clinical Trial**

[The CTS refers to the Clinical Trial Assay (CTA) in figure below]



**Sample Disposition from the 1200.32 Clinical Trial to Bridging Study Results**  
 [The CTS refers to the CTA and the MRS refers to the EGFR Kit in figure below]



**C. Study Population Demographics and Baseline Parameters**

Demographics and baseline clinical characteristics for the 344 patients in the randomized population of the 1200.32 clinical trial are presented in table below. Of those, 271 patients were tested by the EGFR Kit (noted as MRS-tested in table below) and 73 patients were not tested due to no/insufficient tumor samples (noted as MRS-not tested in table below). The 271 patients excluded the 2 non-randomized patients from the 273 patients with valid EGFR Kit results from the CTA positive population. There were no significant differences between patients in the EGFR Kit-tested population and the EGFR Kit-not tested population with respect to their demographic and baseline disease characteristic parameters, with the exception of race group and region, which was expected.

## Demographics and Baseline Characteristics in the EGFR Kit-Tested Population and the EGFR Kit-Not Tested Population

	MRS tested	MRS not tested	Total	p-Value
Total	271 (100.0)	73 (100.0)	344 (100.0)	
Gender [N (%)]+				
Female	175 (64.6)	49 (67.1)	224 (65.1)	0.6852
Male	96 (35.4)	24 (32.9)	120 (34.9)	
Age [years]#				
N	271	73	344	0.4659
Mean	60.1	61.1	60.3	
SD	9.9	10.7	10.1	
Min	28	31	28	
Median	61.0	62.0	61.0	
Max	84	86	86	
Race group [N (%)]*				
Asian	207 (76.4)	42 (57.5)	249 (72.4)	0.0015
Caucasian	59 (21.8)	31 (42.5)	90 (26.2)	
Other	5 (1.8)	0 (0.0)	5 (1.5)	
Region [N (%)]*				
Asia	204 (75.3)	39 (53.4)	243 (70.6)	0.0017
Europe	48 (17.7)	26 (35.6)	74 (21.5)	
North America	2 (0.7)	0 (0.0)	2 (0.6)	
Other	17 (6.3)	8 (11.0)	25 (7.3)	
Smoking status [N (%)]*				
Currently smokes	6 (2.2)	1 (1.4)	7 (2.0)	0.0750
Ex-smoker	87 (32.1)	14 (19.2)	101 (29.4)	
Never smoked	178 (65.7)	58 (79.5)	236 (68.6)	
Alcohol status [N (%)]+				
Drinks - no interf.	79 (29.2)	21 (28.8)	100 (29.1)	0.9203
Non drinker	190 (70.1)	52 (71.2)	242 (70.3)	
Missing	2 (0.7)	0 (0.0)	2 (0.6)	

	MRS tested	MRS not tested	Total	p-Value
Height [cm]#				
N	271	73	344	0.2165
Mean	159.2	160.7	159.5	
SD	9.2	9.3	9.3	
Min	135	141	135	
Median	158.0	160.0	159.0	
Max	190	179	190	
Weight [kg]#				
N	271	73	344	0.3204
Mean	59.82	61.48	60.17	
SD	12.70	12.41	12.64	
Min	31.8	39.3	31.8	
Median	58.00	60.00	58.60	
Max	114.0	94.0	114.0	
Body mass index [kg/m <sup>2</sup> ]#				
N	271	73	344	0.6981
Mean	23.505	23.713	23.549	
SD	4.094	3.919	4.053	
Min	12.90	16.36	12.90	
Median	23.398	23.693	23.424	
Max	40.39	36.59	40.39	
Body surface area [m <sup>2</sup> ]#				
N	271	73	344	0.2265
Mean	1.609	1.639	1.615	
SD	0.192	0.190	0.192	
Min	1.10	1.31	1.10	
Median	1.591	1.621	1.597	
Max	2.32	2.10	2.32	
Baseline ECOG score*				
0	108 (39.9)	25 (34.2)	133 (38.7)	0.1676
1	163 (60.1)	47 (64.4)	210 (61.0)	
2	0 (0.0)	1 (1.4)	1 (0.3)	
Clinical stage at screening [N (%)]+				
IIIB	29 (10.7)	8 (11.0)	37 (10.8)	0.9497
IV	242 (89.3)	65 (89.0)	307 (89.2)	

