OPEN

IDH1/2 Mutations in Patients With Diffuse Gliomas: A Single Centre Retrospective Massively Parallel Sequencing Analysis

Zuzana Sporikova, MSc, Rastislav Slavkovsky, PhD,* Lucie Tuckova, MD,† Ondrej Kalita, MD,‡§ Magdalena Megova Houdova, PhD,* Jiri Ehrmann, MD, PhD,† Marian Hajduch, MD, PhD,* Lumir Hrabalek, MD, PhD,‡ and Miroslav Vaverka, MD, PhD‡*

Downloaded from http://journals.lww.com/appliedimmunohist by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdtwnfKZBYtws= on 12/21/2021

Abstract: Patients below 55 years were genetically studied because the prevalence of isocitrate dehydrogenase 1 (*IDH1*) decreases in older patients and on grounds of cost-effectiveness, as suggested by the World Health Organization (WHO) in 2016. The aim of our study was to use novel massively parallel sequencing (MPS) approaches to examine rare variants of *IDH1/2* in Czech diffuse astrocytic and oligodendroglial tumors (gliomas) patients below 55 years of age who had been immunohistochemically (IHC) diagnosed as IDH1 R132H negative. The IHC IDH1 status (wild type or mutant) of 275 tissue samples was analyzed using antibodies against the IDH1 R132H protein. Sixty-three samples of 55 years old patients with IHC IDH1 WT status were genotyped using two different MPS technologies to detect rare *IDH1* and *IDH2* variants. The tiered IHC (60 positive) and molecular (10 positive) approach thus revealed that 70 of the 275 samples (25%) bore *IDH1*/*IDH2* mutations. The combined molecular and IHC approach thus revealed that 70 of the 275 samples (25%) considered in the study bore *IDH1*/*IDH2* mutations. IHC detection of the IDH1 R132H variant should be routinely complemented with MPS to detect rare *IDH1/2* variants in glioma patients below 55 years of age with negative IHC result of IDH R132H variant.

Key Words: IDH1, IDH2, fast sequencing, immunohistochemistry, diffuse gliomas

(*Appl Immunohistochem Mol Morphol* 2021;00:000–000)

The World Health Organization (WHO) has historically classified tumors of the central nervous system (CNS) based on their histologic features. In 2016, the WHO incorporated selected molecular parameters[1–3] into the tumor classification system, improving diagnosis and patient care. In particular, the use of genetic markers (*IDH1/2*, *ATRX*, *TP53*, and the 1p/19q co-deletion) as diagnostic tools has led to the development of robust and reproducible algorithms that predict patients' survival better than histology alone.

In 2008, exome sequencing studies on glioblastomas identified missense mutations in isocitrate dehydrogenase 1 (*IDH1*),[4] a Krebs cycle gene. This led to the elucidation of common genetic alterations during early formative stages of progressive gliomas and secondary glioblastomas, significantly improving the understanding and classification of gliomas. IDH1 and 2 are important in cellular processes such as the response to glucose, glutamine metabolism, lipogenesis, and the regulation of cellular redox status.[5] Under normal conditions, the IDH enzyme catalyzes the conversion of isocitrate to α-ketoglutarate by decarboxylation. The residues of IDH1 and IDH2 most frequently mutated in gliomas are Arg132 and Arg172, respectively. Mutations of these residues induce neomorphic enzyme activity: instead of the wild type activity, the mutated proteins catalyze the reduction of α-ketoglutarate into the oncometabolite D-2-hydroxyglutate. The aberrant production of D-2-hydroxyglutate leads to a cellular state of malignant transformation involving both epigenetic changes and aberrant differentiation.[6] The presence of IDH1/2 mutations was identified as a marker of survival,[7,8] and these

Received for publication October 22, 2020; accepted October 26, 2021.

