SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Somatic Mutation PCR test

Device Trade Name: Abbott RealTi*m*e IDH1

Device Procode: OWD

Applicant's Name and Address: Abbott Molecular Inc.
1300 E. Touhy Ave
Des Plaines, IL 60018

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P170041

Date of FDA Notice of Approval: July 20, 2018

II. INDICATIONS FOR USE

Abbott RealTi*m*e IDH1 is an *in vitro* polymerase chain reaction (PCR) assay for the qualitative detection of single nucleotide variants (SNVs) coding five IDH1 R132 mutations (R132C, R132H, R132G, R132S, and R132L) in DNA extracted from human blood (EDTA) or bone marrow (EDTA). Abbott RealTi*m*e IDH1 is for use with the Abbott *m*2000rt System.

Abbott RealTi*m*e IDH1 is indicated as an aid in identifying acute myeloid leukemia (AML) patients with an isocitrate dehydrogenase-1 (IDH1) mutation for treatment with TIBSOVO® (ivosidenib).

This test is for prescription use only.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Abbott RealTi*m*e IDH1 labeling.

V. DEVICE DESCRIPTION

Abbott RealTi*m*e IDH1 detects single nucleotide variants (SNVs) coding five IDH1 mutations (R132C, R132H, R132G, R132S, and R132L) by using PCR technology with

homogeneous real-time fluorescence detection. The assay uses human blood or bone marrow aspirate specimens and reports a qualitative result.

The following components comprise the overall device:

1. Abbott RealTi*me* IDH1 Amplification Reagent Kit:

    - Abbott RealTi*me* IDH1 Oligonucleotide Reagent 1: Contains synthetic oligonucleotide forward and reverse primers which amplify the IDH1 R132 region and an endogenous internal control region (IC), and synthetic probes for R132C and R132H mutations and IC in a buffered solution with a reference dye, dNTPs and the preservatives sodium azide and ProClin 950.

    - Abbott RealTi*me* IDH1 Oligonucleotide Reagent 2: Contains synthetic oligonucleotide forward and reverse primers which amplify the IDH1 R132 region and an endogenous internal control region (IC), and synthetic probes for R132G, R132S and R132L mutations and IC in a buffered solution with a reference dye, dNTPs and the preservatives sodium azide and ProClin 950.

    - Abbott RealTi*me* IDH1 DNA Polymerase

    - Abbott RealTi*me* IDH1 Activation Reagent

2. Abbott RealTi*me* IDH1 Control Kit

    The Abbott RealTi*me* IDH1 assay controls are used to establish run validity for the Abbott RealTi*me* IDH1 assay. The Abbott RealTi*me* IDH1 Control Kit consists of the following:

    - Abbott RealTi*me* IDH1 Negative Control: The negative control consists of noninfectious plasmid DNA in a buffered solution with carrier DNA. The plasmid contains the IDH1 internal control (IC) sequence.

    - Abbott RealTi*me* IDH1 Positive Control: The positive control consists of noninfectious plasmid DNA in a buffered solution with carrier DNA. The plasmids contain the following sequences: IDH1 IC, R132H, R132G and R132L mutations.

3. Abbott *m*Sample Preparation System$_{DNA}$ Kit

4. Abbott RealTi*me* IDH1 *m*2000*rt* Application CD-ROM

Software parameters specific to Abbott RealTi*m*e IDH1 are contained in an assay application specification file, which is loaded onto the Abbott *m*2000*rt* instrument by using a CD-ROM disk.

Specimen Preparation

EDTA anticoagulated blood or bone marrow aspirate specimens are processed manually using the Abbott *m*Sample Preparation System$_{DNA}$ Kit. A minimum of 200 µL of EDTA anticoagulated blood or 100 µL of EDTA anticoagulated bone marrow is needed to perform the test. Cells are lysed at an elevated temperature in a lysis buffer containing guanidine isothiocyanate. DNA is captured on magnetic microparticles, and inhibitors are removed by performing a series of washes with wash buffers. The bound genomic DNA is eluted from the microparticles with elution buffer and is ready for PCR amplification. DNA eluates for specimens or controls can be stored at -25 to -15°C, and should not undergo more than 5 freeze/thaw cycles. Storage of thawed DNA eluate must not exceed a cumulative total of 24 hours at 15 to 30°C or 14 days at 2 to 8°C prior to PCR setup.

PCR Amplification and Detection

Abbott RealTi*m*e IDH1 Amplification Reagent Kit include reagents (Oligonucleotide Reagent 1 and Oligonucleotide Reagent 2) that are each manually combined with DNA Polymerase, and Activation Reagent to create 2 unique master mixes. These master mixes are added to 2 separate wells of the Abbott 96 Well Optical Reaction Plate with aliquots of the extracted DNA sample. After manual application of the Abbott Optical Adhesive Cover, the plate is transferred to the Abbott *m*2000*rt* instrument.

Each Abbott RealTi*m*e IDH1 master mix is designed to amplify and detect 2 or 3 IDH1 amino acid mutations as shown in Table 1. In addition, both master mixes are designed to amplify and detect a region of the IDH1 gene outside of codon 132, which serves as an endogenous internal control (IC).

Table 1. IDH1 Mutations Detected by each Master Mix

Master Mix	IDH1 Mutation	SNV
Oligonucleotide 1	R132C	**<u>T</u>GT**
	R132H	**C<u>A</u>T**
Oligonucleotide 2	R132G	**<u>G</u>GT**
	R132S	**<u>A</u>GT**
	R132L	**C<u>T</u>T**

During the amplification reaction on the Abbott *m*2000*rt* instrument, the target DNA is amplified by DNA Polymerase in the presence of primers, deoxyribonucleoside triphosphates (dNTPs), and magnesium chloride ($MgCl_2$). During each subsequent round of thermal cycling, a high temperature is used to melt double-stranded DNA strands, followed by a low temperature where primers anneal to their respective targets and are extended to generate double-stranded DNA products. Exponential amplification of the products is achieved through repeated cycling between high and low temperatures.

Amplification of IDH1 mutation and IC targets takes place simultaneously in the same PCR well.

IDH1 products are detected during the annealing/extension step by measuring the real-time fluorescence signals of the IDH1 mutation and IC-specific probes, respectively. The IDH1 mutation and IC-specific probes are labeled with different fluorophores, allowing their signals to be distinguishable in a single PCR well.

Instrument and Software

The Abbott Realti*m*e IDH1 assay uses the Abbott *m*2000*rt* System with System Software Version 8.0 or higher. The Abbott *m*2000*rt* System is an automated system for performing fluorescence-based PCR to provide quantitative and qualitative detection of nucleic acid sequences.

The operator controls the system through the SCC that is equipped with Abbott *m*2000*rt* software. Results are displayed on the Abbott *m*2000*rt* workstation.

Interpretation of Results

Abbott RealTi*m*e IDH1 is a qualitative assay for which specimen interpretations are reported as "Mutation Detected" or "Not Detected." For specimens with interpretations of "Mutation Detected", the identity of the IDH1 mutation detected is reported.

For each patient sample, 2 PCR reactions are evaluated. For a given reaction to produce a valid result, the IC amplification must pass specifications that are primarily governed by predetermined MaxRatio (MR) and Cycle Number (CN) ranges. Other fluorescence channels in the reaction are used for the detection of specific IDH1 mutations. Amplification curves, if present, in any of the mutant-specific channels must fall within predetermined MR and CN ranges. The presence of an IDH1 mutant allele is determined by a final delta CN (dCN) calculation in which the CN of the IC amplification is subtracted from the CN of the mutant amplification. If the dCN for a particular mutation falls within a predetermined range, the specific IDH1 mutation is reported as “Mutation Detected”.

Additional comparative algorithms are utilized to exclude mutant calls generated from mutant cross-reactivity or multiple mutant detections. Samples with an IDH1 mutation have a result of “XX” (XX = R132C, R132H, R132L, R132G, or R132S) and an interpretation of “Mutation Detected.” When no mutation is detected and the IC from all four reactions for a given sample is valid, the sample result is “Not Detected” and an interpretation of “Not Detected.”

Test Controls

A Positive Control and a Negative Control are required in each run to verify that the sample processing, the amplification, and the detection steps are performed correctly. The

Abbott RealTi*m*e IDH1 controls need to be processed alongside the specimens prior to running the amplification portion of the assay.

The Positive Control is formulated with DNA containing IDH1 mutation and IC sequences. The R132H, R132L, and R132G signals should be detected for the Positive Control. In addition, the IC signal should be detected in both reactions for the Positive Control.

The Negative Control is formulated with DNA containing the IC sequence. Only the IC signal should be detected for the Negative Control in both reactions. IDH1 mutations (R132C, R132H, R132G, R132S, and R132L) should not be detected in the Negative Control. IDH1 mutations detected in the Negative Control are indicative of contamination from other samples or amplified product introduced during sample processing or during preparation of the Abbott 96-Well Optical Reaction Plate.

If the result of the Negative Control or Positive Control is out of range, the run is invalid and a flag is displayed for each specimen.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Currently, there are no other FDA approved IVD medical devices for the detection of IDH1 mutations.

VII. MARKETING HISTORY

The Abbott RealTi*m*e IDH1 assay has not been marketed in the United States or any foreign country.

VIII. PROBABLE ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The risks of the Abbott RealTi*m*e IDH1 test are associated with the potential mismanagement of patients resulting from false results of the assay. The device is used to detect mutations in specimens from AML patients in decisions regarding treatment with ivosidenib. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect IDH1 mutation results, and consequently improper treatment decisions for AML patients. A false positive test result may lead to treatment being administered to a patient who may not benefit, and potentially any adverse side effects associated with treatment. A false negative test result may lead to treatment being withheld from a patient who might have benefitted.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Samples: Bone marrow aspirate and blood were tested in all key analytical studies. For IDH1 mutations, AML clinical specimens were used, when possible; otherwise cell lines

with IDH1 mutations were used. Five separate cell lines were used in the analytical studies, each cell line contained a single IDH1 mutation and represented all 5 IDH1 mutations that can be detected by Abbott RealTi*me* IDH1 including R132C, R132H, R132L, R132G, and R132S. Contrived specimens were either cell lines spiked into blood or bone marrow to target specific mutation percentages, or DNA eluates generated from blood or bone marrow spiked with cell lines were diluted to target specific mutation percentages and gDNA inputs. Functional equivalence studies were used to demonstrate that contrived specimens created using cell lines had comparable performance to clinical specimens in terms of limit of detection and precision (See section IX.4.c Below).

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1. **Comparison to an Orthogonal Method**

The purpose of this study was to demonstrate the accuracy of the Abbott RealTi*me* IDH1 for detection of the 5 IDH1 mutations. A retrospective correlation analysis was performed using a selection of total specimens from patients screened for trial eligibility in two trials. The data set included IDH1 mutation positive patients and IDH1 mutation negative patients, with similar numbers of blood and bone marrow samples (285 blood samples and 271 bone marrow samples). The agreement rates were evaluated by sample type and included analyses of both the subject outcome (detected vs. not-detected) and per specific mutation.

The Abbott RealTi*me* IDH1 assay demonstrated high agreement rates between Abbott RealTi*me* IDH1 and the NGS methods in blood specimens (**Table 2**). The analysis demonstrated a 100.00% (194/194) Positive Percent Agreement (PPA) with a 95% exact CI of (98.12%, 100.00%). The analysis demonstrated a 98.90% (90/91) Negative Percent Agreement (NPA) with a 95% exact CI of (94.03%, 99.97%). The analysis of agreement by specific mutation in **Table 3** demonstrated agreement for 284/285 patient samples.