	MRS tested	MRS not tested	Total	p-Value
Histological classification [N (%)]*				
Adenocarcinoma	265 (97.8)	72 (98.6)	337 (98.0)	1.0000
Other	1 (0.4)	0 (0.0)	1 (0.3)	
Predominantly adenocarcinoma	5 (1.8)	1 (1.4)	6 (1.7)	
EGFR mutation [N (%)]+				
Del 19	129 (47.6)	40 (54.8)	169 (49.1)	0.2161
L858R	115 (42.4)	23 (31.5)	138 (40.1)	
Other	27 (10.0)	10 (13.7)	37 (10.8)	

P-value: + Chi-square test; \* Fisher's exact test; # T-test

Note: Missing alcohol status excluded prior to calculation of p-value

The analysis of demographics and baseline characteristics by treatment arm in the population of 264 EGFR Kit-positive patients was performed and presented in the table below. The 264 patients excluded the 2 non-randomized patients from the 266 patients with EGFR Kit positive results from the CTA positive population. There were no statistically significant differences between the chemotherapy and afatinib treatment groups with respect to any of the demographic or baseline characteristic parameters. The EGFR Kit-positive patient population was balanced between the treatment arms with respect to demographics and baseline disease characteristics.

### Demographics and Baseline Characteristics by Treatment Arm in the CTA/EGFR Kit Double-Positive Patients

	Afatinib 40	Pe500+Cis75	Total	p-Value
Total	178 (100.0)	86 (100.0)	264 (100.0)	
Gender [N (%)]+				
Female	115 (64.6)	56 (65.1)	171 (64.8)	0.9353
Male	63 (35.4)	30 (34.9)	93 (35.2)	
Age [years]#				
N	178	86	264	0.8060
Mean	60.2	59.9	60.1	
SD	10.0	9.7	9.9	
Min	28	37	28	
Median	61.0	61.0	61.0	
Max	84	83	84	
Race group [N (%)]*				
Asian	138 (77.5)	67 (77.9)	205 (77.7)	0.7291
Caucasian	38 (21.3)	17 (19.8)	55 (20.8)	
Other	2 (1.1)	2 (2.3)	4 (1.5)	
Region [N (%)]*				
Asia	135 (75.8)	67 (77.9)	202 (76.5)	0.8745
Europe	29 (16.3)	15 (17.4)	44 (16.7)	
North America	2 (1.1)	0 (0.0)	2 (0.8)	
Other	12 (6.7)	4 (4.7)	16 (6.1)	
Smoking status [N (%)]*				
Currently smokes	4 (2.2)	2 (2.3)	6 (2.3)	1.0000
Ex-smoker	56 (31.5)	27 (31.4)	83 (31.4)	
Never smoked	118 (66.3)	57 (66.3)	175 (66.3)	
Alcohol status [N (%)]+				
Drinks - no interf.	52 (29.2)	24 (27.9)	76 (28.8)	0.8486
Non drinker	125 (70.2)	61 (70.9)	186 (70.5)	
Missing	1 (0.6)	1 (1.2)	2 (0.8)	

	Afatinib 40	Pe500+Cis75	Total	p-Value
Height [cm]#				
N	178	86	264	0.9992
Mean	159.0	159.0	159.0	
SD	9.3	8.4	9.0	
Min	135	138	135	
Median	158.0	158.5	158.0	
Max	190	182	190	
Weight [kg]#				
N	178	86	264	0.1176
Mean	60.51	57.91	59.66	
SD	12.92	12.01	12.67	
Min	32.0	31.8	31.8	
Median	59.25	56.00	58.00	
Max	114.0	93.0	114.0	
Body mass index [kg/m²]#				
N	178	86	264	0.0732
Mean	23.820	22.846	23.503	
SD	4.109	4.157	4.142	
Min	16.38	12.90	12.90	
Median	23.488	22.944	23.424	
Max	40.39	39.21	40.39	
Body surface area [m²]#				
N	178	86	264	0.2194
Mean	1.615	1.585	1.605	
SD	0.194	0.177	0.189	
Min	1.10	1.21	1.10	
Median	1.597	1.568	1.590	
Max	2.32	2.03	2.32	
Baseline ECOG score+				
0	72 (40.4)	31 (36.0)	103 (39.0)	0.4919
1	106 (59.6)	55 (64.0)	161 (61.0)	
Clinical stage at screening [N (%)]+				
IIIB	17 (9.6)	12 (14.0)	29 (11.0)	0.2836
IV	161 (90.4)	74 (86.0)	235 (89.0)	