From the *Institute of Molecular and Translational Medicine; Departments of †Clinical and Molecular Pathology; ‡Neurosurgery, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc; and §Department of Health Care Science, Faculty of Humanities, T. Bata University in Zlin, the Czech Republic.

Ministry of Health of the Czech Republic (grant NV19-04-00281), BBMRI-CZ (grant LM2018125), Palacky University (grant IGA LF_2020_007), Technology Agency of the Czech Republic (grant TE02000058), and the European Regional Development Fund-Project ENOCH (CZ.02.1.01/0.0.0/16_019/0000868), Ministry of Education, Youth and Sport of the Czech Republic (grant LM2018132).

Z.S., R.S., L.T., M.M.H., O.K., L.H., M.V. and J.E.: conceptualization. Z.S., R.S., L.T. and M.M.: methodology. Z.S., R.S., O.K. and L.T.: writing. M.H., O.K., J.E., L.H. and M.V.: supervision. O.K., M.H., and L.H.: funding acquisition.

The authors declare no conflict of interest.

Reprints: Ondrej Kalita, MD, Department of Neurosurgery, Palacky University and University Hospital, Olomouc 77900, Czech Republic (e-mail: ondrej.kalita@fnol.cz).

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.appliedimmunohist.com.

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

mutations have become critical diagnostic tools that are used to guide clinical decision making relating to gliomas.[2]

The incidence of *IDH1* mutations in glioblastomas is ~12%[4] but studies on grade II to III gliomas and secondary glioblastomas found these mutations in ~80% of samples.[9–13] *IDH2* mutations are less common and are mutually exclusive with *IDH1* mutations.[13,14] All *IDH1* and *IDH2* mutations observed in glioblastomas are single amino acid missense mutations at arginine 132 (R132) or the analogous arginine 172 (R172), respectively. The most frequent variant, *IDH1* R132H, is found in over 85% of gliomas[15] and features a heterozygous missense mutation of arginine to histidine (CGT→CAT). This mutation changes the enzyme's active site, reducing its catalytic activity and its affinity for isocitrate.[16]

The detection of R132 and R172 variants has implications for glioma diagnosis,[17] prognosis[14,18] and potentially treatment.[19,20] However, the only variant that can be detected by immunohistochemistry (IHC) is IDH1 R132H; the other variants are currently detected by follow-up genetic sequencing using Sanger or next-generation technology.[2] On the basis of studies examining the effects of variables such as patient age, tumor grade, and IDH1 R132H IHC, the WHO recommended in 2016 that only glioma patients below 55 years of age should undergo sequencing for rare *IDH1* mutations following a negative IDH1 R132H IHC analysis.[2,21,22] Screening for IDH mutations has thus become a key diagnostic tool for brain tumors but is not cost-effective for all patients.[22]

Since the *IDH* mutations status is crucial for diagnostic algorithm for integrated classification of diffuse astrocytic and oligodendroglial tumors, the revelations true positive/negative samples is a necessity these days. Our aim was to reveal samples by massively parallel sequencing (MPS) approaches that were signed as false negative samples by the IHC methodology and if there is a space for reduction of expenses and/or increasing the efficiency of genotyping when using different approaches of next-generation sequencing (NGS) methodology. Our fast *IDH* method is suitable for genotyping of known hotspots for somatic mutations with concordant results validated by the commercially available kit (Nextera XT kit, Illumina).

MATERIAL AND METHODS

Study Group and Tissue Specimens

The study cohort consisted of 275 patients with gliomas who had undergone surgical intervention at the Department of Neurosurgery in Olomouc between the years 2011 and 2017. Formalin-fixed paraffin-embedded (FFPE) sample was collected from each participating patient, and all samples included in the study were validated by a pathologist experienced with CNS tumors.