Table 2. Comparison of Abbott RealTi*me* IDH1 assay with an NGS sequencing results using blood specimens

Abbott RealTi*me* IDH1	NGS Method		Total
	Mutation Detected	Not Detected	
	n	n	n
Mutation Detected	194	1	195
Not Detected	0	90	90
Total	194	91	285

Table 3. Comparison of Abbott RealTi*me* IDH1 Mutation Identity with an NGS method in blood specimens

	NGS Method						Total
	Not Detected	R132C	R132H	R132G	R132S	R132L	
Abbott RealTi*me* IDH 1	n	n	n	n	n	n	n
Not Detected	90	0	0	0	0	0	90
R132C	0	118	0	0	0	0	118
R132H	0	0	49	0	0	0	49
R132G	1	1	0	10	0	0	12
R132S	0	0	0	0	10	0	10
R132L	0	0	0	0	0	6	6
Total	91	119	49	10	10	6	285

The Abbott RealTi*me* IDH1 assay demonstrated high agreement rates between Abbott RealTi*me* IDH1 and the NGS methods in bone marrow specimens (**Table 4**). The analysis demonstrated a 100.00% (193/193) PPA with a 95% exact CI of (98.10%, 100.00%). The analysis demonstrated a 94.87% (74/78) NPA with a 95% exact CI of (87.39%, 98.59%). The analysis of agreement by specific mutation in **Table 5** demonstrated agreement for 267/271 patient samples.

Table 4. Comparison of Abbott RealTi*me* IDH1 assay with an NGS sequencing results using bone marrow specimens

Abbott RealTi*me* IDH1	NGS Method		Total
	Mutation Detected	Not Detected	
	n	n	n
Mutation Detected	193	4	197
Not Detected	0	74	74
Total	193	78	271

Table 5. Comparison of Abbott RealTi*me* IDH1 Mutation Identity with an NGS method using bone marrow specimens

	NGS Method						Total
	Not Detected	R132C	R132H	R132G	R132S	R132L	Total
Abbott RealTi*me* IDH 1	n	n	n	n	n	n	n
Not Detected	74	0	0	0	0	0	74
R132C	0	116	0	0	1	0	117
R132H	2	0	49	0	0	0	51
R132G	2	1	0	10	0	0	13
R132S	0	0	0	0	10	0	10
R132L	0	0	0	0	0	6	6
Total	78	117	49	10	11	6	271

2. **Concordance Between Peripheral Blood and Bone Marrow Results**

The Abbott RealTi*me* IDH1 assay is intended to be used on either blood or bone marrow aspirate. An analysis was performed to show concordance of results between matched blood and bone marrow specimen types tested by Abbott RealTi*me* IDH1.

In this study, specimens from 341 AML patients were tested with the Abbott RealTi*me* IDH1 Assay. Samples types included peripheral blood, peripheral blood mononuclear cell (PBMC) cell pellets, bone marrow aspirate and bone marrow mononuclear cell (BMMC) cell pellets. Specimens were only included in the study if there was at least one peripheral blood or PBMC sample and at least one bone marrow aspirate or BMMC from the same patient. Within-specimen type concordance was first evaluated. For the 127 patients with both peripheral blood and PBMC, 124 (97.6%) demonstrated concordant results (mutation detected vs. not detected) comparing peripheral blood and PBMC. For the 126 patients with both bone marrow aspirate and BMMC, 124 (98.4%) demonstrated concordant results (mutation detected vs. not detected) comparing bone marrow aspirate and BMMC.

Concordance between bone marrow specimens and blood specimens was determined at the subject level ("Mutation Detected" vs. "Not Detected"). Results are shown below in **Table 6** There were seven subjects who had Abbott RealTi*me* IDH1 mutation detected results in bone marrow that were not detected in the paired blood samples. There was one subject who had Abbott RealTi*me* IDH1 mutation (R132C) detected result in blood that was not detected in the bone marrow. The analysis demonstrates concordance between specimen types, with a slightly higher IDH1 mutation positive detection rate observed in bone marrow.

Table 6. Comparing Abbott RealTi*m*e IDH1 assay Results from Blood/PBMC versus Bone Marrow/BMMC

Bone Marrow/BMMC	Blood/PBMC		Total
	Mutation Detected	Not Detected	
	n	n	N
Mutation Detected	203	7	210
Not Detected	1	130	131
Total	204	137	341
Average Positive Agreement = 98.07% (406/414); 95% CI = 96.56 – 99.26% Average Negative Agreement = 97.01% (260/268); 95% CI = 94.82 – 98.7% Overall Agreement = 97.65% (333/341); 95% CI = 95.89 – 99.12%			

3. **Validation of DNA Input**

Quantitation of DNA prior to PCR amplification is not a required step. A fixed amount of diluted eluate (15 µL) from sample extraction is used in each PCR reaction. In order to validate the range of DNA that can be used in the assay, the DNA yield from 277 AML clinical specimens (198 bone marrow aspirate specimens and 79 blood specimens) using the Abbott mSample Preparation SystemDNA was measured. The gDNA input range observed for bone marrow aspirate was 10 ng to 839 ng per PCR reaction. The gDNA input range observed for whole blood was 11 ng to 492 ng per PCR reaction. Table 7 lists the 5%, 25%, 50%, 75%, and 95% quantile for each analysis. To cover this range, the analytical studies were designed to validate 10 ng to 850 ng gDNA input per PCR reaction. Validation of assay performance across a range of DNA is further discussed and shown in Table 11 below.

Table 7. gDNA input range per PCR reaction by Sample Type

	Combined (n=277)	Bone Marrow (n=198)	Whole Blood (n=79)
5 % quantile	18 ng	18 ng	17 ng
25% quantile	48 ng	49 ng	44 ng
50% quantile (median)	84 ng	84 ng	85 ng
75% quantile	132 ng	132 ng	132 ng
95% quantile	337 ng	394 ng	242 ng

4. **Analytical Sensitivity**

a) **Limit of Blank (LoB)**

The Limit of Blank (LoB) was verified by testing 10 normal blood specimens, 10 normal bone marrow aspirate specimens, and 2 blank samples (water). Each specimen or sample was tested in replicates of 4, yielding a total of 88 replicates (40 replicates for whole blood specimens, 40 replicates for bone marrow aspirate specimens, and 8 replicates for blank samples). The overall rate of correct sample interpretation was 100% (88/88) which demonstrated an acceptable rate of true negativity. There was no detectable background amplification for the R132C, R132L, R132S and R132G probe sets. Replicates of R132H show background dCN values in blood specimens (n=36) and bone marrow (n=39). However, all dCN values observed are outside the dCN threshold for detection, therefore all were correctly reported as "Not Detected". Results are summarized in **Table 8**.

Table 8. Mean dCN and SD Calculated for Blood, Bone Marrow, and Blank Samples in the Limit of Blank Study for probe sets that detect each IDH1 mutation.

	Blood (n=40)			Bone Marrow (n=40)			Blank (n=8)		
Mutation	**n***	**Mean dCN**	**SD**	**n***	**Mean dCN**	**SD**	**n***	**Mean dCN**	**SD**
R132H	0/40	14.78	0.838	39/40	14.49	0.557	0/8	NA	NA
R132C	0/40	NA	NA	0/40	NA	NA	0/8	NA	NA
R132L	0/40	NA	NA	0/40	NA	NA	0/8	NA	NA
R132S	0/40	NA	NA	0/40	NA	NA	0/8	NA	NA
R132G	0/40	NA	NA	0/40	NA	NA	0/8	NA	NA

* n = number of reactions that yielded false results. There were 36 total reactions that resulted in the detectable dCN values used to calculate mean and SD but these values are outside the threshold for calling results detected.

b) Limit of Detection (LoD)

The LoD of the Abbott RealTi*m*e IDH1 assay was assessed using eluates generated from IDH1 mutation positive AML clinical specimens and contrived specimens. The LoD for each of the single nucleotide variants (SNVs) coding for IDH1mutations for R132H, R132C, R132L, R132S and R132G was assessed across the anticipated low DNA input and median input (10ng and 85ng, respectively) in two separate studies. Both studies were conducted to allow each of 5 SNVs to be tested using AML clinical specimens in at least 1 study.

i. Limit of Detection Study 1

The IDH1 percent mutation ranged from 0.25% to 10% for 10 ng gDNA input and from 0.13% to 10% mutation for 85 ng gDNA input. IDH1 R132C mutation positive panel members were prepared from blood and bone marrow

aspirate AML clinical specimen eluates. IDH1 R132G, R132H, R132L, and R132S mutation positive panel members were prepared from eluates of IDH1 mutation positive cell lines spiked into blood or bone marrow. Each panel member was tested with 2 Abbott RealTi*m*e IDH1 Amplification Reagent Kit lots with 4 runs per lot over a minimum of 4 days and 5 replicates per run for a total of 40 replicates per panel member per mutation and 200 replicates for the 5 mutations combined. The analysis demonstrated a detection rate of 100% (200/200) at mutation levels of 2% and higher for all IDH1 mutations combined. The analysis demonstrated a detection rate of 98% (196/200) or greater at mutation levels of 1% and higher for all IDH1 mutations combined (Table 9). The data supports a claimed LoD of 1%.

Table 9. Limit of Detection of mutation frequency at 10 ng and 85 ng DNA inputs:

DNA input	Sample Type	Allele Frequency	Detection Rate	
85 ng	Blood	10%	200/200	100%
85 ng	Blood	5%	200/200	100%
85 ng	Blood	2%	200/200	100%
85 ng	Blood	1%	196/200	98%
85 ng	Blood	0.5%	180/200	90%
85 ng	Blood	0.25%	156/200	78%
85 ng	Blood	0.13%	129/200	64.5%
85 ng	Bone Marrow	10%	200/200	100%
85 ng	Bone Marrow	5%	200/200	100%
85 ng	Bone Marrow	2%	200/200	100%
85 ng	Bone Marrow	1%	197/197	100%
85 ng	Bone Marrow	0.5%	191/200	95.5%
85 ng	Bone Marrow	0.25%	165/200	82.5%
85 ng	Bone Marrow	0.13%	129/200	64.5%
10 ng	Blood	10%	200/200	100%
10 ng	Blood	5%	200/200	100%
10 ng	Blood	4%	200/200	100%
10 ng	Blood	2%	200/200	100%
10 ng	Blood	1%	199/200	99.5%
10 ng	Blood	0.5%	166/200	83%
10 ng	Blood	0.25%	72/200	36%
10 ng	Bone Marrow	10%	200/200	100%
10 ng	Bone Marrow	5%	200/200	100%
10 ng	Bone Marrow	4%	200/200	100%
10 ng	Bone Marrow	2%	200/200	100%
10 ng	Bone Marrow	1%	200/200	100%
10 ng	Bone Marrow	0.5%	156/200	78%
10 ng	Bone Marrow	0.25%	61/200	30.5%

ii. **Limit of Detection Study 2**

To confirm the results of the first study with clinical specimens, the analytical sensitivity was assessed using eluates generated from IDH1 mutation positive AML clinical specimens. The IDH1 percent mutation ranged from 0.25% to 5% for 10 ng gDNA input. A 10-member panel was generated for each of 4 SNVs (R132H, R132L, R132G, and R132S) evaluated in this study and was prepared from AML clinical specimen eluates, both blood and bone marrow aspirate. Each panel member was tested with 2 Abbott RealTi*m*e IDH1 Amplification Reagent Kit lots with 4 runs per lot over 4 days and 5 replicates per run for a total of 40 replicates per panel member and 160 replicates for the 4 mutations combined.