P-value: + Chi-square test; \* Fisher's exact test; # T-test  
Note: Missing alcohol status excluded prior to calculation of p-value

## Demographics and Baseline Characteristics by Treatment Arm in the CTA/EGFR Kit Double-Positive Patients (continued)

	Afatinib 40	Pe500+Cis75	Total	p-Value
Histological classification [N (%)]*				
Adenocarcinoma	176 ( 98.9)	82 ( 95.3)	258 ( 97.7)	0.0401
Other	1 ( 0.6)	0 ( 0.0)	1 ( 0.4)	
Predominantly adenocarcinoma	1 ( 0.6)	4 ( 4.7)	5 ( 1.9)	
EGFR mutation [N (%)]*				
Del 19	83 ( 46.6)	43 ( 50.0)	126 ( 47.7)	0.5463
L858R	77 ( 43.3)	38 ( 44.2)	115 ( 43.6)	
Other	18 ( 10.1)	5 ( 5.8)	23 ( 8.7)	

P-value: + Chi-square test; \* Fisher's exact test; # T-test  
 Note: Missing alcohol status excluded prior to calculation of p-value

### D. Safety and Effectiveness Results

#### 1. Safety Results

The safety with respect to treatment with afatinib is not comprehensively addressed in the SSED for the *therascreen*® EGFR RGQ PCR Kit. The evaluation of safety was based on the analysis of adverse events (AEs), clinical laboratory evaluations, physical examinations, and vital signs.

Due to the nature of trial design, the mean treatment time was considerably longer for patients in the afatinib arm than for patients in the chemotherapy arm (335.4 days vs. 85.0 days). Almost all patients experienced at least one AE (afatinib 100.0%; chemotherapy 98.2%). The observed AEs were in general consistent with the known safety profile of either afatinib or chemotherapy and the nature of the disease under investigation. Most common drug-related adverse events were diarrhea (95%), rash/acne (89%) and paronychia (57%) with afatinib, and nausea (66%), decreased appetite (53%) and vomiting (42%) with chemotherapy. There were 13 deaths (5.7%) reported due to on-treatment AEs in the afatinib arm, four of which were considered to be related to afatinib by the investigator. The reported terms for these cases were sepsis, dyspnea, acute respiratory distress syndrome, and death. Altogether three deaths (2.7%) were reported due to on-treatment AEs in the chemotherapy arm, none of which was considered to be treatment related by the investigator. Drug-related serious adverse events (SAEs) were reported for 14.4% of patients in both treatment arms. Refer to the drug label for more information.

#### 2. Effectiveness Results

##### a. Overall Efficacy

The trial objective was to compare the efficacy and safety of afatinib monotherapy with chemotherapy as first-line treatment for the NSCLC patients whose tumors harbor EGFR mutations (i.e., exon 19 deletions, exon 21 L858R substitution, and “other” EGFR mutations). The primary evaluation of efficacy in this trial was based on the randomized set, i.e., all randomized patients, regardless of whether treated or not. Based on the CTA test results, 345 patients were in the randomized set (afatinib 230 patients; chemotherapy 115 patients). The proposed companion diagnostic test for afatinib is the *therascreen*® EGFR RGQ

PCR Kit for selection of patients with EGFR mutations. QIAGEN performed retrospective testing of tissue specimens from patients from the 1200.32 clinical trial using the *therascreen*® EGFR RGQ PCR Kit.

The overall efficacy results are summarized in figures and table below. The primary efficacy outcome was progression-free survival (PFS) as assessed by an independent review committee (IRC). Other efficacy outcomes included objective response rate (ORR) and overall survival (OS). EGFR mutation status was prospectively determined for screening and enrollment of patients by a Clinical Trial Assay (CTA). Tumor samples from 264 patients (178 randomized to afatinib and 86 patients randomized to chemotherapy) were tested retrospectively by the companion diagnostic *therascreen*® EGFR RGQ PCR Kit. A statistically significant improvement in PFS as determined by the IRC was demonstrated for patients randomized to afatinib compared to those randomized to chemotherapy, in both the overall CTA+ population and the EGFR Kit+/CTA+ population.