IHC

The 1 to 2 μm thick tissue sections were pretreated using the system PT Link (Agilent) at 97°C, pH9 for 20 minutes to ensure epitope retrieval. Hydrogen peroxide was used to block endogenous peroxidase activity. The sections were subsequently treated for with primary antibody, Anti-IDH1 R132H, clone H09 (Dianova, Hamburg, Germany), dilution 1 : 100 for 20 minutes at room temperature. EnVision Flex+, Mouse, High pH (Agilent DAKO) was used to amplify the signal of primary antibody. After the application of the secondary antibody EnVision, Flex/HRP (Agilent DAKO) for 20 minutes, the reaction was finally visualized using DAB+ Substrate Chromogen System (Agilent DAKO).

Fluorescence In Situ Hybridization (FISH)

The 1p/19q gene co-deletion was detected by FISH, which was performed in accordance with the manufacturer's protocol for FFPE tissue sections (IntellMed Ltd., Olomouc, Czech Republic). The locus-specific identifiers 1p36.3 and 19q13 were used for chromosome copy number enumeration. At least 100 nonoverlapping nuclei were selected for assessment in each sample using fluorescence microscopy.

IDH1 and *IDH2* Genotyping by Next-Generation Sequencing

The protocols used for DNA extraction from FFPE tissue sections and *IDH1* R132 and *IDH2* R172 genotyping are provided in the supplementary material and methods (Supplemental Digital Content 1, http://links.lww.com/AIMM/A328). Two NGS-based methods were used to increase the reliability of the results. Also, the genotyping was repeated in case of discordant results by 2 methods. Briefly, the first commercial method included amplification of specific regions and preparation of library using tagmentation (Nextera XT kit, Illumina). The second Fast method included multiplex amplification comprising *IDH1* and *IDH2* reactions with specific primers containing overhangs required for sequencing and this process is followed by amplicon purification. These primers ensures skipping the process of tagmentation and indexing resulting in substantial time-saving.

RESULTS

IHC

A total of 275 patient (mean age = 60.2 y) samples were histologically evaluated by the pathologist according to the 2016 CNS WHO recommendations by the pathologist, therefrom 11 samples were unable to be IHC examined for *IDH* R132H (Table 1).[2] The samples were then subdivided according to IHC R132H positivity (mutated) or negativity (WT). The data were stratified by tumor subtype; R132H immunoreactivity was observed in 60 of 275 samples (22%).

FISH

No generally accepted cut-off values suitable for analytical validation of 1p or 19q deletions detection in oligodendroglioma have been reported[23] therefore we set the cut-off at 20% of nuclei harboring only 1 copy. FISH analysis indicated the presence of the 1p/19q co-deletion in 6 of 8 IHC R132H positive samples, one sample could not be repeatedly analyzed probably because of tissue processing error.

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

TABLE 1. Histologic Subtypes of Diffuse Gliomas Included in Our Single Center Study Conducted Between 2011 and 2017, Showing Subgroup Characteristics

Diagnosis	Grade	N/Total (%)	M/F	Age Mean/Range (y)
IHC-IDH R132H WT				
Oligodendroglioma	II	1/10 (10%)	0/1	9.3
1p/19q co-deleted		0	0	—
Anaplastic oligodendroglioma	III	4/12 (33.3%)	2/2	54.1 (30.3-54.1)
Diffuse astrocytoma	II	17/40 (42.5%)	10/7	43.0 (22.8-76.1)
Anaplastic astrocytoma	III	22/35 (62.9%)	9/13	64.6 (33.2-81.6)
Glioblastoma	IV	162/178 (91.0%)	104/58	61.8 (23.7-84.3)
IHC-IDH R132H mutated				
Oligodendroglioma	II	9/10 (90%)	6/3	49.3 (33.1-73.0)
1p/19q co-deleted		6/8 (75%)	4/2	51.7 (34.3-73.0)
Anaplastic oligodendroglioma	III	8/12 (66.7%)	3/5	49.6 (33.1-68.7)
Diffuse astrocytoma	II	22/40 (55%)	10/12	42.3 (23.1-70.8)
Anaplastic astrocytoma	III	9/35 (25.7%)	5/4	40.0 (27.6-56.7)
Glioblastoma	IV	12/178 (6.7%)	7/5	47.9 (33.8-75.2)

F indicates female; M, male; WT, wild type.