The analysis demonstrated a detection rate of 100% (160/160) at mutation levels of 1% and higher for the 4 IDH1 mutations combined. Refer to Table 10 for results.

Table 10. Limit of Detection of mutation frequency at 10 ng for R132H, R132L, R132G and R132S using eluates prepared from AML clinical specimens

DNA input	Sample Type	Allele Frequency	Detection Rate	
10 ng	Blood	5%	160/160	100%
10 ng	Blood	2%	160/160	100%
10 ng	Blood	1%	160/160	100%
10 ng	Blood	0.5%	131/160	81.9%
10 ng	Blood	0.25%	46/160	28.8%
10 ng	Bone Marrow	5%	160/160	100%
10 ng	Bone Marrow	2%	160/160	100%
10 ng	Bone Marrow	1%	160/160	100%
10 ng	Bone Marrow	0.5%	123/160	76.9%
10 ng	Bone Marrow	0.25%	51/160	31.9%

c) **Functional Equivalence Study Comparing Clinical Specimens to Contrived Specimens**

The data generated for Limit of Detection study 1 and Limit of Detection study 2 were reanalyzed to evaluate functional equivalency between IDH1 mutation-positive AML blood/bone marrow specimens (clinical specimens) and blood/bone marrow specimen spiked with IDH2 mutation-positive cell lines (contrived specimens). Both limit of detection and precision performance using a fixed DNA input amount of 10 ng and mutation percentages of 5%, 2%, 1%, 0.5% and 0.25% were evaluated. The results from clinical versus contrived specimens for SNVs R132H, R132S, R132G, and R132L showed similar detection rates, dCN values and standard deviation of dCN values when comparing each dilution. The study supports the conclusion that performance is not unduly overestimated when the cell lines are used.

5. Validation of IDH1 Mutation Detection a Across a Low to High Range of DNA Input Levels

A specific input of genomic DNA is not specified for the Abbott RealTi*m*e IDH1. This study evaluated the detection of 2% mutation levels across a range of gDNA input amounts that span the 5th and 100th percentile of gDNA concentrations that are expected to be used for this assay.

For each IDH1 SNV, the following gDNA inputs were tested with percent mutation near the LoD (2% mutation level) in both blood and bone marrow aspirate: 1 ng, 25 ng, 200 ng, 400 ng, and 850ng. Five panel members were prepared per mutation for each sample type (blood and bone marrow), yielding 10 panel members per mutation and a total of 50 panel members. Each panel member was tested in triplicate with a single lot of the Abbott RealTi*m*e IDH1 Amplification Reagent Kit.

All panel members reported "Mutation Detected" at a 100% (15/15) detection rate when total gDNA input levels were 25 ng, 200 ng, 400 ng and 850 ng per reaction. When the gDNA input level was 1 ng per reaction, an overall detection rate of 0% (0/15) was observed due to IC cycle number being nonreactive or greater than the maximum for non-detected replicates, as expected for the low input level. Refer to **Table 11** for results.

Table 11. Abbott RealTi*m*e IDH1 2% Mutation Detection across range of DNA Input Levels

Sample Type	gDNA Input per Reaction (ng)	Detection Rate (%)	
Blood	1	0/15	0%
Blood	25	15/15	100%
Blood	200	15/15	100%
Blood	400	15/15	100%
Blood	850	15/15	100%
Bone Marrow	1	0/15	0%
Bone Marrow	25	15/15	100%
Bone Marrow	200	15/15	100%
Bone Marrow	400	15/15	100%
Bone Marrow	850	15/15	100%

The analysis demonstrated a detection rate of 100% at mutation levels of approximately 2% when the total gDNA input was approximately 25 ng, 200 ng, 400 ng and 850 ng per reaction for all mutations tested. Labeling indicates that the assay has been validated across the range of 10 ng to 850 ng. This statement supported by the totality of the studies Limit of Detection 1, Limit of Detection 2

and Characterization of IDH1 Mutation Detection at DNA Input Levels Across and Below Assay Range.

6. **Characterization of dCN Across a Range of Mutation Levels at Fixed DNA Input**

To demonstrate the linearity of the Abbott RealTi*m*e IDH1 assay across the range of mutant allele fractions, a series of dilutions with varying concentrations of mutant DNA in a background of wild-type DNA at a fixed DNA input (50ng) from both blood and bone marrow specimens was evaluated for all mutations. Synthetic DNA coding for R132C, R132L, and R132S were diluted in whole blood eluate and R132H and R132G were diluted in bone marrow eluate. Each of the 5 IDH1 mutations were tested with IDH2 percent mutation levels of 50%, 20%, 10%, 5%, 2.5%, and 1.25%, while gDNA input was held constant at 50 ng. Three replicates of each of the prepared panel members were tested. One lot of amplification reagents and one Abbott *m*2000*rt* instrument was used.

Correlation and linear regression analyses evaluated the association of $\log_2$ percent mutation with dCN for each of the 5 IDH2 mutations. The slope of the regression lines ranged from -0.95 to -1.04 (an ideal value = -1.0) with R^2 values all above 0.998. These data demonstrated that at fixed DNA input, the amplification efficiency was acceptable.

7. **Characterization of dCN Across a Range of DNA Inputs at a Fixed Mutation Level**

A study was conducted to demonstrate the amplification efficiency of the control relative to the mutant is linear across a range of DNA concentrations and consistency of the dCN values. The mutant allele fraction was held constant. Synthetic DNA coding for R132C, R132L, and R132S were diluted in whole blood eluate and R132H and R132G were diluted in bone marrow eluate. The percent mutation level for each plasmid was held constant at 5% while gDNA input was tested at 200 ng, 100 ng, 50 ng, 20 ng, 10 ng, and 5 ng. Three replicates of each of the prepared panel members were tested. One lot of amplification reagents and one Abbott *m*2000*rt* instrument was used.

Linear regression analyses evaluated the association of $\log_2$ DNA input with dCN for each of the 5 IDH1 mutations. When the percent mutation level was held constant, the slope of the regression lines ranged from -.001 to 0.003 (an ideal value = 0.0). These data were used to calculate amplification efficiency. The amplification efficiency was calculated based on CN values ranged from 94.44 to 104.88% for all mutations.

These data demonstrated that the assay has acceptable amplification efficiency and linearity for each probe set.

8. Analytical Specificity

a) Primer and Probe Specificity
An in silico analysis was performed to evaluate primer and probe specificity using publicly available sequence and mutation databases. The Basic Local Alignment Search Tool (BLAST) was used to search for potential cross-reactive sequences associated with the Abbott RealTi*m*e IDH1 assay primers and probes. The BLAST search results showed that the primers and probes are specific for the IDH1 gene. Amplification/detection of unintended human genes is not expected.

b) Cross Reactivity and Exclusivity
This study evaluated the rate of correct sample interpretation for each Abbott RealTi*m*e IDH1 target using a panel representing IDH1 SNVs targeted by the assay, IDH1 SNVs not targeted by the assay, and wild type genomic DNA. Panel members were prepared such that each PCR reaction contained a total input of approximately 85 ng of wild type human placental (HP) DNA, or wild type HP DNA plus plasmid DNA resulting in a percent mutation of approximately 25% to 50% in 85 ng of total DNA. Panel Members included wild type HP DNA (R132) and wild type HP DNA plus one of 7 DNA plasmids coding for each of the 5 IDH1 SNVs targeted by the assay (R132C, R132H, R132L, R132G, and R132S) or 2 IDH1 SNVs not targeted by the assay (R132P, R132V). The prepared panel members were directly tested using the Abbott RealTi*m*e IDH1 Amplification Reagent Kit. Forty replicates per panel member were tested.

The wild type HP DNA panel member reported “Not Detected” with a 100% (40/40) rate of correct sample interpretation. Five panel members targeted by the assay each reported “Mutation Detected” with a 100% (40/40) rate of correct sample interpretation, which included the correct identity of the IDH1 mutation. Two panel members not targeted by the assay (R132P mutation, and R132V mutation) reported “Not Detected” with a 100% (40/40) rate of correct sample interpretation.

The analysis of the underlying dCN value was performed. **Table 12** summarizes the mean dCN, SD, and number of replicates (n) that provided detectable dCNs for the mean and SD calculations for each allele. The dCN values for R132H are observed in all 40 replicates of the wild type and all 40 replicates of the samples containing the R132P plasmid while dCN values for R132G are observed for all 40 replicates of the samples containing the R132P plasmid. However, the final sample results have the correct interpretation because for a sample to be called mutation detected, it must meet three threshold criteria. These three criteria consist of the dCN, MR, and Max CN thresholds. Even though, R132P met the dCN threshold criteria for R132H, it does not meet the MR threshold criteria for R132H. The assay is not designed to detect R132P.These data indicate that the

probes specifically detect the R132 alleles of IDH1 that they are designed to detect.

Table 12. Mean dCN and SD Calculated for Probes to the R132 Alleles of IDH1 for each sample with Plasmids Containing Specific IDH1 mutation.

	R132H			R132C			R132L			R132S			R132G		
Mutation	**n#**	**Mean**	**SD**	**n#**	**Mean**	**SD**	**n#**	**Mean**	**SD**	**n#**	**Mean**	**SD**	**n#**	**Mean**	**SD**
WT	38	13.9	0.84	0			0			0			0		
R132H	40	2.4	0.09	0			0			0			0		
R132C	0			40	3.8	0.2	0			0			0		
R132L	0			0			49	1.25	0.6	0			0		
R132S	0			0			0			40	1.8	0.05	0		
R132G	0			0			0			0			40	1.76	0.1
R132P	40	5.09	0.35	0			0			0			0		
R132V	0			0			0			0			40	3.43	0.2

#n = number of reactions out of 40 that resulted in the detectable dCN values used to calculate mean and SD. Shaded cells indicate results for the matched mutations and probe sets designed to detect those mutations.

9. Potentially Interfering Substances

a) Potential interfering substances from clinical specimens

Potentially interfering substances that may be found in clinical specimens were added to IDH1 mutation negative and IDH1 R132H mutation positive blood and bone marrow aspirate specimens. For the IDH1 mutation positive panel members, a R132H positive cell line was spiked in blood and bone marrow targeting a 6% mutation level. A total of 3 replicates of each test condition and each sample were evaluated for the presence of potential interference. Test conditions included control diluents (one for bilirubin and one for all other substances) or 6 potentially interfering substances at study specified concentrations (hemoglobin [2-3 g/L], albumin [120-140 g/L], bilirubin [324-366 µM], triglycerides [37-40.7mM], R(-)-2-hydroxyglutarate (2-HG) [100,000 ng/mL], and EDTA [17 µM]). The analysis demonstrated the percent agreement was 100% (3/3) for each sample at each test condition. No interference of the Abbott RealTi*m*e IDH1 assay was observed in the presence of the potentially interfering substances for all IDH1 mutation positive and negative samples for either blood or bone marrow aspirate specimens.

b) Ethanol Carryover

The purpose of this study was to characterize the robustness of the Abbott RealTi*m*e IDH1 assay to residual ethanol carryover in sample preparation eluate. Specimens for the Abbott RealTi*m*e IDH1 assay are processed manually using the Abbott *m*Sample Preparation System $_{DNA}$ to isolate DNA. The operator is instructed to place the tubes on the magnetic capture stand, use a pipette tip to carefully remove the ethanol wash buffer from the tube, and to remove the liquid as completely as possible. This is followed by a 13 ± 1 minute heated drying step to evaporate residual ethanol. This study assesses the consequences of residual ethanol left behind at these steps due to inadequate pipetting or incomplete drying on Abbott RealTi*m*e IDH1 assay performance.

