SVOA Neurology

ISSN: 2753-9180

ScienceVolks Open Access

Research Article

Evaluation Of Surrogate Markers to Define *H3 G34R/V*-Mutant Gliomas

Samasuk Thammachantha[1]* and Chinnachote Teerapakpinyo[2]

[1] *Department of Pathology, Neurological Institute of Thailand (NIT), Bangkok, Thailand.*

[2] *Chulalongkorn GenePRO Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.*

***Corresponding Author:** Samasuk Thammachantha, Department of Pathology, Neurological Institute of Thailand (NIT), Bangkok, Thailand.*

DOI: https://doi.org/10.58624/SVOANE.2024.05.0126

***Received:** January 09, 2024* ***Published:** January 29, 2024*

Abstract

Background: *H3 G34*-mutant glioma was a new high-grade glioma, characterized by mutation of histone3.3 codon34. Currently, standard gene sequencing methods are helping in tumor diagnosis, but less expensive and faster H3 G34R/V staining method has not been studied yet in Thailand.

Objectives: The purpose of this research is to find surrogate makers for diagnosing this tumor by uncomplicated immunohistochemical method.

Methods: Formalin-fixed and paraffin-embedded tissue samples of 30 diffuse hemispheric gliomas were collected H3 G34R, H3 G34V, Olig2, p53 were evaluated using immunohistochemistry. Comparisons of immunohistochemical method and sequencing standard technique were made. Cost-effectiveness was analyzed.

Results: Two cases of *H3 G34*-mutant gliomas were confirmed by mutation analysis. One case was positive for H3 G34V antibody (1/1) while another case was negative for H3 G34R (0/1). The cost-effectiveness showed that the sequencing technique prolonged turnaround time but gave a cost saving 23 USD (17%).

Conclusions: G34R and G34V antibodies are not sensitive surrogate markers for diagnosing *H3.3 G34*-mutant gliomas. However, more samplings are yet to be tested in the future.

***Keywords:** Glioma, H3 G34R/V, Olig2, p53*

Introduction

Mutations in histone *H3 G34* are a key to define new diffuse high-grade gliomas (HGG), which are called "Diffuse hemispheric glioma, *H3 G34*-mutant" by the recent 2021 World Health Organization (WHO) classification [1, 2]. These tumors occur most often in cerebral hemisphere and primarily affect adolescents and young adults. The molecular alterations found in these gliomas are mutations of glycine 34 in histone H3, either substituted by Arginine(G34R) or Valine(G34V). Histomorphology of these tumors usually shows high-grade features with abundant mitotic figures and variable necrosis [3, 4]. However, a subset of them associated with primitive neuronal component and astroblastoma have been reported [5-7].

Immunohistochemical profiles of these tumor characterized by positive H3 G34R/V, positive p53 (88%) and Olig2 negative (100%) [8, 9]. However, accuracy of H3 G34R/V immunohistochemical study was inconsistency. In this study, the authors aim to validate commercial antibodies (H3 G34R and H3 G34V), as well as supporting antibodies (Olig2 p53), to compare with standard sequencing method.

Materials and Methods

Tissue samples

Thirty diffuse gliomas in the hemispheric location of Thai population were collected from the Department of Pathology, Neurological Institute of Thailand (NIT) from January 2017 to March 2021. Only cases of patients under the age of 55 were included. Mean age at diagnosis was 36.6 years (range from 4-55 years) Regarding to previous WHO classification of CNS tumors (2016), all gliomas were classified into 3 gradings; 6 were diffuse astrocytomas, 9 were anaplastic astrocytomas and 15 were glioblastomas (GBM). Using of tissue was approved by Institutional Review Board (IRB) of NIT(number#64042, 19 Jan 2022)

Histopathology

All tumors were reviewed by neuropathologist (ST). Formalin-fixed, paraffin-embedded (FFPE) blocks of corresponding cases were cut at 4 μm thick. Immunohistochemical stains (IHC) were manually performed by 2 experienced technicians. Primary antibodies including H3 G34R (RM240, 1: 100, RevMAb Biosciences, CA, USA), H3 G34V (RM307, 1:100, RevMAb Biosciences, CA, USA), Olig-2 (HL1072, 1:100, Genetex, Irvine, CA, USA), p53 (DO-7, 1:100; Dako, Carpinteria, CA, USA). Appropriate positive controls were included. H3 G34R, H3 G34V, Olig2 and p53 positivity was defined when >10% of tumor nuclei showed intense staining.