Sequencing of IHC IDH1 WT Patients Below 55 Years of Age

To maximize cost-effectiveness rising from low prevalence of *IDH* mutations in patients 55 years or above,[22] only tumors from patients under the age of 55 with WT IDH1 according to IHC (n = 63) were sequenced (Table 2).

The sequencing results indicated that 10 of the 63 samples (16%) were either *IDH1* R132 or *IDH2* R172 mutated. The 2 sequencing methods gave consistent results for all 10 positive samples; 2 samples could not be successfully analyzed by at least 1 of the methods (Table 3). As expected, we detected rare mutations in *IDH1* (R132S and R132C were present in 3 samples). In addition, 3 samples were found to carry rare *IDH2* mutations in codon 172 that were not detectable by IHC. Surprisingly, 4 samples were genotyped as being *IDH1* R132H positive even though the IHC data indicated that all samples chosen for sequencing contained only WT IDH. Two samples could not be analyzed because of low input DNA quality and poor PCR amplification of targeted regions. Furthermore 1 sample of 11 that could not be IHC analyzed and fulfilled the age criteria was unequivocally signed as IDH R132H mutated (Table 3, case No. 8).

Quality Control of Sequencing Assay

The typical NGS assays consist of > 30 PCR cycles that include more than billion-fold amplification of targeted DNA segments followed by manipulation of amplicons, leading to concern for amplicon contamination and repeatability of results. In Table 4 we present the results analysis or different types of controls for fast *IDH*. To increase the contamination possibility, we ranked samples for processing using regular alternation pattern (wt or negative alternating with *IDH1* R132H mutation positive) as shown in Table 4. For negative control we used heavily fragmented (< 100 bp) low amount of DNA (~1 ng/μL) isolated from blood of donor, or no template controls where no template was added, however, base calling and data processing were handled as usual. We did not observed any contamination as wt samples contained 0.17% of variant c.395G > A (n = 6), which is expected overall error rate caused by library preparation followed by Illumina based sequencing.[24] For no template/fragmented DNA we observed on average less than one c.395G > A variant bearing read and 3 c.395G > A reads were observed at maximum (n = 12). This would translate in <0.3% contamination when assuming 1000 or more reads are required for the processing. Also we observed high repeatability and reproducibility of c.395G > A containing sample. VAF was equal to 32.1% ± 0.6% (n = 8, average ± SD).

TABLE 2. Characteristics of Patients Aged 55 Years or Below Selected for Sequencing to Detect *IDH* Mutations in Codons 132 and 172

Diagnosis	Grade	M/F	Age Mean/Range (y)	Mutated *IDH* Codons R132 and 172 /Total
Oligodendroglioma	II	0/1	9	0/1 (0%)
1p/19q co-deleted		0	—	0
Anaplastic oligodendroglioma	III	1/2	46 (30-54)	2/3 (67%)
Diffuse astrocytoma	II	5/5	35 (23-53)	6/10 (60%)
Anaplastic astrocytoma	III	4/2	43 (33-54)	2/6 (33%)
Glioblastoma	IV	26/17	48 (24-55)	2/43 (5%)

F indicates female; IDH; M, male.

