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Detection of *IDH1* and *IDH2* Mutations in Patients with Acute Myeloid Leukemia Using Novel, Highly Sensitive Real-Time PCR Assays with Rapid Turnaround Time

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Abstract

Isocitrate dehydrogenase 1 and 2 (*IDH1*/*IDH2*) mutations are frequent in acute myeloid leukemia (AML). Here we describe the qualitative polymerase chain reaction (PCR)-based Abbott Real*time IDH1* and *IDH2* assays, which detect single nucleotide variants coding for five *IDH1* and nine *IDH2* mutations. We evaluated the sensitivity and specificity of the Abbott Real-Time *IDH1*/*IDH2* assays and conducted a workflow analysis that compared them with PCR-based Sanger and next-generation sequencing (NGS) assays in blood or bone marrow specimens from 100 AML patients. Sanger sequencing and NGS detected *IDH* mutations with variant allele sensitivity limits of 20% and 10%, respectively. The Abbott Real-Time *IDH1*/*IDH2* assays demonstrated 100% sensitivity and 95% specificity *vs.* Sanger sequencing and detected mutations at the 1% level. Low-level *IDH2* mutations in five samples were detected by the Abbott Real-Time *IDH2* assay but not by Sanger sequencing. Turnaround time (TAT) based on a workflow analysis showed Abbott Real-Time *IDH1*/*IDH2* assay results were available in three business days *vs.* eight days for Sanger sequencing and 15 days for NGS. Higher sensitivity and rapid TAT for detecting *IDH* mutations may improve identification of patients with lower mutant-*IDH* burden and allow for quicker administration of the FDA-approved IDH inhibitors, Ivosidenib and Enasidenib.

Keywords: *IDH1*; *IDH2*; Mutation; Isocitrate dehydrogenase; Acute myeloid leukemia; Real-time; Next generation sequencing

Introduction

Genetic profiling of acute myeloid leukemia (AML) reveals distinct molecular subgroups that inform disease classification and prognostic stratification [1]. Dysregulated metabolism is one of the most common features of cancer cells [2]. The isocitrate dehydrogenases 1 and 2 (*IDH1* and *IDH2*) play prominent roles in cellular metabolism. *IDH1* is present in the cytoplasm and peroxisome and is involved in lipid metabolism and glucose sensing [3]. *IDH2* resides in the mitochondria and is a component of the Krebs cycle, involved in the regulation of oxidative respiration [3]. IDH1 and IDH2 enzymes catalyze oxidative decarboxylation of isocitrate to alpha-ketoglutarate (αKG). Mutations in genes encoding IDH1 and IDH2 proteins are typically mutually exclusive, likely due to their common underlying biochemical mechanism and physiological consequences [4]. Both *IDH1* and *IDH2* mutations produce neomorphic activity resulting in the conversion of αKG to an oncometabolite, 2-hydroxyglutarate (2-HG) [5]. *IDH1* and *IDH2* mutations can co-operate with other gene mutations and molecular defects to promote leukemogenesis [6]. Expression of mutant IDH1 or IDH2 proteins is sufficient to block differentiation of hematopoietic cells *in vitro* and *in vivo* [7-9]. Differentiation block can be reversed by hindering these mutations [7,9,10]. *IDH* mutations are acquired early in AML pathogenesis and may exist in the founding clone [6]. *IDH1* mutations affect codon 132 and have been reported to occur in 7-14% of adult and 1% of pediatric AML cases [11-13]. *IDH2* mutations affect codon 140 or codon 172 and are observed in 8-19% of adult and 1-2% of pediatric AML cases [12-14].