DNA extraction and gene panel study

On hematoxylin and eosin(H&E) stained FFPE sections, representative areas of tumor sampling were outlined for micro-dissection. DNA was isolated using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The amount and the quality of DNA was evaluated by Nanodrop 1000 (Thermo Scientific, Massachusetts). Molecular analysis was performed on polymerase chain reaction (PCR) followed by direct sequencing.

H3, *IDH1* and *IDH2* mutation analysis

H3 mutation analysis was determined in all tumors by pyrosequencing method at Chulalongkorn GenePRO Center(CT). Because of all mutations in *H3* and *IDH* were mutually exclusive [10-12]. *H3*-mutant glioma cases were not been tested for *IDH* sequencing. Only *H3*-wild type tumors were further analyzed by *IDH* mutation analysis (*IDH1* and *IDH2)*. (Fig .1)

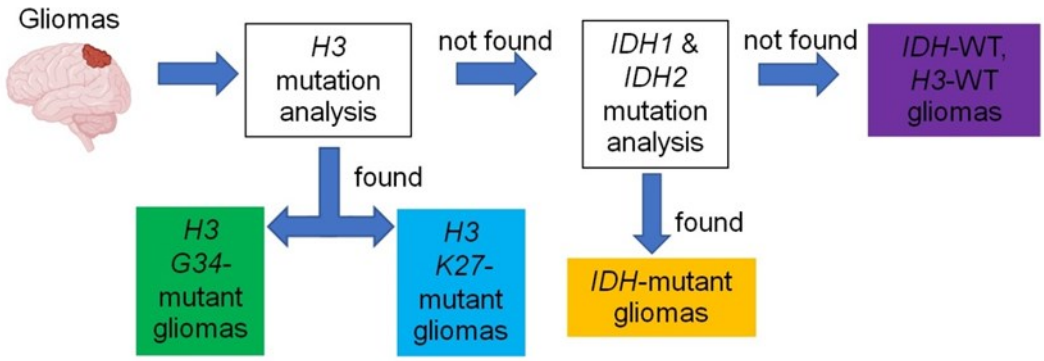

Figure 1. *Flow chart of mutation analysis.*

A mutation analysis of *H3* was performed by DNA extraction process. Validated specific PCR protocol was used and modified with some modification and bidirectional sequencing to detect G34R/V point mutation. Each sample was analyzed by licensed sequencher (version 5.3). A mutation reported conforms to HGVS guidelines on mutation nomenclature (www.hgvs.org) and named according to the reference sequence NM_002107.7 [13, 14].

After *H3* mutation analysis, mutations in *IDH1* codon 132 and *IDH2* codon 172 were screened by PCR and bidirectional sequencing. Then samples were processed by licensed sequencher (version 5.3), HGVS guidelines and named according to the reference sequence NM_005896.4(*IDH1*) and NM_002168.4(*IDH2*).

Results

Histopathologic Findings

Two cases (Case#23 and#24) of *H3 G34*-mutant gliomas were of anaplastic astrocytomas (WHO 2016). Both cases demonstrated high-grade features such as high cellularity, increased mitotic activity (average 5/10 HPF). None of *H3 G34* -mutant gliomas showed low-grade histologic finding.