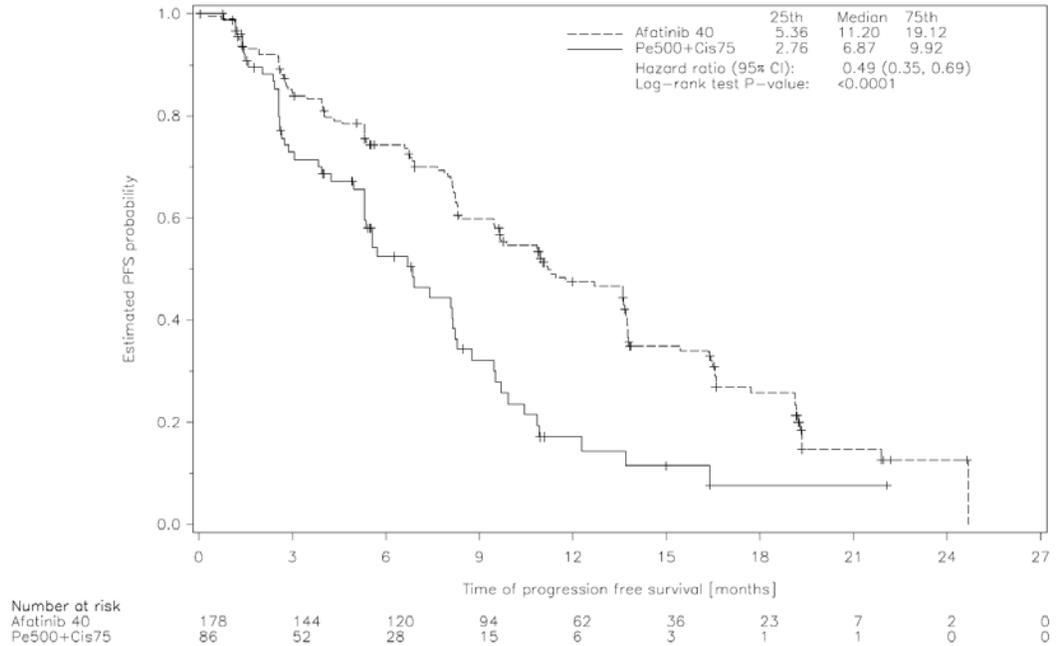
Analysis of the EGFR Kit +/CTA+ subset (n=264) revealed that those patients treated with afatinib had a significant increase in PFS time (median PFS 11.2 vs. 6.9 months) and are less likely to have an event of progressive disease or death (HR= 0.49, 95 % CI [0.35; 0.69], p<0.0001) than patients treated with chemotherapy. The observed clinical benefit in the subset of patients tested with the EGFR Kit was comparable to that observed in the full study population (n=345).

**Clinical Benefit of Patients Tested with the *therascreen*® EGFR RGQ PCR Kit in the 1200.32 Clinical Trial Population**

Parameter	EGFR Kit+/CTA+ Population n = 264		CTA+ Population n = 345	
	Chemotherapy n = 86	Afatinib n = 178	Chemotherapy n = 115	Afatinib n = 230
<b>Progression-free Survival (PFS)</b>				
<b>Number of Deaths or Progressions, N (%)</b>	53 (61.6%)	120 (67.4%)	69 (60.0%)	152 (66.1%)
<b>Median PFS (Months)</b>	6.9	11.2	6.9	11.1
<b>Median PFS 95% CI</b>	[5.3, 8.2]	[9.7, 13.7]	[5.4, 8.2]	[9.6, 13.6]
<b>Hazard Ratio</b>	0.49		0.58	
<b>Hazard Ratio 95% CI</b>	[0.35, 0.69]		[0.43, 0.78]	
<b>P-Value (stratified log-rank test*)</b>	<0.0001		<0.001	

\*Stratified by EGFR mutation status and race.