Recent regulatory approvals of targeted therapies for treatment of AML highlight the importance of detecting pathogenic mutations that can drive disease progression and lead to relapse. Ivosidenib (TIBSOVO®; Agios Pharmaceuticals, Inc., Cambridge, MA) and enasidenib (IDHIFA®; Celgene Corporation, Summit, NJ) are approved for treatment of adult patients with relapsed or refractory (R/R) AML with an *IDH1* or *IDH2* mutation, respectively. A recent study of 80 patients with mutant-*IDH1* or -*IDH2* AML in morphologic remission after standard chemotherapy showed 40% of patients had persistent *IDH1* or *IDH2* mutations during remission, and those patients had an increased risk of relapse at one year of follow-up compared with patients without persistent *IDH* mutations after chemotherapy (59% *vs.* 24%, respectively; $p<0.01$) [15]. Importantly, in that study, *IDH* mutational burden during remission was not significantly associated with AML relapse: Patients with *IDH1*/*IDH2* variant allele frequencies (VAFs) below 10% had similar risk of relapse as those with higher mutational burden [15].

Timeliness is key to choosing appropriate and optimal treatments for patients with AML [16]. While most AML patients start therapy within four days after initial diagnosis, treatment decisions for patients with R/R AML may require even more rapid therapeutic decision making [16].

Two new companion diagnostic tests, the Abbott Real-Time *IDH1* and Abbott Real-Time *IDH2* assays (Abbott Molecular, Inc.; Des Plaines, Illinois), were recently developed for detection of *IDH1* and *IDH2* mutations, to identify patients with AML who may benefit from treatment with targeted IDH inhibitors. The Abbott Real-Time assays are qualitative *in vitro* polymerase chain reaction (PCR) tests

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that detect single nucleotide variants (SNV) coding *IDH1* and *IDH2* mutations.

The objectives of the current analyses were to comprehend the turnaround time (TAT) for results of the three different mutation testing modalities for *IDH1* and *IDH2* (Abbott Real-Time PCR-based *IDH1* and *IDH2* assays, Sanger sequencing, and next generation sequencing [NGS]) *via* workflow analysis. Additionally, we compared the sensitivity of these three methods for detecting mutant *IDH1* and *IDH2*.

Methods

This study was approved by the Medical College of Wisconsin Institutional Review Board. Blood or bone marrow aspirates from 100 patients with AML were received by the Blood Center of Wisconsin (BCW, now Versiti; Milwaukee, Wisconsin) as part of a diagnostic workup. Samples were analyzed for presence of *IDH* mutations using the Abbott Real-Time *IDH1* and *IDH2* assays, or BCW PCR-based Sanger sequencing assay, and a 30-gene NGS HemeOnc panel (BCW).

The Abbott Real-Time *IDH* assays target five *IDH1* mutations at codon 132 (R132H, R132C, R132L, R132G, and R132S) and nine *IDH2* mutations at codons 140 and 172 (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W). The BCW PCR-based Sanger sequencing assay and NGS panel detect mutations in exon 4 of *IDH1* and *IDH2* with VAF sensitivity limits of 20% and 10%, respectively. NGS was utilized to resolve discordant results between the Real-Time and Sanger methods.

Clinical specimens were blinded (ie, the type of *IDH* mutation was not identified) to study investigators. DNA was extracted from 100 clinical specimens using the sample preparation system (Abbott Molecular, Inc.) or a QIAGEN DNA extraction kit (QIAGEN; Venlo, Netherlands). A set of commercially available controls (*IDH1*-R132H/*IDH2*-R172K, *IDH1*-R132C/*IDH2*-R140Q) with different mutant VAF levels (20%, 5%, 1% and 0.25%) were also tested using the four assays mentioned above.

Abbott real-time *IDH1/IDH2* reagent preparation and reaction plate assembly

Details regarding sample handling and preparation and assay procedures are available at https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170041C.pdf, https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170005C.pdf.