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

TABLE 3. Patient Characteristics and Genotyping Results Obtained Using the FastIDH Method and the Nextera XT Library Prep Kit

Case No.	Age (y)	Sex	Side	Location	Subtype	WHO Grade	*IDH* Nextera Variant	MAF, %	*IDH* Fast Variant	MAF, %
1	53	F	Right	Frontal	Anaplastic oligodendroglioma	III	*IDH2* R172K	56	*IDH2* R172K	48
2	30	M	Left	Frontal	Diffuse (fibrillar) astrocytoma	II	*IDH2* R172M	59	*IDH2* R172M	46
3	32	M	Left	Frontal	Diffuse (fibrillar) astrocytoma	II	*IDH1* R132C	14	*IDH1* R132C	13
4	33	F	Left	Frontal	Anaplastic astrocytoma	III	*IDH1* R132C	64	*IDH1* R132C	48
5	33	F	Left	Frontal	Diffuse (fibrillar) astrocytoma	II	*IDH1* R132S	35	*IDH1* R132S	34
6	33	F	Left	Frontal	Diffuse (fibrillar) astrocytoma	II	*IDH1* R132H	23	*IDH1* R132H	29
7	35	M	Left	Temporal	Diffuse astrocytoma	II	*IDH1* R132H	15	*IDH1* R132H	35
8	38	M	Left	Temporal	Diffuse (gemistocytar) astrocytoma	II	*IDH1* R132H	26	*IDH1* R132H	28
9	54	M	Left	Temporal	Anaplastic oligoastrocytoma	III	*IDH2* R172K	50	*IDH2* R172K	38
10	47	F	Right	Frontal	Diffuse astrocytoma	II	*IDH1* R132H	15	*IDH1* R132H	15
11	45	M	Right	Frontal	Glioblastoma	IV	Not analyzable		Not analyzable	
12	53	M	Right	Temporal	Glioblastoma	IV	wt		Not analyzable	

Nucleotide substitutions in our samples for *IDH* mutations: *IDH1* R132H-c.395G > A; *IDH1* R132C-c.394C > T, *IDH1* R132S-c.394C > A, *IDH2* R172M-c.515G > T, *IDH2* R172K-c.515G > A.

F indicates female; IDH, isocitrate dehydrogenase 1; M, male; MAF, mutation allelic fraction in the sample; wt, wild type.

TABLE 4. Quality Control for FastIDH Assay

Run ID	Index	Sample	Result (If Available)	c.395A Count (R132H)	Total Read Count	VAF c.395G > A (R132H)
FR124	i30	Neg. contr. fr. DNA		0	0	NA
FR124	i31	Positive control 1	*IDH1* R132H	1990	6078	32.7%
FR124	i32	Neg. contr. fr. DNA		1	7	NA
FR124	i33	Positive control 1	*IDH1* R132H	1695	5294	32.0%
FR124	i35	Neg. contr. fr. DNA		0	0	NA
FR124	i36	Positive control 1	*IDH1* R132H	1853	5663	32.7%
FR124	i38	Wt control 1	wt	5	4258	0.1%
FR124	i41	Neg. contr. fr. DNA		3	4	NA
FR124	i42	Positive control 1	wt	2352	7490	31.4%
FR122	i30	Wt control 2	wt	7	3569	0.2%
FR122	i32	Positive control 1	*IDH1* R132H	1290	3994	32.3%
FR122	i36	Wt control 2	wt	3	1268	0.2%
FR122	i38	Positive control 1	*IDH1* R132H	1539	4829	31.9%
FR122	i36	Wt control 2	wt	7	4761	0.1%
FR122	i42	Positive control 1	*IDH1* R132H	1651	5077	32.5%
FR122	i31	Neg. contr. no template		0	15	NA
FR122	i33	Neg. contr. no template		0	0	NA
FR122	i35	Neg. contr. no template		0	0	NA
FR120	i30	Neg. contr. no template		1	5	NA
FR120	i31	Neg. contr. no template		0	7	NA
FR120	i32	Neg. contr. no template		0	0	NA
FR120	i33	Neg. contr. no template		0	1	NA
FR120	i35	Neg. contr. no template		0	0	NA
FR120	i36	Positive control 2	*IDH1* R132H	5744	14602	39%
FR120	i38	Positive control 1	*IDH1* R132H	4702	15031	31%
FR120	i41	Wt control 3	wt	26	17907	0.1%
FR120	i42	Wt control 4	wt	18	15300	0.1%
				Average c.395A count	Aver. total read count	VAF average +-SD
		DH1 R132H positive control 1 (n = 8)		2134	6131	32.1% ± 0.6%
		Wt controls (n = 6)		8	12259	0.17% ± 0.04%
		Negative control: no template/fr. DNA (n = 12)		0.2	3.1	NA

Data from 3 independent runs were used.