The ethanol wash buffer is approximately 75% ethanol. The impact of residual ethanol in sample preparation eluate on the robustness of the Abbott RealTi*m*e IDH1 assay was tested by comparing target containing 3% ethanol to target without ethanol (nominal). A final concentration of approximately 3% ethanol was chosen for this study by assuming a worst-case scenario of 20 µl residual ethanol wash buffer after the removal and evaporation steps. After these steps, 500 µl elution buffer is added. In this worst-case scenario, the final concentration of ethanol would be: (20 µl (75% ethanol)) / 520 µl = 2.8%.

IDH1 mutant plasmid targets (R132C, R132H, R132G, R132H and R132S) were diluted to 5% mutation in 20 ng total gDNA input. The source of gDNA used for this study was human placental gDNA. Ethanol (EtOH) was added to a sample of each target type to yield approximately 3% ethanol.

For each of the five mutants, a total of 3 replicate PCR reactions of each sample with ethanol and a total of 3 replicates without ethanol (nominal) were tested in one run.

The results of this study support that Abbott RealTi*m*e IDH2 is robust to the presence of residual ethanol in sample and control eluates.

10. Potentially Interfering Drugs

Potentially interfering drugs that may be found in clinical specimens were added to IDH1 mutation negative and IDH1 R132C mutation positive blood and bone marrow aspirate specimens. For the IDH1 mutation positive panel members, a R132C positive cell line was spiked in blood and bone marrow targeting a 6% mutation level. A total of 3 replicates of each test condition (control or Drug Pool 1 to 4) for each specimen was evaluated. The control condition tested specimens with no potentially interfering drugs added, whereas Drug Pool 1 to 4 tested specimens with added pooled drug combinations as described in Table 13.

Table 13. Abbott RealTi*m*e IDH2 Potentially Interfering Drug Pools			
Pool 1	**Pool 2**	**Pool 3**	**Pool 4**
Vancomycin Hydrochloride	Levofloxacin	Linezolid	Meropenem
Busulfan	Acyclovir	Arsenic Trioxide	Azacytidine
Decitabine	Cyclophosphamide	Cytarabine	Daunorubicin Hydrochloride
Hydroxyurea	Doxorubicin Hydrochloride	Etoposide	Fludara
Mitoxantrone Hydrochloride	Idarubicin Hydrochloride	Lomustine	Melphalan
Everolimus	Vincristine Sulfate	Erythropoietin	Filgrastim
Dexamethasone	Mercaptopurine	Mycophenolate Mofetil	Tacrolimus
	Prednisone	enasidenib	ivosidenib

Each drug was tested at three times the peak serum concentration (CMAX), recommended dose as listed in the drug package inserts, or recommended concentration per Clinical and Laboratory Standards Institute EP07-A21. The analysis demonstrated the percent agreement was 100% (3/3) for each sample and test condition.

No interference of the Abbott RealTi*m*e IDH1 assay was observed in the presence of the potential interfering drugs for all IDH1 mutation positive and negative samples for either blood or bone marrow aspirate specimens.

11. <u>Potentially Interfering Microorganisms</u>

Potentially interfering microorganisms were added to IDH1 mutation negative and IDH1 R132C mutation positive blood and bone marrow aspirate specimens. For the IDH1 mutation positive panel members, a R132C positive cell line was spiked in blood and bone marrow targeting a 6% mutation level. A total of 3 replicates of each of 5 test conditions (control and Microbe Pools 1 to 4) were evaluated. The microbe pools are shown in Table 14.

Table 14. Abbott RealTi*m*e IDH2 Potentially Interfering Microbe Pools			
Pool 1	**Pool 2**	**Pool 3**	**Pool 4**
Staphylococcus aureus (MRSA; COL)	*Streptococcus agalactiae*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*
Staphylococcus aureus (CoNS)	*Streptococcus pneumoniae*	*Enterobacter cloacae*	*Acinetobacter baumannii*
Staphylococcus epidermidis	*Klebsiella pneumoniae*	*Aspergillus terreus*	varicella-zoster virus

Escherichia coli	*Candida albicans*	influenza A H3	respiratory syncytial virus type A
Serratia marcescens	herpes simplex virus type 1 MacIntyre		respiratory syncytial virus type B
cytomegalovirus	herpes simplex virus type 2 MS		

Potentially interfering microorganisms were chosen based on clinical presentation in AML patients. The analysis demonstrated the percent agreement was 100% (3/3) for each sample and test condition.

No interference or cross reactivity of the Abbott RealTi*m*e IDH1 assay was observed in the presence of the potentially interfering microorganisms for all IDH1 mutation positive and negative samples tested with either blood or bone marrow aspirate specimens.

12. Precision: Within-Laboratory Repeatability

The within-laboratory repeatability was evaluated by two operators using an 8-member panel. The panel contained IDH1 mutation negative blood and bone marrow aspirate specimens as well as blood and bone marrow aspirate specimens spiked with R132C or R132G positive cell lines targeting a 6% mutation level. Positive mutations were chosen such that the panel contained a representative mutation amplified by each of the two master mixes. Each operator tested the panel using 2 lots of grouped reagents–2 lots of Abbott mSample Preparation SystemDNA, Abbott RealTi*m*e IDH1 Amplification Reagent Kits, and Abbott RealTi*m*e IDH1 Control Kits–for 4 runs per lot over 4 days. Each run consisted of 2 replicates for each panel member for a total of 32 replicates per panel member (16 replicates per lot). Testing was conducted using 2 m2000rt instruments. Each reagent lot was assigned to its own instrument.

The Abbott RealTi*m*e IDH1 Assay demonstrated a percent agreement of 100% (95% CI 89, 100) with expected results for each panel overall **(Table 15).**

Table 15. Abbott RealTi*me* IDH1 Repeatability Overall Percent Agreement

Mutation Status	Sample Type	Agreement with Expected Result (Number of Replicates) Yes	No	Percent Agreement (%)	95% Confidence Interval
WT	Blood	32	0	100	(89 - 100)
WT	Blood	32	0	100	(89 - 100)
R132C	Blood	32	0	100	(89 - 100)
R132G	Blood	32	0	100	(89 - 100)
WT	Bone Marrow	32	0	100	(89 - 100)
WT	Bone Marrow	32	0	100	(89 - 100)
R132C	Bone Marrow	32	0	100	(89 - 100)
R132D	Bone Marrow	32	0	100	(89 - 100)

The mean and SD of dCN values and the within-run, between-run, between-lot and between-operator variability are shown in Table 16 The mean and SD of IC CN values and the within-run, between-run, between-lot and between-operator variability for Positive and Negative Controls as well as the mean and SD of mutant CN values and the within-run, between-run, between-lot and between-operator variability for Positive Controls are shown in Tables 17. The results met their acceptance criteria and demonstrated acceptable precision.

Table 16. Abbott RealTi*me* IDH1 Repeatability Precision Analysis of dCN Values

				Within-Run Variability	Between-Run Variability	Between-Lot Variability	Between-Operator Variability	Total
Mutation Status	Sample Type	N	Mean dCN	SD	SD	SD	SD	SD
R132C	Blood	32	9.32	0.310	0.248	0.111	0.080	0.420
R132G	Blood	32	6.00	0.163	0.000	0.097	0.058	0.190
R132C	Bone Marrow	32	7.83	0.154	0.174	0.283	0.283	0.366
R132G	Bone Marrow	32	5.60	0.170	0.000	0.000	0.008	0.170

Table 17. Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls

Panel	Value	N	Mean	Within-Run Variability SD	Between-Run Variability SD	Between-Lot Variability SD	Between-Operator Variability SD	Total SD
IDH1 Negative Control	OLIGO1 IC CN	16	23.32	0.631	0.591	0.242	0.343	0.961
	OLIGO2 IC CN	16	22.01	0.224	0.000	0.077	0.114	0.262
IDH1 Positive Control	OLIGO1 IC CN	16	23.23	0.746	0.268	0.245	0.185	0.830
	OLIGO2 IC CN	16	22.02	0.173	0.000	0.068	0.000	0.186
	R132H CN	16	26.08	0.688	0.370	0.000	0.205	0.808
	R132L CN	16	24.04	0.305	0.000	0.212	0.040	0.371
	R132G CN	16	24.18	0.195	0.116	0.035	0.079	0.243

13. **Reproducibility Studies**

A. Within-Laboratory Lot-to-Lot Reproducibility

The within-laboratory lot-to-lot reproducibility was evaluated using 3 unique lots of Abbott mSample Preparation SystemDNA kits and 3 unique lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kits. An 8-member panel contained IDH1 mutation negative blood and bone marrow specimens as well as blood and bone marrow specimens spiked with R132C or R132G positive cell lines targeting a 6% mutation level. Positive mutations were chosen such that the panel contained a representative mutation amplified by each of the two master mixes. For each panel member, 10 replicates were prepared using 3 unique lots of Abbott mSample Preparation SystemDNA for a total of 30 extractions per panel member. Each extraction was tested using 3 unique lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kit for a total of 9 unique sample preparation/amplification kit combinations. Three m2000rt instruments were used, one for each lot of Abbott RealTi*m*e IDH1 Amplification Reagent Kit. Thus, for each panel member, a total of 90 replicates were tested.

Test result for mutation positive panel members demonstrated 89/90 (98.9%) agreement rates for each R132C and R132G in blood and 90/90 (100%) agreement rates for R132C and R132G in bone marrow. Mutation negative panel member included 2 blood and 2 bone marrow specimens. All mutation negative panel members demonstrated 90/90 (100%) agreement rates with

expected results. The percent agreement by Abbott mSample Preparation SystemDNA lot was 97% or greater for each panel member. The percent agreement by Abbott RealTi*m*e IDH1 Amplification Reagent Kit lot was 97% or greater for each panel member.

The within-laboratory lot-to-lot reproducibility, the mean and SD of dCN values and the between-replicate, between-amplification lot and between-sample prep lot variability are shown in **Table 18**. The mean and SD of IC CN values and the between-replicate, between-amplification lot and between-sample prep lot variability for Positive and Negative Controls as well as the mean and SD of mutant CN values and the between-replicate, between-amplification lot and between-sample prep lot variability for Positive Controls is shown in **Table 19.**

Table 18. Analysis of dCN Values for Mutation Positive Panel Members

				Between-Replicate Variability	Between-Amplification Lot Variability	Between-Sample Prep Lot Variability	Total
Mutation Status	**Sample Type**	**N**	**Mean**	**SD**	**SD**	**SD**	**SD**
R132C	Blood	89*	8.39	0.309	0.363	0.981	1.051
R132G	Blood	89*	6.16	0.160	0.198	0.096	0.272
R132C	Bone Marrow	90	6.92	0.301	0.270	0.291	0.441
R132G	Bone Marrow	90	5.61	0.157	0.149	0.320	0.376

*One replicate was Not Detected and no CN value was calculated and excluded from analysis since dCN value was not generated.