**Kaplan-Meier Curve of PFS by Independent Review by Treatment Group (EGFR Kit+/CTA+ Population)**



**b. Drug Efficacy in EGFR Kit Mutation Detected (MD) Population**

The EGFR Kit MD population included two sub-populations in the 1200.32 clinical trial. One population defined as EGFR Kit+/CTA+ had clinical outcome (see Section D-2-a above). The other population defined as EGFR Kit+/CTA- had no clinical outcome because the *therascreen*<sup>®</sup> EGFR RGQ PCR Kit was not used to select patients for the 1200.32 clinical trial. Thus, additional efficacy analyses were conducted to consider patients who were not included in the 1200.32 clinical trial because they were tested negative by the CTA, but could have been tested positive by the EGFR Kit (i.e., EGFR Kit+/CTA-). The clinical outcome of the EGFR Kit+/CTA- sub-population was imputed as described below.

There were 264 EGFR Kit+/CTA+ patients included in the bridging study, PPA which is  $Pr(CTA+|EGFR\ Kit+)$  was estimated by the ratio  $264/(264+d_0)$ , where  $d_0$  stands for the number of EGFR Kit+/CTA- subjects with missing clinical outcomes. Using the point estimate of the PPA and its 95% confidence interval (95% CI), the value of  $d_0$  is estimated to be 10.26 and the upper bound of the two-sided 95% CI is 18.95.

Analyses performed were stratified by EGFR mutation status and race and adjusted for patient's baseline presence of bone metastases; because bone metastases status was not balanced between the treatment and control arms based on EGFR Kit test results. In the hypothetical

scenario, patients who are EGFR Kit+/CTA- in the treatment arm (afatinib) were assigned as PFS events at the baseline (Day 1); patients who are EGFR Kit+/CTA- in the control arm (chemotherapy) were censored for PFS at Month 11 (Day 335, the median PFS for afatinib). Using point estimate based on d0 (n=11), median PFS times (95% CI) were 11.0 (8.3, 13.6) for the afatinib arm and 6.9 (5.3, 8.8) for the chemotherapy arm, with a hazard ratio (95% CI) of 0.56 (0.39, 0.79) and corresponding p-value of 0.0009. Results from all of the hypothetical analyses were generally similar to those from the primary efficacy analysis.

c. **Sensitivity Analysis (Imputation Analysis to Missing EGFR Kit Data)**

To assess the variability of estimated afatinib effect in EGFR Kit positive patients and the robustness of study conclusions to missing data under different missing data mechanisms or imputation methods, the following analyses of the primary clinical endpoint (PFS) were conducted to address missing test results. The case analyses referred below were defined as one of the following:

- (a) all patients were censored for PFS at Month 11 (Day 335, the median PFS for afatinib);
- (b) patients who are EGFR Kit+/CTA- in the treatment arm (afatinib) were assigned as PFS events at the baseline (Day 1); patients who are EGFR Kit+/CTA- in the control arm (chemotherapy) were censored for PFS at Month 11 (Day 335, the median PFS for afatinib).

Analysis of PFS was performed stratifying by EGFR mutation status and race and adjusting for patient's baseline presence of bone metastases. The results from the 50 imputations were combined using PROC MIANALYZE.

*Missing-at-Random (MAR) analysis:*

These analyses use the combined data from three sets of patients:

- 1) EGFR Kit+/CTA+ patients (N=264)
- 2) EGFR Kit un-evaluable, CTA+ (N for EGFR Kit+ varies from 73 to 65 dependent upon multiple imputation of EGFR Kit result from 73 patients with EGFR Kit un-evaluable results)
- 3) EGFR Kit+/CTA- patients (N varies from 13 to 14 for the point estimate of d0 and from 24-25 for the upper bound of the 95% CI of d0; dependent upon N in 2) above)

Using the point estimates of d0 for the number of EGFR Kit+/CTA- patients (i.e., group 3) above), for the case (a) analysis a hazard ratio (95% CI) of 0.52 (0.38, 0.72) and corresponding p-value of <0.0001 was

observed. For the case (b) analysis, a hazard ratio (95% CI) of 0.60 (0.44, 0.82) and corresponding p-value of 0.0015 was observed.

Similarly, using the upper bound of 95% CI for d0, for the case (a) analysis a hazard ratio (95% CI) of 0.54 (0.39, 0.74) and corresponding p-value of 0.0001 was observed. For the case (b) analysis a hazard ratio (95% CI) of 0.70 (0.51, 0.95) and corresponding p-value of 0.0217 was observed.