Briefly, the Abbott Real-Time *IDH1* or *IDH2* oligonucleotide reagents were each manually combined with DNA polymerase and an activation reagent to create unique "master mixes" that amplify and detect two or three *IDH1*/*IDH2* amino acid mutations, and can detect sequences in regions outside of codon 132 (*IDH1*) or codons 140 and 172 (*IDH2*), to serve as endogenous internal controls (IC) (Table 1) [17,18]. Detection of the IC ensures that enough target material is available (target adequacy) and appropriate amplification of the IC ensures that the process has been performed correctly (process control). These master mixes are added to two separate wells of a 96-well optical reaction plate along with aliquots of the extracted DNA sample. The plate is transferred to the Abbott *m*2000 Real-Time instrument (Abbott Molecular, Inc.).

Target DNA is amplified by DNA polymerase in the presence of the activation agent, which includes primers, deoxyribonucleoside triphosphates (dNTPs), and magnesium chloride ($MgCl_2$). Amplification of *IDH1*, *IDH2*, and IC targets takes place simultaneously. *IDH1*/*IDH2* are detected during the annealing/extension step by measuring the real-time fluorescence signals of the mutant-*IDH1*, mutant-*IDH2*, and IC-specific probes, which are labelled with different fluorophores to allow their signals to be distinguishable in a single PCR well.

Master Mix	*IDH* Mutation	Single Nucleotide Variants*
IDH1 Oligonucleotide 1	R132C	TGT
	R132H	CAT
IDH1 Oligonucleotide 2	R132G	GGT
	R132S	AGT
	R132L	CTT
IDH2 Oligonucleotide 1	R140Q	CAG
	R140L	CTG
IDH2 Oligonucleotide 2	R140G	GGG
	R140W	TGG
IDH2 Oligonucleotide 3	R172K	AAG
	R172M	ATG
IDH2 Oligonucleotide 4	R172G	GGG
	R172S	AGT and AGC
	R172W	TGG

*The codon with the mutant nucleotide underlined.

Table 1: *IDH* mutations detected by each master mix.

Positive and negative controls are used in each run to verify that sample processing, amplification, and detection are performed correctly. Positive controls are formulated with DNA containing *IDH1*-R132H, *IDH1*-R132L, *IDH1*-R132G, *IDH2*-R140Q, *IDH2*-R140W, *IDH2*-R172K, or *IDH2*-R172W mutations, along with the IC signals, which should be detected in the positive controls. The negative controls are formulated with DNA containing only IC sequences, and thus only the IC signal should be detected in these samples.

Sanger and NGS testing

The BCW Sanger sequencing and NGS assay methods followed standard laboratory-developed test procedures. BCW Sanger sequencing follows the PCR-based bidirectional Sanger sequencing targeting *IDH1* exon 4 including mutations at codon 132 (R132H, R132C, R132L, R132G, and R132S) and *IDH2* exon 4 including mutations at codons 140 and 172 (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W). The sensitivity, specificity, and TAT based on workflow analysis for the BCW Sanger sequencing assay are 20%, 99%, and eight business days, respectively. The BCW NGS HemeOnc panel contains 30 genes, including *IDH1* and *IDH2*, targeted for myeloid malignancies; however, for this study, the data were analyzed only for *IDH1* and *IDH2*. The sensitivity, specificity, and TAT based on workflow analysis for the NGS HemeOnc panel are 10%, 99%, and 15 business days, respectively.

Workflow analysis

The workflow analysis to determine TAT of the Abbott Real-Time *IDH1* and *IDH2* assays included assessment of the times, from the receipt of the specimen, to perform assay procedures (eg, DNA extraction, amplification, detection, data collection), lab reporting, and delivery of test results to a treating physician.