For negative control with fragmented DNA we used ultrasound fragmented donor blood DNA with median fragment size <100 bp.

For no template control on DNA library was added to the sequencer in order to see the contamination based on index hopping and/or sequencer overflow.

DNA isolated from glial tumor tissue with known *IDH1/2* status was used as positive control and wt control.

IDH indicates isocitrate dehydrogenase 1; NA, not analyzable; VAF, variant allele fraction; wt, wild type.

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

DISCUSSION

The integration of phenotypic and genotypic parameters in the classification of CNS tumors has improved diagnosis. The frequency of the *IDH1* R132H variant is reported to be ~90% (it was 91% in our studied population), and this mutant can be detected by IHC.[25] IHC detection of the IDH1 R132H variant was performed using the DIA-H09 antibody, for which the expected true positive rate is 88% to 99%. If we assume that all samples found to be IDH1 R132H positive by IHC would also be *IDH1* R132H positive by sequencing, the concordance rate of the sequencing and IHC methods would be 94% (60/64). A number of other *IDH1* variants are known, including R132C (whose frequency in glioma patients is reportedly around 3%; in the studied population, it was 3%), R132S (frequency in this work: 1%), and R132G and R132L (whose reported frequencies are both around 1%; neither was detected in this work). *IDH2* variants are less common, with R172K being observed in 3% of glioma patients (3% in our study), R172M (1% in our study), R172W (none in our study) and R172S (none in our study) having frequencies of ~1% each.[26] Our data agree with the previously reported stratification and frequency of *IDH* mutations in gliomas.

IDH1 status determination is crucial for diagnosis and selecting an appropriate treatment strategy. Typically, the first step in treating a glioma is to perform the safest radical resection that will provide enough tumor tissue for reliable diagnosis. Regardless of tumor grade, any glioma expressing wild type IDH should be regarded as a glioblastoma and treated with aggressive chemoradiotherapy according to the Stupp protocol. The treatment of gliomas expressing mutated variations of *IDH* should be guided by the presentation of clinical and molecular features. For radically resected low-grade tumors exhibiting both the 1p/19q co-deletion and an *IDH* mutation, one might even consider omitting oncotherapy altogether and simply recommend watchful follow-up.[27]

As noted above, accurate determination of *IDH* status is vital for selecting effective treatment strategies for diffuse glioma patients and thus for their prognosis. The financial burden of diagnosing gliomas is increasing because of the multitude and complexity of current laboratory methods. WHO recommends testing all IDH negative samples from patients below 55 years old, in our study it was 63 patients of 275 (23%). %). IHC was analytically false negative for IDH1 R132H in 4 specimens, and an additional 6 specimens had clinical false negative IHC by virtue of alternate *IDH1/2* mutations. The data validate the notion that while IHC is the standard method for detecting IDH, genetic sequencing should also be used to confirm negative IHC results and minimize the risk of false negatives. Using 2 different molecular approaches, this work confirmed the high incidence of *IDH1* variants in glioma patients below 55 years old.

The IHC determination of *IDH1* R132H mutation failed in 11/275 (4%) samples included in our cohort. This failure might be caused by laboratory errors or possible inadequate tissue handling. Therefore, second step control incorporating sequencing is fundamental for crucial genes involved in the molecular-histologic definition of gliomas. This 2-step procedure could be applied also for other genes examined in gliomas like alpha-thalassemia/mental retardation syndrome X-linked (*ATRX*) or telomerase reverse transcriptase (*TERT*).