The results remain robust for both case scenarios, and consistent with the primary efficacy analysis.

*Sensitivity analysis:*

These analyses use the combined data from three sets of patients:

- 1) EGFR Kit+/CTA+ patients (N=264)
- 2) EGFR Kit un-evaluable, CTA+ (N for EGFR Kit+ varies from 46 to 31 dependent upon multiple imputation of EGFR Kit result from 73 patients with EGFR Kit un-evaluable results)
- 3) EGFR Kit+/CTA- patients (N varies from 12 to 13 for the point estimate of d0 and from 22-23 for the upper bound of the 95% CI of d0; dependent upon N in 2) above)

Using the point estimate of d0 for the number of EGFR Kit+/CTA- patients (i.e., group 3) above), for the case (a) analysis a hazard ratio (95% CI) of 0.51 (0.36, 0.71) and corresponding p-value of <0.0001 was observed. For the case (b) analysis a hazard ratio (95% CI) of 0.58 (0.42, 0.81) and corresponding p-value of 0.0015 was observed.

Similarly, using the upper bound of 95% CI for d0, for the case (a) analysis a hazard ratio (95% CI) of 0.51 (0.36, 0.72) and corresponding p-value of <0.0001 was observed. For the case (b) analysis a hazard ratio (95% CI) of 0.67 (0.48, 0.93) and corresponding p-value of 0.0153 was observed.

The results remain robust for both case scenarios, and consistent with the primary efficacy analysis.

### **3. Subgroup Analyses**

To determine whether the samples available for testing using the *therascreen*<sup>®</sup> EGFR RGQ PCR Kit were representative of the randomized population in the 1200.32 clinical trial (n=345), analyses of PFS by treatment arm were performed in patients with and without EGFR Kit results.

### Analyses of PFS for Patients With and Without EGFR Kit Results

	PFS Median [95% CI]		HR [95% CI]
	Afatinib	Chemotherapy	Statistics Between Treatment Arms
<b>EGFR Kit-Tested (n=272)</b>	11.0 [9.5, 13.6] (n=184)	6.9 [5.3, 8.2] (n=88)	0.50 [0.36, 0.70] p<0.0001
<b>EGFR Kit-not Tested (n=73)</b>	11.1 [8.9, 16.5] (n=46)	8.3 [5.2, 16.4] (n=27)	0.77 [0.35, 1.69] p=0.5099

Although the estimated PFS time of the 27 patients without EGFR Kit test results (PFS = 8.3) in the chemotherapy arm is comparable to that of 184 patients with EGFR Kit test results in the afatinib treatment arm (PFS = 11.0), the two-sided 95% confidence interval (95% CI) of median PFS for the 27 chemotherapy patients without EGFR Kit results is consistent with the 95% CI of the 88 chemotherapy patients with EGFR Kit results. These 27 patients also represent a small sample size for the chemotherapy patients without EGFR Kit result, and a difference in PFS for chemotherapy patients with EGFR Kit results and without EGFR Kit results was not observed (p=0.427). Sensitivity analyses conducted above including patients without EGFR Kit results further demonstrated robustness of the PFS efficacy outcome.

#### **E. Financial Disclosures**

The 1200.32 bridging study was conducted retrospectively at a single testing site in Belgium, and exempt from the requirements for Investigational Device Exemption as defined in Title 21 of the Code of Federal Regulations (21 CFR), 812.2(c)(3). The investigational product was not used in the diagnosis or treatment of patients. The applicant has adequately disclosed the financial interest/arrangements with clinical investigator. The information provided does not raise any questions about the reliability of the data.

### **XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION**

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation.