Results

The Abbott Real-Time *IDH1* and *IDH2* assays demonstrated 100% sensitivity and 95% specificity for detecting *IDH1* and *IDH2* mutations compared to the BCW Sanger sequencing assay. Five of 100 samples with low-level *IDH2* mutations (< 20% VAF) were missed by the BCW Sanger sequencing assay but were detected as mutation-positive in the

Sample	Real Time Assay		BCW Sanger Assay		BCW NGS HemeOnc Panel	
	IDH1	***IDH2***	***IDH1***	***IDH2***	***IDH1***	***IDH2***
Abb-011	ND	R140Q detected	ND	ND	ND	ND
Abb-031	ND	R140Q detected	ND	ND	ND	*R140Q (3.0%) detected
Abb-060	R132H detected	R140Q detected	R132H detected	ND	R132H (32.48%) detected	*R140Q (8.1%) detected
Abb-096	ND	R140Q detected	ND	ND	ND	ND
Abb-097	ND	R140Q detected	ND	ND	ND	ND
BCW, BloodCenter of Wisconsin; ND, not detected.						
*The variant (raw data) was seen below the assay 10% sensitivity limit.						

Table 2: Results of five discordant samples showing Abbott real time PCR assay for *IDH1/IDH2* as a highly sensitive assay compared to BCW Sanger sequencing and the NGS HemeOnc panel.

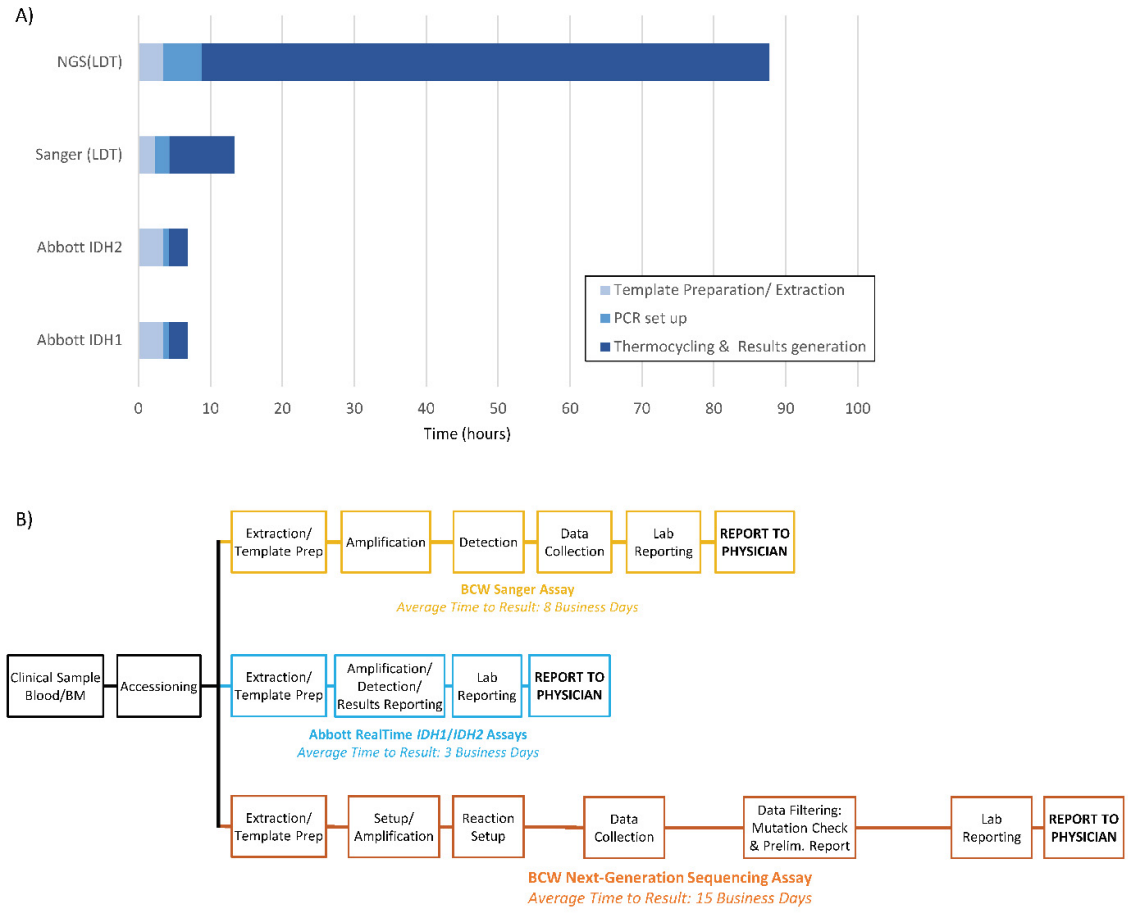

Figure 1: A) Assay processing time and B) Turnaround time (TAT) for real time *IDH1/IDH2* assays, Sanger sequencing, and next-generation sequencing.