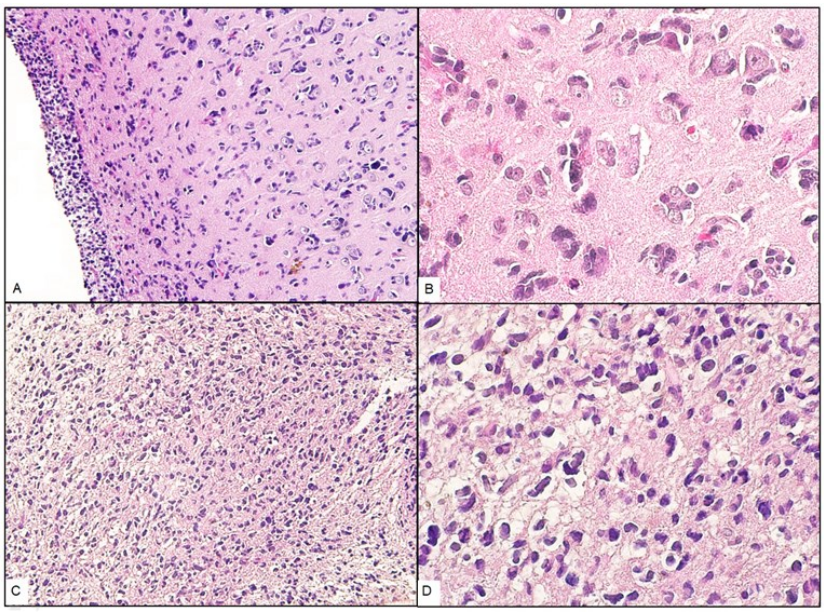

Figure 2. *Case#23 (A) showed diffuse infiltrating glial neoplastic cells and subpial accumulation. (B) Perivascular and perineuronal satellitosis were noted. (A: H&E; 200xmagnification, B: H&E; 600xmagnification). Case#24 (C) showed increased cellularity. (D) Tumor cells with hyperchromatic elongated nuclei. Focal perinuclear halo was seen. (C: H&E; 200xmagnification, D: H&E; 400xmagnification).*

G34R and G34V immunohistochemical study

The number of positive G34R and G34V cases per total tested cases were 0 of 30(0%) and in 1 of 30(3%), respectively. Positive staining of both G34R and G34V were not seen in non-H3 mutant gliomas.

Comparison of results of IHC and mutational analyses

Case#2 and #17 had inadequate DNA to perform *H3* and *IDH* mutation analysis. Total of 28 samples were studied, *H3* mutation was found in 4 of 28 cases (17%), two cases were *H3 K27M* mutation, one was *H3 G34R* and *G34V* each. *H3* mutation analysis of case#23 revealed GGG>GTG mutation (c.104G>T; G34V) while case#24 harbored mutation GGG-AGG (c.103G>A; G34R). Only G34V antibody had the concordant result with mutation analysis, while G34R had discordant result (false negative) between IHC and mutation analysis.

24 of *H3*-wild type tumors were analyzed for *IDH* mutation, and *IDH*-mutant gliomas were found in 6 of 24 cases (25%). All were *IDH1 R132H* mutant.

All *H3 G34*-mutant gliomas showed loss of Olig2 staining in (2/2) as well as p53 staining (2/2). (Figure 2.) Loss of Olig2 staining could be found in other gliomas including *H3 K27M* mutant gliomas (case#8). While p53 mutation was found in 1 case (case#21) of *H3 K27M* mutant gliomas also.