After recent classification of tumors of the CNS and the development of MPS techniques, it starts to be feasible to use a glioma-tailored customized gene panels for understanding the molecular background.[28,29] In our work, we showed that precision medicine information is increased even by testing *IDH1* and *IDH2* genes only. Our homemade Fast IDH MPS method shows concordant results as Nextera XT based method. For library preparation FAST IDH includes just 1 step (PCR amplification with purification, ~3 h in total) in contrast to Nextera XT method (1. PCR amplification with purification, 2. tagmentation and 3. indexing PCR amplification with purification, ~7 h in total) and the likelihood of the technical error is and hands-on time thus reduced. Overall Fast IDH method provides higher cost efficiency because of faster sample processing and will be licenced to a company to be available world-wide. Because of the high cost of sequencing chemistry we used in our laboratory the smallest flow-cell (MiSEQ nano) with pricing 380 to 500 euros to achieve cost efficiency of the method. Usually in routine diagnostics 2 to 4 samples for *IDH1/2* genotyping are sequenced in each run (once a week or every second week). Therefore it is necessary to combine *IDH1/2* sequencing with other sequencing genotyping methods such as *KRAS*, *NRAS*, *BRAF* (colon cancer), EGFR and *BRAF* (lung cancer), or *BRCA1/2* (ovarian cancer). When sequencing 16 samples, the cost sequencing is around 30 euros per sample for both methods. About 15 euros per sample (half of total costs) includes library preparation, work cost along with running costs. Library preparation using Nextera XT costs around 50 euro per sample. When the fastIDH kit is commercially available, expected costs per sample are 30 to 60 euros. Total cost of *IDH1/2* sequencing would nowadays thus be about or even under 100 euro. This opens the question over the current validity of the model published by DeWitt in 2017[22] which is based on 1800 USD (~1500 euros) as NGS costs. Although it might sound controversial, we assume that nowadays even tumors of patients over 55 years should be analyzed by MPS and gradually NGS could replace IDH IHC entirely. This data suggests that more than two-thirds of costs are saved and less common mutations and false negatives are revealed with propriate efficacy. Limitation of our work is that we did not sequenced IHC wt samples of patients over 55 to see if there are any noncanonical or false negative samples.

CONCLUSION

The correct identification of diffuse gliomas is crucial for identifying appropriate tailored therapies. Gliomas are classified using the 2016 WHO system, which is based on the presence of validated biomarkers including IDH mutations and the 1p/19 co-deletion. We have shown that rare variants of *IDH1/2* occur in patients with CNS tumors, corroborating

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

previous findings. Our laboratory performs DNA sequencing of tumors identified as IDH1-negative by IHC in patients below 55 years old, and we recommend this approach to other laboratories interested in precise molecular diagnostics.