Abbott Real-Time *IDH2* assay. These five discordant samples were subsequently tested by NGS, which detected *IDH2* mutations in two of the samples (Table 2).

The Abbott Real-Time *IDH1* and *IDH2* assays detected all of the commercially available *IDH1* and *IDH2* mutation controls down to the 1% level (with some variability at the 0.25% level) (Table 2). In contrast, the Sanger method was sensitive to 20% mutant-*IDH2* VAF.

TAT workflow analysis showed that the results from the Abbott Real-Time *IDH1/IDH2* assays were available in three business days from the time of sample receipt in a clinical testing lab, compared with eight business days for the Sanger sequencing assay and 15 business days for the NGS assay (Figure 1). The longer TATs for the latter two assays mainly reflect additional time needed for sequential steps involving amplification, thermocycling/detection, and data collection and filtering; whereas, the Abbott Real-Time assays perform these processes simultaneously.

Discussion

The availability of effective treatments for a disease like AML with few therapeutic options reinforces the need for rapid diagnosis of pathogenic targets that are treatable. Indeed, the prognostic and

therapeutic implications of *IDH* mutations in AML led to the recent recommendation by the College of American Pathologists and the American Society of Hematology to test for *IDH1* and *IDH2* mutations during the diagnostic workup for AML [19]. Moreover, paired diagnosis and relapse samples from patients with newly diagnosed mutant-*IDH* AML show these mutations can persist after induction chemotherapy [20-22]. The presence of persistent *IDH* mutations as measurable residual disease (MRD) during morphologic remission is a harbinger of relapse and is associated with poorer overall survival [15,23].

Targeted therapy with Ivosidenib and Enasidenib has shown promising results in patients with mutant-*IDH* R/R AML in clinical trials [24,25]. In a phase 1/2 trial of Enasidenib monotherapy, median overall survival among 214 patients with mutant-*IDH2* R/R AML treated with Enasidenib was 8.8 months, which compares favorably to median OS in similar patients treated with a variety of other salvage treatment regimens (~3.5 months) [26,27]. Similarly, in a phase 1/2 study of Ivosidenib monotherapy in 125 patients with mutant-*IDH1* R/R AML, ivosidenib was associated with a median overall survival of 8.8 months [25].

Conclusion

Our results demonstrate that the Abbott Real-Time *IDH1*/*IDH2* assays reliably detect *IDH1* and *IDH2* mutations at VAF levels as low as 1% in patients with R/R AML. The Abbott Real-Time *IDH1*/*IDH2* assays were more sensitive than the PCR-based Sanger sequencing assay and NGS panel, which had sensitivity limits of 20% and 10%, respectively. As noted, *IDH1*/*IDH2* VAFs less than 10%, which is below the limits of detection with Sanger and NGS methodologies, can significantly increase relapse risk and may warrant treatment with an *IDH* inhibitor. The higher sensitivity of the Abbott Real-Time *IDH1*/*IDH2* assays could potentially improve the ability to diagnose new and relapsed patients with AML who have a lower *IDH* mutational burden. Moreover, the more rapid TAT for the Abbott Real-Time *IDH* assay results could help clinicians to make quicker decisions regarding optimal therapeutic treatment options for patients with AML.

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Declaration of Interest

Dash DP, Wise L, Knier A, Boretsky M, Simons J, Berchanskiy D and Indig MDA have no relevant financial interest to report. Joseph AM is employed by Abbott Molecular, Inc.

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