Table 19. Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls

Panel	Value	N	Mean	Between-Replicate Variability SD	Between-Amplification Lot Variability SD	Between-Sample Prep Lot Variability SD	Total SD
IDH1 Negative Control	OLIGO1 IC CN	36	22.71	0.466	0.234	0.176	0.532
	OLIGO2 IC CN	36	21.78	0.306	0.281	0.124	0.434
IDH1 Positive	OLIGO1 IC CN	36	22.61	0.555	0.210	0.155	0.613

Table 19. Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls							
Panel	**Value**	**N**	**Mean**	**Between-Replicate Variability SD**	**Between-Amplification Lot Variability SD**	**Between-Sample Prep Lot Variability SD**	**Total SD**
Control	OLIGO2 IC CN	36	21.82	0.176	0.243	0.077	0.310
	R132H CN	36	25.20	0.513	0.179	0.105	0.553
	R132L CN	36	24.04	0.214	0.221	0.050	0.311
	R132G CN	36	24.18	0.205	0.154	0.049	0.261

B. Within-Laboratory Specimen Handling Reproducibility

The within-laboratory specimen handling reproducibility of Abbott RealTi*m*e IDH1 was evaluated using two operators.

The panel included three mutation positive AML blood specimens (R132C, R132H and R132S), three mutation positive AML bone marrow aspirate specimens (R132C, R132H and R132S), two mutation positive contrived blood specimens (R132L and R132G) and two mutation positive contrived bone marrow aspirate specimens (R132L and R132G), prepared from normal blood or bone marrow spiked with IDH1 mutation positive cell line targeting 6% mutation level.

Each operator tested the panel using the same lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kit and Abbott RealTi*m*e IDH1 Control Kit and unique lots of Abbott mSample Preparation SystemDNA. Each operator performed 1 run per day over a total of 5 days. A run consisted of sample preparation and PCR. Two (2) replicates of each panel member were tested in each run, for a total of 20 replicates per panel member (10 replicates per operator). One (1) replicate of each control was tested in each run, for a total of 10 replicates per control (5 replicates per operator). Testing was conducted using two *m2000rt* instruments. Each Abbott *m*Sample Preparation System$_{DNA}$ lot was assigned to its own instrument.

In the within-laboratory specimen handling reproducibility, all IDH1 mutation positive panel members were reported as "Mutation Detected" with 100% (10/10) agreement for each panel member by operator, and an overall agreement of 100% (20/20) for each panel member. The mean and SD of dCN values and the within-run, between-run and between-operator variability are shown in **Table 20.** The mean and SD of IC CN values and the between-run and between-operator variability for Positive and Negative Controls as well as the mean and

SD of mutant CN values and the between-run and between-operator variability for Positive Controls are shown in **Table 21.**

Table 20. Specimen Handling Reproducibility: Overall Variability of dCN for mutation positive samples

Mutation Status	Sample Type	N	Mean (dCN)	Within-Run Variability SD	Between-Run Variability SD	Between-Operator Variability SD	Total SD
R132C	Blood	20	3.75	0.13	0.188	0.237	0.329
R132H	Blood	20	2.86	0.074	0.039	0	0.084
R132S	Blood	20	3.33	0.108	0.045	0	0.117
R132L	Blood	20	4.07	0.173	0.11	0.347	0.403
R132G	Blood	20	4.91	0.156	0.065	0.239	0.292
R132C	Bone Marrow	20	3.97	0.068	0.189	0.441	0.485
R132H	Bone Marrow	20	3.23	0.051	0.083	0	0.098
R132S	Bone Marrow	20	2.53	0.033	0.128	0.09	0.159
R132L	Bone Marrow	20	4.17	0.11	0.172	0.24	0.315
R132G	Bone Marrow	20	4.47	0.112	0.091	0.193	0.241

Table 21. Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls

Panel	Value	N	Mean	Between-Run Variability SD	Between-Operator Variability SD	Total SD
IDH1 Negative Control	OLIGO1 IC CN	10	23.28	0.443	0.530	0.691
	OLIGO2 IC CN	10	22.00	0.162	0.000	0.162
IDH1 Positive Control	OLIGO1 IC CN	10	23.27	0.556	0.366	0.666
	OLIGO2 IC CN	10	22.05	0.230	0.000	0.230
	R132G CN	10	24.60	0.229	0.000	0.229
	R132H CN	10	26.24	0.411	0.466	0.621
	R132L CN	10	24.40	0.225	0.000	0.225

C. Eluate (External Site) Reproducibility

Reproducibility of the Abbott RealTi*m*e IDH1 assay was evaluated at 3 external sites by testing DNA eluate extracted from IDH1 wild type (WT) mutation negative and IDH1 mutation positive blood and bone marrow specimens. The panel members included 5 IDH1 SNVs and wild type specimens targeting 25 ng and 85 ng gDNA input levels. IDH1 mutation positive panel members were prepared to target a mutation percentage of approximately 2% or 15%. The mutation positive panel members were prepared from eluates of IDH1 mutation positive cell lines spiked into blood or bone marrow. Panel members were tested using 3 unique lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kit. At each site, 2 technologists performed 2 amplification/detection runs over a minimum of 5 days. Each run included 3 replicates of each panel member yielding a total of 180 replicates for each panel member for all 3 sites combined. Valid test results were observed for 780/780 (100%) of replicates at site 1, 766/780 (98.2%) of replicates at site 2 and 778/780 (99.7%) of replicates at site 3. The agreement analysis is summarized in **Table 22.** The eluate reproducibility analysis demonstrated 100% agreement for each panel member at Site 1 and Site 3. All the non-agreements came from Site 2 where a range of 76% to 100% agreement rates were observed for each panel member. For Site 2, there were 100% agreement rates for R132L (in bone marrow), R132S (in bone marrow), R132G (in bone marrow), R132S (in blood), R132G (in blood) and WT (in blood). Agreement rates for R132H (in bone marrow) was 86%, for R132C (in bone marrow) was 91%, for R132H (in blood) was 98%, for R132C (in blood) was 76%, and for WT (in bone marrow) was 98%.

Table 22. External Site (Eluate) Reproducibility Agreement Analysis

		Agreement Status			
Mutation	Specimen Type	Yes	No	Percent Agreement (%)	95% Confidence Interval
R132H	Bone Marrow	169*	8	95	(91, 98)
R132C	Bone Marrow	173*	5	97	(94,99)
R132L	Bone Marrow	178*	0	100	(98, 100)
R132S	Bone Marrow	177*	0	100	(98, 100)
R132G	Bone Marrow	178*	0	100	(98, 100)
R132H	Blood	179	1	99	(97, 100)
R132C	Blood	164*	14	92	(87, 96)
R132L	Blood	180	0	100	(98, 100)
R132S	Blood	180	0	100	(98, 100)
R132G	Blood	179*	0	100	(98, 100)
WT	Bone Marrow	179	1	99	(97, 100)
WT	Blood	179*	0	100	(98, 100)

*Invalid replicates excluded

For the Eluate Reproducibility study, the mean and SD of dCN values and the within-run, between-run, between-lot, between-operator and between-site variability are shown in **Table 23.** Samples that were incorrectly classified as "not detected" in this study did not have dCN values calculated and could not be included in the variability of dCN analysis. The mean and SD of IC CN and mutant CN values and the between-run, between-lot, between-operator and between-site variability for Positive and Negative Controls are shown in **Table 24**.

Table 23. Eluate Reproducibility: Overall Variability of dCN for mutation positive samples and CN of Internal Controls (IC) for mutation negative samples.

Mutation	Specimen Type	Value	N	Mean	Within-Run SD	Between-Run SD	Between-Lot SD	Between-Operator SD	Between-Site SD	Total SD
R132H	Bone Marrow	dCN	169[a]	6.05	0.351	0.243	0.061	0.218	0.298	0.567
R132C	Bone Marrow	dCN	173[b]	6.43	0.693	0.547	0.220	0.498	0.628	1.213
R132L	Bone Marrow	dCN	178[c]	5.65	0.307	0.132	0.000	0.076	0.418	0.541
R132S	Bone Marrow	dCN	177[d]	3.41	0.116	0.046	0.140	0.057	0.304	0.361
R132G	Bone Marrow	dCN	178[c]	6.48	0.224	0.155	0.056	0.150	0.130	0.342
R132H	Blood	dCN	179[e]	3.54	0.307	0.549	0.000	0.550	0.000	0.835
R132C	Blood	dCN	164[f]	8.33	0.527	0.212	0.000	0.000	0.738	0.931
R132L	Blood	dCN	180	2.71	0.267	0.240	0.000	0.159	0.710	0.811
R132S	Blood	dCN	180	5.04	0.295	0.152	0.043	0.071	0.382	0.513
R132G	Blood	dCN	179[g]	5.62	0.296	0.112	0.121	0.086	0.179	0.392
WT	Bone Marrow	OLIGO1 IC CN	179[h]	19.60	0.761	0.294	0.289	0.216	0.434	0.992
WT	Bone Marrow	OLIGO2 IC CN	179[h]	19.47	0.515	0.413	0.000	0.000	0.662	0.935
WT	Blood	OLIGO1 IC CN	179[g]	19.23	1.496	0.000	0.548	0.499	0.345	1.704

Table 23. Eluate Reproducibility: Overall Variability of dCN for mutation positive samples and CN of Internal Controls (IC) for mutation negative samples.

Mutation	Specimen Type	Value	N	Mean	Within -Run SD	Between -Run SD	Between -Lot SD	Between-Operator SD	Between -Site SD	Total SD
WT	Blood	OLIGO2 IC CN	179[g]	19.57	0.873	0.706	0.000	0.000	1.357	1.761

Note: "Not Detected" replicates were excluded since dCN values were not generated.
[a]8 replicates were Not Detected, 3 replicates were invalid.
[b]5 replicates were Not Detected, 2 replicates were invalid.
[c]2 replicates were invalid.
[d]3 replicates were invalid.
[e]1 replicate was Not Detected.
[f]14 replicates were Not Detected, 2 replicates were invalid.
[g]1 replicate was invalid.
[h]1 replicate was Mutation Detected.

Table 24. Eluate Reproducibility: Analysis of Oligo Internal Controls (IC) CN and mutant CN values for Positive and Negative Controls

Panel	Value	N	Mean	Between-Run SD	Between-Lot SD	Between-Operator SD	Between-Site SD	Total SD
IDH1 Negative Control	OLIGO1 IC CN	60	23.13	0.642	0.000	0.000	0.658	0.919
	OLIGO2 IC CN	60	22.02	0.134	0.039	0.156	0.328	0.389
IDH1 Positive Control	R132H CN	60	25.75	0.612	0.000	0.478	0.629	0.999
	R132L CN	60	23.81	0.158	0.066	0.085	0.409	0.451
	R132G CN	60	24.57	0.159	0.092	0.060	0.398	0.443
	OLIGO1 IC CN	60	22.78	0.927	0.000	0.206	0.355	1.014
	OLIGO2 IC CN	60	21.82	0.568	0.000	0.167	0.294	0.661

The agreement rate at site 2 was lower than expected. In order to determine whether this was due to circumstances unrelated to the assay, and to obtain performance with a clinical specimen set from patients and not cell lines, a second multi-center Eluate Reproducibility study with DNA from blood or bone marrow aspirate AML clinical specimens will be conducted postmarket as a condition of approval. This study will evaluate the analytical performance of the

Abbott RealTi*me* IDH1 assay using clinical specimens and re-evaluate cross-site performance.