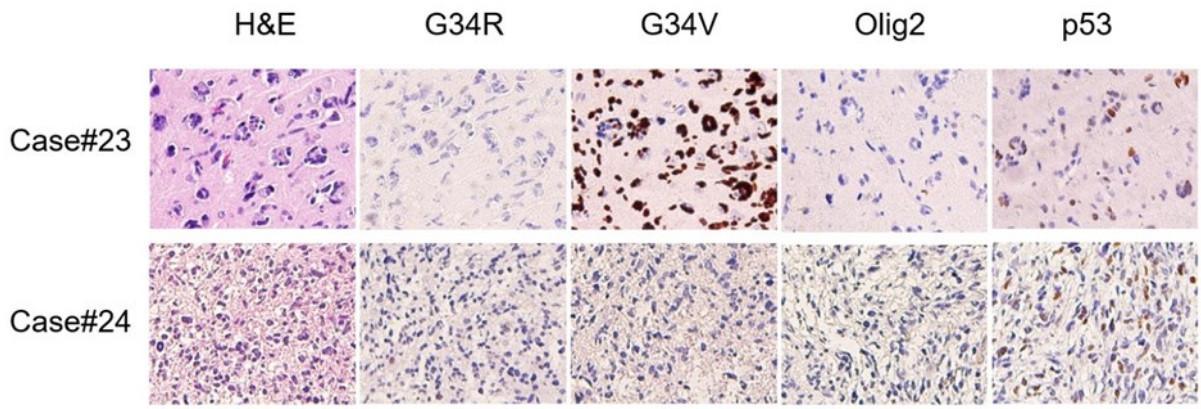

Figure 3. *Histomorphology and IHC characters of H3 G34-mutant glioma.*

Discussions

According to the past WHO classification, *H3 G34*-mutant gliomas were previously diagnosed as "High-grade gliomas" include anaplastic astrocytoma, GBM and primitive neuroepithelial tumor (PNET) [4]. The current 2021 WHO classification of CNS tumors classified "Diffuse hemispheric glioma, *H3 G34*-mutant" into grade 4 which associated with better prognosis compared to classical GBM [8, 15, 16]. Moreover, these tumors tend to occur in the younger patients (age <30 years) [17, 18]. In our study, only two cases were *H3 G34*-mutant by our mutation analysis (18 and 23 year of age). Histologically, these two cases were anaplastic astrocytoma. Neither low-grade feature nor primitive neuronal component was found.

Table 1. Summary of clinical data and immunohistochemical profiles for 30 cases of diffuse glioma.

Patient #	Age Dx	Gender	Path Dx	G34R	G34V	Olig2	p53	*H3* analysis	*IDH* analysis
1	53	M	GBM	-	-	-	-	-	-
2	50	F	GBM	-	-	-	-	Failed	NP
3	28	F	GBM	-	-	+	-	-	-
4	41	M	GBM	-	-	-	-	-	-
5	43	F	GBM	-	-	-	-	-	-
6	52	M	GBM	-	-	-	-	-	-
7	39	M	GBM	-	-	+	-	-	-
8	33	M	GBM	-	-	-	-	*K27M*	NP
9	4	F	GBM	-	-	+	-	-	-
10	50	F	GBM	-	-	+	-	-	-
11	41	F	GBM	-	-	-	+	-	-
12	55	F	GBM	-	-	+	-	-	-
13	54	M	GBM	-	-	-	-	-	-
14	48	M	GBM	-	-	+	-	-	-
15	31	M	GBM	-	-	-	-	-	-
16	51	M	AA	-	-	+	-	-	-
17	40	F	AA	-	-	+	-	Failed	NP
18	47	M	AA	-	-	-	-	-	-
19	35	M	AA	-	-	+	-	-	-
20	34	M	AA	-	-	+	-	-	*IDH1* R132H
21	12	F	AA	-	-	+	+	*K27M*	NP
22	34	F	AA	-	-	+	-	-	*IDH1* R132H
23	18	M	AA	-	+	-	+	*G34V*	NP

24	23	F	AA	-	-	-	+	*G34R*	NP
25	36	F	DA	-	-	-	-	-	-
26	6	M	DA	-	-	+	-	-	-
27	29	M	DA	-	-	+	-	-	*IDH1* R132H
28	39	F	DA	-	-	+	-	-	*IDH1* R132H
29	34	M	DA	-	-	+	-	-	*IDH1* R132H
30	39	M	DA	-	-	+	-	-	*IDH1* R132H

Age Dx – Age at diagnosis (years), M – Male, F – Female, Path Dx – Pathological diagnosis, GBM – Glioblastoma, AA – Anaplastic astrocytoma, DA – Diffuse astrocytoma, Failed – DNA not enough, NP – not performed.

The Immunohistochemical result shows that *H3 G34V*-mutant glioma(case#23) is positive for H3 G34V antibody, but *H3 G34R*-mutant glioma(case#24) are negative for H3 G34R antibody. (Table 2.) Previous study of Salloum et al, claimed that *G34V* mutation was extremely uncommon (G34R:G34V mutation ratio was 8:1) [19]. Another study in Asian population, Wang et al found that ratio of G34R:G34V mutation was 6.5:1 [20]. In our study, the ratio was 1:1 but there was limited by sample size. Moreover, there was no single positive case for G34R antibody, we cannot confirm the G34R antibody's reliability because the result might be the possibility of antibody denaturation.

Table 2. G34R and G34V IHC expression in literature.