REFERENCES

1. Perry A. WHO's arrived in 2016! An updated weather forecast for integrated brain tumor diagnosis. *Brain Tumor Pathol*. 2016;33:157–160.
2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*. 2016;131:803–820.
3. Wesseling P, Capper D. WHO 2016 Classification of gliomas. *Neuropathol Appl Neurobiol*. 2018;44:139–150.
4. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science*. 2008;321:1807–1812.
5. Reitman ZJ, Yan H. Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism. *J Natl Cancer Inst*. 2010;102:932–941.
6. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462:739–744.
7. Chen J-R, Yao Y, Xu H-Z, et al. Isocitrate dehydrogenase (IDH)1/2 mutations as prognostic markers in patients with glioblastomas. *Medicine (Baltimore)*. 2016;95:e2583.
8. Okita Y, Narita Y, Miyakita Y, et al. IDH1/2 mutation is a prognostic marker for survival and predicts response to chemotherapy for grade II gliomas concomitantly treated with radiation therapy. *Int J Oncol*. 2012;41:1325–1336.
9. Balss J, Meyer J, Mueller W, et al. Analysis of the IDH1 codon 132 mutation in brain tumors. *Acta Neuropathol*. 2008;116:597–602.
10. Bleeker FE, Lamba S, Leenstra S, et al. *IDH1* mutations at residue p. R132 ($IDH1^{R132}$) occur frequently in high-grade gliomas but not in other solid tumors. *Hum Mutat*. 2009;30:7–11.
11. Kang MR, Kim MS, Oh JE, et al. Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. *Int J Cancer*. 2009;125:353–355.
12. Sanson M, Marie Y, Paris S, et al. Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. *J Clin Oncol*. 2009;27:4150–4154.
13. Yan H, Parsons DW, Jin G, et al. *IDH1* and *IDH2* mutations in gliomas. *N Engl J Med*. 2009;360:765–773.
14. Hartmann C, Meyer J, Balss J, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta Neuropathol*. 2009;118:469–474.
15. Watanabe T, Vital A, Nobusawa S, et al. Selective acquisition of IDH1 R132C mutations in astrocytomas associated with Li-Fraumeni syndrome. *Acta Neuropathol*. 2009;117:653–656.
16. Xu X, Zhao J, Xu Z, et al. Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity. *J Biol Chem*. 2004;279:33946–33957.
17. Camelo-Piragua S, Jansen M, Ganguly A, et al. A sensitive and specific diagnostic panel to distinguish diffuse astrocytoma from astrocytosis: chromosome 7 gain with mutant isocitrate dehydrogenase 1 and p53. *J Neuropathol Exp Neurol*. 2011;70:110–115.
18. Reuss DE, Mamatjan Y, Schrimpf D, et al. IDH mutant diffuse and anaplastic astrocytomas have similar age at presentation and little difference in survival: a grading problem for WHO. *Acta Neuropathol*. 2015;129:867–873.
19. Wang F, Travins J, DeLaBarre B, et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science*. 2013;340:622–626.
20. Schumacher T, Bunse L, Pusch S, et al. A vaccine targeting mutant IDH1 induces antitumour immunity. *Nature*. 2014;512:324–327.
21. Chen L, Voronovich Z, Clark K, et al. Predicting the likelihood of an isocitrate dehydrogenase 1 or 2 mutation in diagnoses of infiltrative glioma. *Neuro Oncol*. 2014;16:1478–1483.
22. Dewitt JC, Jordan JT, Frosch MP, et al. Cost-effectiveness of IDH testing in diffuse gliomas according to the 2016 WHO classification of tumors of the central nervous system recommendations. *Neuro Oncol*. 2017;19:1640–1650.
23. Pinkham MB, Telford N, Whitfield GA, et al. FISHing tips: what every clinician should know about 1p19q analysis in gliomas using fluorescence in situ hybridisation. *Clin Oncol*. 2015;27:445–453.
24. Fox E, Reid-Bayliss KS, Emond MJ, et al. Accuracy of next generation sequencing platforms. *Next Gener Seq Appl*. 2014;1:1000106.
25. Lee SC. Diffuse gliomas for nonneuropathologists: the new integrated molecular diagnostics. *Arch Pathol Lab Med*. 2018;142:804–814.
26. Arita H, Narita Y, Yoshida A, et al. IDH1/2 mutation detection in gliomas. *Brain Tumor Pathol*. 2015;32:79–89.
27. Weller M, van den Bent M, Tonn JC, et al. European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. *Lancet Oncol*. 2017;18:e315–e329.
28. Zacher A, Kaulich K, Stepanow S, et al. Molecular diagnostics of gliomas using next generation sequencing of a glioma-tailored gene panel. *Brain Pathol*. 2017;27:146–159.
29. Na K, Kim HS, Shim HS, et al. Targeted next-generation sequencing panel (TruSight Tumor 170) in diffuse glioma: a single institutional experience of 135 cases. *J Neurooncol*. 2019;142:445–454.

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.