14. Analytical Carryover

The purpose of this study was to evaluate the Abbott RealTi*me* IDH1 assay for carryover when high IDH1 mutation positive samples are tested adjacent to IDH1 mutation negative samples (wild type, no IDH2 mutation) or blank samples containing no template.

Potential carryover from high IDH1 mutation positive samples to IDH1 mutation negative samples and blank samples was evaluated by testing replicates of alternating samples within the same sample preparation run and amplification-detection run. Six runs were performed, 3 runs alternating 11 high IDH2 mutation positive and 11 IDH2 mutation negative samples and 3 runs alternating 11 high IDH2 mutation positive and 11 blank samples for a total of 132 samples.

High IDH1 mutation positive samples were prepared by adding IDH1 R132C and R132G mutation positive cell lines to normal blood or bone marrow aspirate specimens to achieve approximately 25% mutation level. The IDH1 mutation negative samples were blood or bone marrow aspirate from normal donors; the blank sample was water.

No carryover was detected for the Abbott RealTi*me* IDH2 assay when high IDH2 mutation positive samples were tested adjacent to IDH2 mutation negative samples or blank samples containing no template.

15. Guardband Studies

a) PCR Master Mix and Sample Volume Robustness

The purpose of this study was to characterize the robustness of the Abbott RealTi*me* IDH1 assay to variations in amplification reaction volume. The Abbott RealTi*me* IDH1 assay procedure requires the operator to manually load 45 µL of master mix to be combined with 15 µL of target into wells of a 96-well plate, followed by a visual inspection for proper volume after the addition of the master mix and the target. The visual check allows the operator to catch inaccurate pipetting of larger volumes, but will likely not allow the visualization of small volume changes. However, it is possible that an incorrect amount of master mix or sample could be added to the plate due to operator error or pipette miscalibration. This study addressed the effects of variations in master mix and target volumes on Abbott RealTi*me* IDH1 assay performance.

To characterize the robustness of the Abbott RealTi*me* IDH1 assay to variations in master mix and target sample volumes, the following test conditions were run:

- Increase of master mix volume by 10%
- Decrease of master mix volume by a range from 10% to 40%
- Increase or decrease of target sample volume by 30%

Each condition was assessed with Abbott RealTi*me* IDH1 Positive Control (PC), Abbott RealTi*me* IDH1 Negative Control (NC), and five mutant plasmid targets (R132C, R132G, R132H, R132L, and R132S). All mutant plasmids were diluted to 5% in a total gDNA input of 20 ng per reaction (15µl). The source of gDNA used for this study was human placental gDNA.

All conditions tested provided valid, correct results. This study supports that the Abbott RealTi*me* IDH2 assay is robust to changes in both master mix volume and target sample volume.

b) <u>PCR Master Mix and Sample Volume Robustness</u>

The purpose of this study was to characterize the robustness of the Abbott RealTi*me* IDH1 assay to variations in amplification reaction volume. The Abbott RealTi*me* IDH1 assay procedure requires the operator to manually add 45 µL of master mix and 15 µL of sample into wells of a 96-well plate, followed by a visual inspection for proper volume after the addition of the master mix and the sample. The visual check allows the operator to check for inaccurate pipetting of larger volumes, but will likely not allow the visualization of small volume changes. However, it is possible that an incorrect amount of master mix or sample could be added to the plate due to operator error or pipette miscalibration. This study addressed the effects of variations in master mix and target volumes on Abbott RealTi*me* IDH1 assay performance.

To characterize the robustness of the Abbott RealTi*me* IDH1 assay to variations in master mix and target sample volumes, the following test conditions were run:

- Increase of master mix volume by 10%
- Decrease of master mix volume by a range from 10% to 40%
- Increase or decrease of sample volume by 30%

The results of this study support that Abbott RealTi*me* IDH2 is robust to changes in both master mix volumes and target sample volume.

c) <u>PCR Cycle Temperature and Duration Robustness</u>

The purpose of this study was to characterize the robustness of the Abbott RealTi*me* IDH1 assay to variations in *m*2000*rt* instrument PCR cycle temperature and duration. Abbott RealTi*me* IDH1 has set specifications for PCR cycle temperature and cycle duration. However, alterations in *m*2000*rt* instrument performance may result in different PCR cycling parameters, which may impact assay performance. This study addresses the effects of variations in

PCR cycle temperature and duration on Abbott RealTi*me* IDH1 assay performance.

The following test conditions were run to characterize the robustness of the Abbott RealTi*me* IDH2 assay to variations in PCR cycle temperature and duration:

- Increase or decrease all cycle temperatures by 1.0°C
- Increase or decrease all cycle durations by 5.0 seconds
- A combination of 1.0°C and 5.0 second changes (increases and decreases)

Each test condition was evaluated with wild type gDNA adn five IDH1 mutant plasmt targets (R132C, R132H, R132G, R132H and R132S). The results of this study support that Abbott RealTi*me* IDH2 is robust to changes in ± 1.0°C in PCR cycle temperature and ± 5 seconds in PCR cycle duration.

16. <u>Stability Studies</u>

a) <u>Abbott RealTi*me* IDH1 Amplification Reagent Stability</u>

The stability of the Abbott RealTi*me* IDH1 Amplification Reagent was assessed at various time points after storage under the following conditions: intended storage condition at -15ºC to -25ºC; inverted storage at -15ºC to -25ºC; and storage after simulating transport and temperature extreme conditions which consisted of ≥ 30°C for ≥ 4 hours followed by three cycles of ≥ 48 hours in a container with dry ice and ≥ 30°C for ≥ 4 hours, then stored at the intended storage condition. Three lots Abbott RealTi*me* IDH1Amplification Reagent Kit were tested for stability. The 3 lots have 3 distinct production lots of IDH1 Oligonucleotide Reagents 1, 2, Activation Reagent, and DNA Polymerase. 3 lots were used for the intended storage condition and 1 lot was used for each of the other two conditions.

The IDH1 Positive Control, IDH1 Negative Control and 12 panel members tested at each timepoint. The 12 panel member consisted of blood and bone marrow specimens without IDH1 mutations, and blood and bone marrow specimens representing each of the IDH1 mutations targeted by Abbott RealTi*me* IDH2 assay. The panels consist of DNA eluates that were generated from IDH1 mutation positive cell lines spiked in blood or bone marrow at either a low positive (6% mutation frequency) or high positive (25% mutation frequency). All panel members were tested with 6 replicates at each time point that they are evaluated.

Stability at the intended storage condition was evaluated by performing functional testing at day 0, month 6, month 7, month 12, month 18 and month 19 for the intended use condition. To date, real-time stability testing for the Abbott

RealTi*me* IDH1 Amplification Reagent has been completed through month 12 and met acceptance criteria. These data currently support a 11-month stability claim.

Stability at the inverted storage condition was tested at day 0, month 2, month 6, month 12, month 18 and month 19. To date the inverted condition has been completed through month 12 and met acceptance criteria. The storage after simulating transport and temperature extreme conditions was tested at day 0, day 7, month 6, month 7, month 10, month 12, month 18 and month 19. To date, the simulating transport and temperature extreme conditions has been tested to month 12 and met acceptance criteria.

b) Abbott RealTi*me* IDH1 Control Kit and Abbott *m*Sample Preparation $\text{System}_{\text{DNA}}$ Stability

The stability of the Abbott RealTi*me* IDH1 Control Kit and Abbott *m*Sample Preparation $\text{System}_{\text{DNA}}$ kit was assessed at various time points under the following conditions: intended storage condition -15°C to -25°C; inverted storage at -15°C to -25°C; and storage after simulating transport and temperature extreme conditions which consisted of ≥ 30°C for ≥ 4 hours followed by three cycles of ≥ 48 hours in a container with dry ice and ≥ 30°C for ≥ 4 hours, then stored at the intended storage condition. The stability of 3 lots of Abbott RealTi*me* IDH1 Control Kit and 3 lots of Abbott *m*Sample Preparation $\text{System}_{\text{DNA}}$ Kit were assessed. Three lots of each kit were used for the intended storage condition and 1 lot was used for each of the other two conditions.

IDH1 Positive Control (PC) and IDH1 Negative Control (NC) were tested for each storage condition, at each time point, in this study. The PC and NC are tested with 6 replicates at each time point that they are evaluated. To evaluate the stability of the Abbott *m*Sample Preparation $\text{System}_{\text{DNA}}$ kit, in addition to testing PC and NC, a panel of blood/bone marrow samples were tested at baseline (one run for each of the three lots of sample preparation kits). The panel consists of 4 blood samples and 4 bone marrow aspirate samples. The 4 specimens for each sample type were as follows: 2 specimens that are IDH1 mutation negative, 1 specimen with the IDH1 R132C mutation, and 1 specimen with the IDH1 R132S mutation. This panel will be tested at least one month past the final expiration date.

Stability at the intended storage condition for the Abbott RealTi*me* IDH1 Control kit was evaluated by performing functional testing at day 0, month 4, month 6, month 10 and month 12, and will be evaluated at month 18 and month 19 for the intended use condition. To date, real-time stability testing for the Abbott RealTi*me* IDH1 Control Kit has been completed through month 12 and met acceptance criteria. These data currently support a 11-month stability claim.

Stability of the Abbott RealTi*m*e Control kit was evaluated at the inverted condition has been tested at day, month 2, month 6, month 10, month 12 and will be tested atmonth 18 and month 19. To date the inverted condition has been completed through month 12 and met acceptance criteria. The storage after simulating transport and temperature extreme conditions was tested at day 0, day 7, month 6, month 9, and month 12 and will be tested at month 18 and month 19. Studies have met acceptance criteria for tested time points.

Stability at the intended storage condition for the Abbott *m*Sample Preparation $System_{DNA}$ kit was evaluated by performing functional testing at day, month 2, month 6, month 10, month 12 and month 13 and will be tested at month 18 and month 19. Studies have met acceptance criteria for tested time points.

c) Activated Master Mix Use and Storage and Assembled Plate Stability (section 2.13)

The storage stability of activated master mix and assembled PCR plate when used with the Abbott RealTi*m*e IDH1 assay were assessed using a control condition, and 3 test conditions. The control condition (Condition A) was performed at ambient room temperature (15°C to 30°C) without delay between the master mix preparation and addition to the 96-well PCR plate. The run for the control condition was initiated immediately after the completion of assay set-up. For the activated master mix stability part of this study, activated master mix was stored at 2°C to 8°C for 9 days (test Condition B) or at -20°C (± 5°C) for 64 days with 6 freeze/thaw cycles (test Condition C) before the completion of assay set-up and run initiation. For the assembled plate stability part of this study, test condition (D) had an intentional delay of 135 minutes after master mix preparation and addition to the 96-well PCR plates.