Previous literature	Percentage
Haque et al (UK, 2017) [21].	11/11 (100%)
Onishi et al (Japan, 2020) [22].	3/3 (100%)
Cheng et al (China, 2020) [23].	3/3 (100%)
Gianno et al (Italy, 2021) [24].	7/9 (78%)
Present study: H3 G34R H3 G34V	 0/1(0%) 1/1 (100%)

Additional antibodies in our study were Olig2 and p53, both *H3 G34*-mutant gliomas showed loss of Olig2 expression and strong p53 nuclear staining. Unfortunately, our study is limited by samples of these tumors in order to calculate reliable statistical profile. So, G34R and G34V antibodies are not sensitive surrogate markers for diagnosing *H3 G34*-mutant gliomas (Table 2), we recommended that using of these novel antibodies should be combined with other additional antibodies such as Olig2 and p53.

Regarding to the cost, advantages of the G34R and G34V IHC are not only faster result (1 day compared to 7 days of mutation test) but also a reduced cost. In our country, the cost per case is 40 USD (for both G34R and G34V IHC) compared to 57 USD (for mutation test). In our study, the cost of tests needed to diagnosed 2 cases of *H3 G34*-mutant gliomas (case#23 and #24) would be 137 USD compared to 114 USD of mutation analysis. (Fig 4.) We believe that mutation analysis alone (method 2) would give a cost saving 23 USD (17%), compare with combined IHC and mutation test in method 1. However, the test can vary significantly among labs and countries.

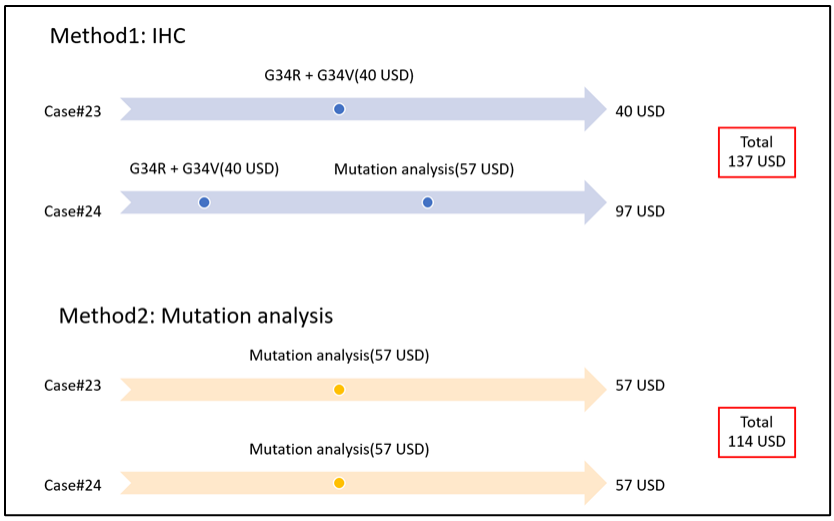

Figure 4. *Compared costs between IHC and mutation analysis for diagnosis of H3 G34R/V gliomas in our study (2 cases).*

Conclusions

The 2021 CNS WHO classification revealed many new gliomas, which require varieties of molecular testing. However, this gives a big difficulty in countries with limited laboratory resources. This study aims to improve turn-around time, reducing the workup costs and provide practical diagnostic guideline regarding to *H3 G34*-mutant glioma. Up to now, this is the first reported study of novel *H3 G34*-mutant gliomas in South East Asia, using of immunohistochemical methods. However, this is a single center-based study, we believe that the multi-center data provides more accuracy of these antibodies to generate future H3 G34 immunohistochemical testing.