### **XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES**

#### **A. Effectiveness Conclusions**

The clinical benefit of the *therascreen*® EGFR RGQ PCR Kit was demonstrated in a retrospective analysis of patients enrolled in the Phase 3 study, the 1200.32 clinical trial, in which the EGFR mutation was determined using a CTA. Overall, a statistically significant efficacy benefit for GILOTRIF™ (afatinib) vs. chemotherapy was observed

in the subset of NSCLC patients whose tumors had EGFR exon 19 deletions or exon 21 (L858R) substitution mutation, as detected by the *therascreen*® EGFR RGQ PCR Kit. The observed clinical benefit in the subset of patients tested with the *therascreen*® EGFR RGQ PCR Kit was comparable to that observed in the intended-to-treat study population. Additional case scenario analysis to consider discordant results between CTA and the EGFR Kit, as well as sensitivity analysis to impute missing data, consistently support an improvement of PFS in patients with these specific EGFR mutations. Safety and efficacy of GILOTRIF™ (afatinib) have not been established in patients whose tumors have L861Q, G719X, S768I, exon 20 insertions, and T790M mutations, which are also detected by the *therascreen*® EGFR RGQ PCR Kit.

Analytical performance studies with the *therascreen*® EGFR RGQ PCR Kit demonstrated an ability to detect the exon 19 deletions or exon 21 L858R substitution mutations with an analytical sensitivity of detecting 16.87% mutation and 5.97% mutation in NSCLC FFPE clinical specimens, respectively. Reproducibility and repeatability studies supported consistent assay performance in detecting these specific EGFR mutations. Correlation to the reference methods in the detection of EGFR mutations showed a PPA of 99.4% and a NPA of 86.6% for Sanger sequencing.

## **B. Safety Conclusions**

The adverse effects of the device are based on data collected in the clinical study conducted to support PMA approval as described above. As an *in vitro* diagnostic test, the *therascreen*® EGFR RGQ PCR Kit involves testing on formalin-fixed, paraffin embedded (FFPE) NSCLC tissue sections. These tissue sections are routinely removed as part of the diagnosis of NSCLC by pathologists. The test, therefore, presents no additional safety hazard to the patient being tested.

The risks of the *therascreen*® EGFR RGQ PCR Kit 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 GILOTRIF™ (afatinib) with inappropriate expectation of therapeutic benefit and experience side effects. A patient with a false negative result may be excluded from treatment with GILOTRIF™ (afatinib) and not experience the potential therapeutic benefit.

## **C. Benefit-Risk Conclusions**

The probable benefits of the device are based on data collected in the clinical study conducted to support PMA approval as described above. The clinical benefit of the *therascreen*® EGFR RGQ PCR Kit was demonstrated in a retrospective analysis of efficacy and safety data obtained from NSCLC patients in the Phase 3 study, the 1200.32 clinical trial. Of the 345 patients enrolled into the 1200.32 clinical trial, 264 cases (77% of the study population, including 178 patients from the afatinib arm and 86

patients from the chemotherapy arm) were retested retrospectively by the *therascreen*® EGFR RGQ PCR Kit. Analysis of the 264 subset revealed that those patients treated with afatinib were less likely to have an event of progressive disease or death (HR= 0.49, 95 % CI [0.35; 0.69], p<0.0001) than patients treated with chemotherapy. Sensitivity analyses assessing the impact of discordant and missing EGFR Kit data on PFS showed consistent results with the primary analysis.

The risks of the *therascreen*® EGFR RGQ PCR Kit are associated with the potential mismanagement of patients resulting from false results of the test. The device is a key part of diagnostic evaluation for NSCLC in decisions regarding treatment with afatinib. There is currently no FDA-approved test for the selection of candidate metastatic NSCLC patients for treatment with afatinib.

In conclusion, given the available information above, the data support the use of the *therascreen*® EGFR RGQ PCR Kit as an aid in selecting NSCLC patients for afatinib treatment based on a *therascreen*® EGFR RGQ PCR Kit “Mutation Detected” result, 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 1200.32 clinical trial support the utility of the *therascreen*® EGFR RGQ PCR Kit as an aid in selecting patients with NSCLC for whom GILOTRIF™ (afatinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated. GILOTRIF™ (afatinib) demonstrated significant improvement in PFS compared with chemotherapy in NSCLC patients with EGFR mutations (i.e., exon 19 deletion or exon 21 L858R substitution mutations) identified with the *therascreen*® EGFR RGQ PCR Kit.

### **XIII. CDRH DECISION**

CDRH issued an approval order on July 12, 2013. The final conditions of approval can be found in the approval order.

The applicant’s manufacturing facilities were inspected on August 28, 2012 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 GILOTRIF™ (afatinib) for additional information related to use of the drug.

Post-approval Requirements and Restrictions: See approval order.