Storage stability of activated master mix and assembled PCR plate was assessed using a 12-member panel. Panel members contained the 5 IDH1 mutations targeted by the assay (R132C, R132H, R132L, R132G and R132S) and wild type (no IDH1 mutation) in blood and bone marrow. IDH1 mutation positive eluates were generated by adding an IDH1 mutation positive cell line to normal blood or bone marrow specimens, isolated using the Abbott *m*Sample Preparation System $_{DNA}$, and diluted to target 6% mutation and 10 ng total gDNA input. Wild type panel members were created by diluting eluates generated from clinical AML specimens negative for IDH2 mutations to target 10 ng total gDNA. All panel members were tested in triplicate for all conditions.

All replicates for both bone marrow specimens and blood specimens showed 100% agreement with the expected result for all storage conditions. Changes for dCN, mutant CN and IC CN were evaluated for all conditions. The range of dCN mean differences for mutation positive panel members was -0.48 to 0.54, with the largest difference being 0.54 for Condition C. This study demonstrated acceptable assay performance across the conditions that were tested.

d) Clinical Specimen Stability

Clinical specimen stability was evaluated by testing prospectively collected clinical AML blood specimens (n=8, including 1 with the R132H mutation and 1 with the R132C mutation) and AML bone marrow aspirate specimens (n=6, including 1 with the R132 H mutation).

After fresh collection, specimens were shipped frozen on dry ice for up to 24 hours, placed at -20°C upon receipt, and then thawed and aliquotted for the testing. The control condition (Condition A) was performed by performing DNA extraction immediately upon receipt. The control condition was compared to the following storage conditions: storage at 15°C to 30°C for 50 hours (Condition B); Storage at 2°C to 8°C for 8 days (Condition C); Storage at -20°C for 32 days with 4 freeze thaw cycles (Condition D); and store on dry ice for 9 days (Condition E). Each condition was tested in triplicate except for a single bone marrow aspirate sample, which was only tested in duplicate for conditions A, C, D and E, due to the limited sample volume.

All replicates for both bone marrow specimens and blood specimens were in 100% agreement with the expected result for all storage conditions. Variation in dCN, mutant CN and IC CN were also evaluated. This study demonstrated that the assay provided acceptable results on clinical specimens that were stored under the tested conditions.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The safety and effectiveness of the Abbott RealTi*m*e IDH1 assay were demonstrated through testing of specimens from patients enrolled in Study AG120-C-001 (ClinicalTrials.gov Identifier: NCT02074839).

A. Study Design

Study AG120-C-001 was an open-label, single-arm, international, multicenter clinical trial of TIBSOVO (ivosidenib) of 174 adult patients with relapsed or refractory Acute Myeloid Leukemia (R/R AML) and one of 5 IDH1 mutations in codon R132 who were assigned to receive a 500-mg daily dose until disease progression, development of unacceptable toxicity, or undergoing hematopoietic stem cell transplantation. Both peripheral blood and bone marrow specimens were obtained from patients for testing. Specimen types for patients that were retrospectively confirmed with the Abbott RealTi*m*e IDH1 assay were peripheral blood, peripheral blood mononuclear cells, bone marrow aspirate, and bone marrow aspirate mononuclear cells, based on availability. Clinical testing was performed at three geographically diverse sites.

1. Clinical Inclusion and Exclusion Criteria

a. Inclusion Criteria for Specimen Testing

- Specimens had to have sufficient sample volume for testing.
- The specimen had to be handled or stored in accordance to the Abbott RealTi*m*e IDH1 Clinical Brochure.
- Specimens were collected with appropriate informed consent in study AG120-C-001.

b. Inclusion Criteria for Patient Enrollment in AG120-C-001 (abbreviated)

- Relapsed or refractory AML
- IDH1 mutation detected in blood or bone marrow specimen.
- Subjects must be amenable to serial BM sampling, peripheral blood sampling, and urine sampling during the study.
- Eastern Cooperative Oncology Group PS 0 to 2.
- Platelet count ≥ 20,000/µL (transfusions to achieve this level are allowed). Subjects with a baseline platelet count of < 20,000/µL due to underlying malignancy are eligible with Medical Monitor approval.
- Subjects must have adequate hepatic function
- Subjects must have adequate renal function
- Subjects must be recovered from any clinically relevant toxic effects of any prior surgery, radiotherapy, or other therapy intended for the treatment of cancer.
- Women cannot be pregnant or breastfeeding
- None of the following cardiac conditions: New York Heart Association Class III or IV congestive heart failure, left ventricular ejection fraction < 40%, history of myocardial infarction within the 6 months prior to screening, uncontrolled angina pectoris, history of severe ventricular arrhythmias, or QTcF ≥ 450 msec
- No systemic anticancer therapy or radiotherapy within 14 days prior to the first dose of TIBSOVO® (hydroxyurea allowed for control of peripheral leukemic blasts in subjects with WBC > 30,000/µL)
- Provide informed consent
- To be included in the efficacy analysis:
  - Patient whose first dose was less than 6 months before the data cutoff date
  - Patients who were assigned to receive 500 mg ivosidenib daily

2. Follow-up Schedule

Disease assessment and other clinical assessments were conducted according to protocol during the trial. Patients were to be followed for a minimum of six, 28-day cycles of TIBSOVO® or discontinued study therapy in order to be included in the analysis.

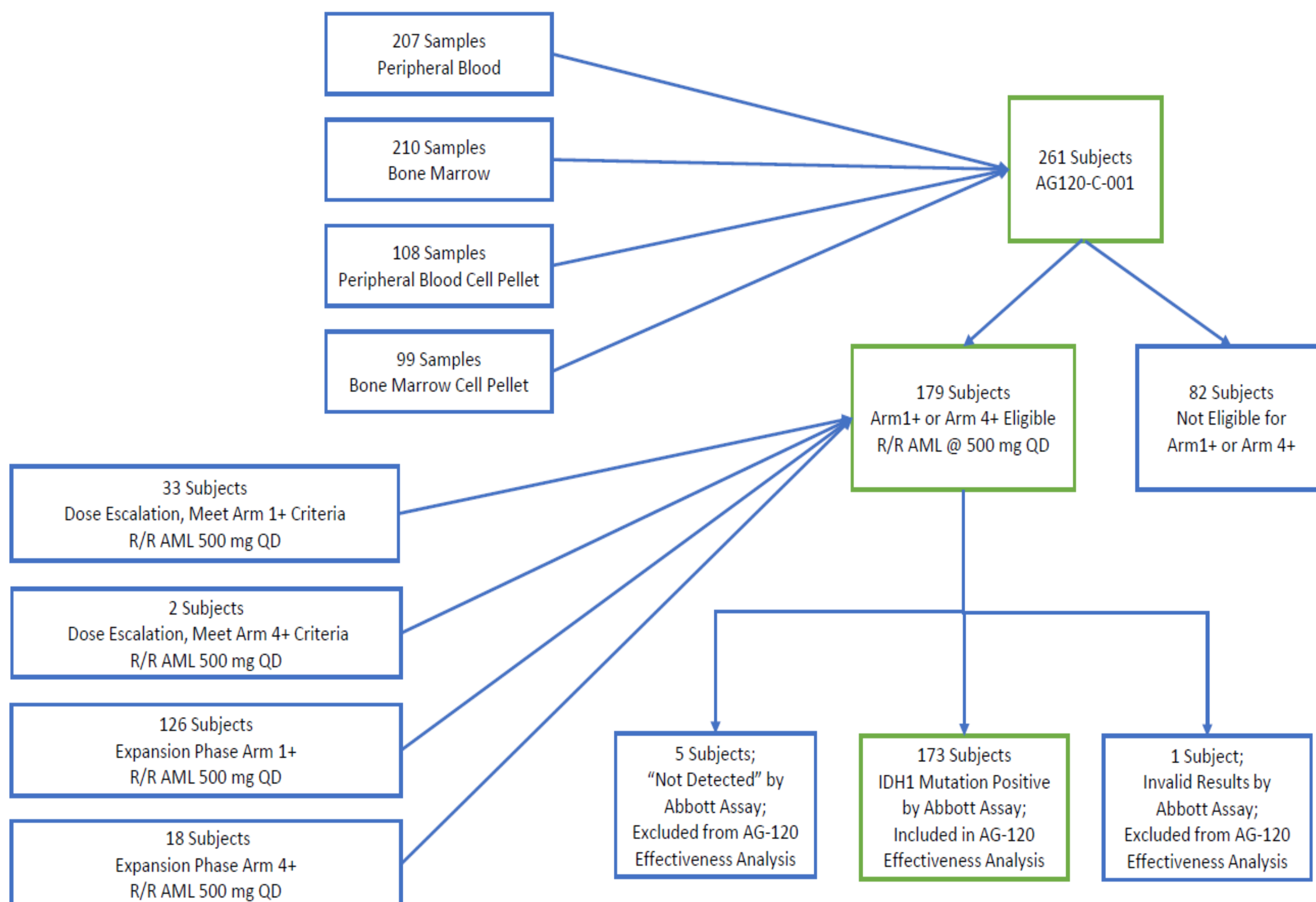
3. Clinical Endpoints

Efficacy for TIBSOVO® was established on the basis of the rate of complete remission (CR) plus complete remission with partial hematologic recovery (CRh), and the duration of CR+CRh, and the rate of conversion from transfusion dependence to transfusion independence.

B. Accountability of PMA Cohort

Study AG120-C-001 included 174 patients in the efficacy analysis of TIBSOVO®. To be included in the efficacy analysis, the Abbott RealTi*m*e IDH1 assay had to detect an IDH1 mutation in either blood or bone marrow and the patient had to receive a 500 mg daily dose of TIBSOVO®.

A total of 261 subjects with relapsed or refractory AMLwere tested as part of AG120-C-001 to determine eligibility for the trial. Both peripheral blood and bone marrow specimens were obtained from each patient. Eighty-two (of 261) subjects did not meet the eligibility criteria that required the starting daily dose of TIBSOVO® to be 500 mg. The 179 patients were tested with the Abbot RealTi*m*e IDH1 assay resulting in 174 patients with detectable IDH1 mutations and 5 patients without detectable IDH1 mutations. The 174 patients with detectable IDH1 mutation were included in the therapeutic efficacy analysis of TIBSOVO®. Total disposition of specimens and specimen types collected from patients is shown in Figure 1 below.



C. <u>Study Population Demographics and Baseline Parameters</u>

The demographics and baseline characteristics of the 174 patients used for efficacy analysis within study AG120-C-001 are shown in **Table 25**. The median age of study participants was 67 years and predominantly white (62%). Enrollment according to gender was equivalent. The predominant IDH1 positive mutation was R132C (59%) followed by R132H (25%). Each of the five IDH1 mutations that are detected by the Abbott RealT*ime* IDH1 assay were represented in patients enrolled into the trial.

Table 25. Baseline Demographic and Disease Characteristics in Enrolled Subjects with Relapsed or Refractory AML	
Demographic and Disease Characteristics	**TIBSOVO (500 mg daily) N=174**
Demographics	
Age (Years) Median (Min, Max)	67 (18, 87)
Age Categories, n (%)	
<65 years	63 (36)
≥65 years to <75 years	71 (41)
≥75 years	40 (23)
Sex, n (%)	
Male	87 (50)
Female	86 (50)
Race, n (%)	
White	108 (62)
Black or African American	10 (6)
Asian	6 (3)
Native Hawaiian/Other Pacific Islander	1 (1)
Other/Not provided	49 (28)
Disease Characteristics	
ECOG PS, n (%)	
0	36 (21)
1	97 (56)
2	39 (22)
3	2 (1)
IDH1 Mutation Variant, n (%)[a]	
R132C	102 (59)
R132H	423(25)
R132G	12 (7)
R132S	10 (6)
R132L	7 (4)
Cytogenetic Risk Status, n (%)	
Intermediate	104 (60)
Poor	47 (27)
Missing/Unknown	23 (13)
Relapse Type, n (%)	
Primary refractory	64 (37)
Refractory relapse	45 (26)
Untreated relapse	65 (37)

Relapse Number, n (%)	
0	64 (37)
1	83 (48)
2	21 (12)
≥3	6 (3)
Prior Stem Cell Transplantation for AML, n (%)	40 (23)
Transfusion Dependent at Baseline,[b] n (%)	110 (63)
Median Number of Prior Regimens (Min, Max)	2 (1, 6)
Type of AML, n (%)	
De Novo AML	116 (67)
Secondary AML	58 (33)

ECOG PS: Eastern Cooperative Oncology Group Performance Status.
[a] Using confirmatory Abbott RealTi*me* IDH1 assay testing results
[b] Patients were defined as transfusion dependent at baseline if they received any transfusion occurring within 56 days prior to the first dose of TIBSOVO®.

D. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with TIBSOVO® (ivosidenib) was addressed during the review of the NDA and is not addressed in detail in this SSED. Refer to Drugs@FDA for safety information on TIBSOVO.

Abbott RealTi*me* IDH1 assay involves testing on blood or bone marrow aspirate samples. These specimens are routinely removed as part of the practice of medicine for the diagnosis of AML and therefore sample collection presents no additional safety hazard to the patient being tested.

3. Effectiveness Results

The effectiveness of the Abbott RealTi*me* IDH1 assay was demonstrated through testing of specimens from patients enrolled in Study AG120-C-001 (ClinicalTrials.gov Identifier NCT02074839). Study AG120-C-001 was an open-label, single-arm, multicenter clinical trial of TIBSOVO (ivosidenib) of 174 adult patients with relapsed or refractory Acute Myeloid Leukemia (R/R AML) and one of 5 IDH1 mutations in codon R132, who were assigned to receive a 500 mg daily dose. IDH1 mutations were identified by a local or central diagnostic test and retrospectively confirmed using the Abbott RealTi*me* IDH1 assay. TIBSOVO was given orally at a starting dose of 500 mg daily until disease progression, development of unacceptable toxicity, or undergoing hematopoietic stem cell transplantation. Twenty-one of the 174 patients (12%) went on to stem cell transplant following TIBSOVO treatment.

Blood and bone marrow aspirate specimens from R/R AML patients being considered for treatment were tested. Patients with an IDH1 mutation positive result were eligible for enrollment in the drug trial if they met other eligibility criteria. Patients with an IDH1 mutation negative result at screening were ineligible for drug trial enrollment. Conclusions supporting TIBSOVO efficacy were based on patients with IDH1 mutation positive results as detected by the companion diagnostic Abbott RealTi*m*e IDH1 assay.

Efficacy for TIBSOVO was established on the basis of the rate of complete remission (CR) plus complete remission with partial hematologic recovery (CRh), the duration of CR+CRh, and the rate of conversion from transfusion dependence to transfusion independence. The efficacy results are shown in **Table 26**. The median follow-up was 8.3 months (range, 0.2 to 39.5 months) and median treatment duration was 4.1 months (range, 0.1 to 39.5 months).

Table 26. Efficacy Results in Patients with Relapsed or Refractory AML

Endpoint	**TIBSOVO (500 mg daily) N=174**
CR[a] n (%)	43 (24.7)
95% CI	(18.5, 31.8)
Median DOR[b] (months)	10.1
95% CI	(6.5, 22.2)
CRh[c] n (%)	14 (8.0)
95% CI	(4.5, 13.1)
Median DOR (months)	3.6
95% CI	(1.0, 5.5)
CR+CRh[d] n (%)	57 (32.8)
95% CI	(25.8, 40.3)
Median DOR (months)	8.2
95% CI	(5.6, 12.0)

CI: confidence interval

[a] CR (complete remission) was defined as <5% blasts in the bone marrow, no evidence of disease, and full recovery of peripheral blood counts (platelets >100,000/microliter and absolute neutrophil counts [ANC] >1,000/microliter).

[b] DOR (duration of response) was defined as time since first response of CR or CRh to relapse or death, whichever is earlier.

[c] CRh (complete remission with partial hematological recovery) was defined as <5% blasts in the bone marrow, no evidence of disease, and partial recovery of peripheral blood counts (platelets >50,000/microliter and ANC >500/microliter).

[d] CR+CRh rate appeared to be consistent across all baseline demographic and baseline disease characteristics with the exception of number of prior regimens.

For patients who achieved a CR or CRh, the median time to CR or CRh was 2 months (range, 0.9 to 5.6 months). Of the 57 patients who achieved a best response of CR or CRh, all achieved a first response of CR or CRh within 6 months of initiating TIBSOVO.

Among the 110 patients who were dependent on red blood cell (RBC) and/or platelet transfusions at baseline, 41 (37.3%) became independent of RBC and platelet transfusions during any 56-day post baseline period. Of the 64 patients who were independent of both RBC and platelet transfusions at baseline, 38 (59.4%) remained transfusion independent during any 56-day post baseline period.

3. Pediatric Extrapolation

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

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 3 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Concordance between blood and bone marrow specimens was demonstrated in Section IX a.2. The results demonstrated and support the claim that either bone marrow specimens or peripheral blood specimens can be tested with the Abbott RealTi*m*e IDH1 assay to determine whether TIBSOVO is an appropriate treatment for R/R AMLpatients.

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The Abbott RealTi*m*e IDH1assay supports the accurate qualitative detection of single nucleotide variants (SNVs) coding five IDH1 mutations (R132C, R132H, R132G, R132S and R132L) in DNA extracted from human bone marrow (EDTA) or blood (EDTA) in patients with relapsed or refractory AML. The analytical studies support that the assay can accurately identify the specific IDH1 mutations in blood or bone marrow specimens of patients with AML.

The clinical effectiveness of the Abbott RealTi*m*e IDH1 assay was demonstrated in Study AG120-C-001 was an open-label, single-arm, international, multicenter clinical trial of TIBSOVO (ivosidenib) of 174 adult patients with relapsed or refractory Acute Myeloid Leukemia (R/R AML) and one of 5 IDH1 mutations in codon R132 who were assigned to receive a 500-mg daily dose until disease progression, development of unacceptable toxicity, or undergoing hematopoietic stem cell transplantation. Of the 174 patients identified as having an IDH1 mutation by the Abbott RealTi*m*e IDH1 assay, 57 (32.8%) exhibited complete remission or complete remission with partial hematological recovery.

B. Safety Conclusions

The probable risks of the Abbott RealTi*m*e IDH1 assay 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 test results, and consequently improper patient management decisions for relapse and refractory AML. A false positive test result may lead to TIBSOVO (ivosidenib) treatment in a patient who is not expected to benefit, and suffer from any potential adverse side effects associated with treatment. A false negative test result may lead to TIBSOVO (ivosidenib) treatment not being administered to a patient who may benefit from this drug. In a clinical study, the adverse event profile was determined to be acceptable.

The analytical accuracy comparison of the device was performed to next-generation sequencing (NGS). The results demonstrated high levels of agreement for both blood and bone marrow and to NGS which serves to mitigate risk.

To address, the slight difference observed between bone marrow and blood, the package insert contained the following statement: “(there was a) slightly higher IDH1 mutation positive detection rate observed in bone marrow.”

These findings from analytical accuracy and comparison of concordance between blood and bone marrow, the requirement for post-market studies, along with clinical consequences of misclassification, contribute to the moderate uncertainty about the

risks associated with this device. The patient perspective on device related risk was not collected in this study.

C. Benefit-Risk Determination

The probable benefits of the Abbott RealTi*me* IDH1 assay were demonstrated through testing of specimens from patients enrolled in Study AG120-C-001 (ClinicalTrials.gov Identifier NCT02074839). Study AG120-C-001 was an open-label, single-arm, multicenter clinical trial of TIBSOVO (ivosidenib) of 174 adult patients with relapsed or refractory Acute Myeloid Leukemia (R/R AML) and one of 5 IDH1 mutations in codon R132, who were assigned to receive a 500-mg daily dose. IDH1 mutations were identified by a local or central diagnostic test and retrospectively confirmed using the Abbott RealTi*me* IDH1 assay. TIBSOVO was given orally at a starting dose of 500 mg daily until disease progression, development of unacceptable toxicity, or undergoing hematopoietic stem cell transplantation. Twenty one of the 174 patients (12%) went on to stem cell transplant following TIBSOVO treatment.

The baseline demographic and disease characteristics were examined; the study participants were predominantly white (62%) with a median age of 67 years. All 5 IDH1 mutations in codon R132 were detected among patients enrolled into the trial. The predominant IDH1 positive mutation was R132C (59%) followed by R132H (25%).

Blood and bone marrow aspirate specimens from R/R AML patients being considered for treatment were tested. Patients with an IDH1 mutation positive result were eligible for enrollment in the drug trial if they met other eligibility criteria. Patients with an IDH1 mutation negative result at screening were ineligible for drug trial enrollment. Conclusions supporting TIBSOVO efficacy were based on patients with IDH1 mutation positive results as detected by the companion diagnostic Abbott RealTi*me* IDH1 assay.

Efficacy for TIBSOVO was established based on the rate of complete remission (CR) plus complete remission with partial hematologic recovery (CRh), the duration of CR+CRh, and the rate of conversion from transfusion dependence to transfusion independence. The median follow-up was 8.3 months (range, 0.2 to 39.5 months) and median treatment duration was 4.1 months (range, 0.1 to 39.5 months). For the 57 (of 174) patients who achieved a CR or CRh, to CR or CRh was 2 months (range, 0.9 to 5.6 months). Of the 57 patients who achieved a best response of CR or CRh, all achieved a first response of CR or CRh within 6 months of initiating TIBSOVO.

Among the 110 patients who were dependent on red blood cell (RBC) and/or platelet transfusions at baseline, 41 (37.3%) became independent of RBC and platelet transfusions during any 56-day post baseline period. Of the 64 patients who were independent of both RBC and platelet transfusions at baseline, 38 (59.4%) remained transfusion independent during any 56-day post baseline period.

There is probable clinical benefit of the device based on the analytical performance of the device and supportive clinical evidence as described above, which also serves as the

basis for the CDER drug approval. Due to the nature of the analytical data provided, a post-marketing commitments was obtained for a three-site reproducibility study with clinical samples from peripheral blood and bone marrow. Thus, in all, given the nature of the clinical study and results from the analytical validation, there is a moderate degree of uncertainty regarding the benefit. There is overall probable benefit of this device and supportive magnitude, probability and duration of response, as demonstrated by the CR, CRh rates and rates of transfusion dependence detailed above. The patient perspective on device related benefit was not collected in this study.

1. Patient Perspectives

   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for 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.

XIV. CDRH DECISION

CDRH issued an approval order on July 20, 2018. The final conditions of approval cited in the approval order are described below.

The applicant will provide an eluate reproducibility (external-site) study to test clinical specimens from AML patients with an IDH1 mutation. Testing of blood and bone marrow from AML patients with an IDH1 mutation using an appropriate study design is needed for a more robust characterization of the eluate reproducibility of your device. The results from these studies will be included in the labeling.

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

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications,

Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.