Acknowledgments

Thank you Dr. Shanop Shuangshoti (Chulalongkorn GenePRO Center, Faculty of Medicine, Chulalongkorn University, Bangkok) for advice and useful comments; Mr. Kittinan Kumsa-ard and Ms. Asama Tukanghan(NIT) for processing and validation of H3 G34R, H3 G34V, Olig2, p53 antibodies.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Louis DN, Wesseling P, Aldape K, et al. cIMPACT-NOW update 6: new entity and diagnostic principle recommendations of the cIMPACT-Utrecht meeting on future CNS tumor classification and grading. Brain Pathol. 2020;30(4):844-856. doi: 10.1111/bpa.12832
2. Morris M, Driscoll M, Henson JW, et al. Low-Grade Gemistocytic Morphology in H3 G34R-Mutant Gliomas and Concurrent K27M Mutation: Clinicopathologic Findings. J Neuropathol Exp Neurol. 2020;79(10):1038-1043. doi: 10.1093/jnen/nlaa101
3. Schwartzentruber J, Korshunov A, Liu XY, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature. 2012;482:226-31.
4. Gessi M, Gielen GH, Hammes J, et al. H3.3 G34R mutations in pediatric primitive neuroectodermal tumors of central nervous system (CNSPNET) and pediatric glioblastomas: possible diagnostic and therapeutic implications?. J Neurooncol. 2013;112:67–72.
5. Wood MD, Halfpenny AM, Moore SR. Applications of molecular neuro-oncology - a review of diffuse glioma integrated diagnosis and emerging molecular entities. Diagn Pathol. 2019;14(1):29. doi: 10.1186/s13000-019-0802-8
6. Andreiuolo F, Lisner T, Zlocha J, et al. H3F3A-G34R mutant high grade neuroepithelial neoplasms with glial and dys-

7. Yoshimoto K, Hatae R, Sangatsuda Y. et al. Prevalence and clinicopathological features of H3.3 G34-mutant high-grade gliomas: a retrospective study of 411 consecutive glioma cases in a single institution. Brain Tumor Pathol. 2017;34, 103–112.

8. Korshunov A, Capper D, Reuss D, et al. Histologically distinct neuroepithelial tumors with histone 3 G34 mutation are molecularly similar and comprise a single nosologic entity. Acta Neuropathol. 2016;131(1):137–46.

9. Picart T, Barritault M, Poncet D, et al. Characteristics of diffuse hemispheric gliomas, H3 G34-mutant in adults. Neurooncol Adv. 2021;3(1):vdab061. doi: 10.1093/noajnl/vdab061

10. Wu G, Broniscer A, McEachron TA, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat Genet. 2012;44(3):251-253. doi: 10.1038/ng.1102

11. Pathak P, Jha P, Purkait S, et al. Altered global histone-trimethylation code and H3F3AATRX mutation in pediatric GBM. J Neurooncol. 2015;121(3):489-497. doi: 10.1007/s11060-014-1675-z

12. Fontebasso AM, Liu X-Y, Sturm D, Jabado N. Chromatin remodeling defects in pediatric and young adult glioblastoma: a tale of a variant histone 3 tail. Brain Pathol Zurich Switz. 2013;23(2):210-216. doi: 10.1111/bpa.12023

13. Gessi M, Gielen GH, Hammes J, et al. H3.3 G34R mutations in pediatric primitive neuroectodermal tumors of central nervous system (CNS-PNET) and pediatric glioblastomas: possible diagnostic and therapeutic implications? J Neurooncol. 2013;112(1):67-72. doi: 10.1007/s11060-012-1040-z

14. Gielen GH, Gessi M, Hammes J, et al. H3F3A K27M mutation in pediatric CNS tumors: a marker for diffuse high-grade astrocytomas. Am J Clin Pathol. 2013;139(3):345-9. doi: 10.1309/AJCPABOHBC33FVMO

15. WHO Classification of Tumours Editorial Board. Central nervous system tumours. Lyon (France): International Agency for Research on Cancer; 2021. (WHO Classification of Tumours Series, 5th ed.; vol. 6). https://publications.iarc.fr/601

16. Lee J, Solomon DA, Tarik T. The Role of Histone Modifications and Telomere Alterations in the Pathogenesis of Diffuse Gliomas in Adults and Children. J Neurooncol. 2017; 132(1): 1–11. doi: 10.1007/s11060-016-2349-9

17. Sturm D, Witt H, Hovestadt V, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell. 2012;22(4):425-37. doi: 10.1016/j.ccr.2012.08.024

18. Ichimura K, Nishikawa R, Matsutani M. Molecular markers in pediatric neuro-oncology. Neuro Oncol. 2012;14 (Suppl 4):iv90-9.

19. Salloum R, McConechy MK, Mikael LG, et al. Characterizing temporal genomic heterogeneity in pediatric high-grade gliomas. Acta Neuropathol Commun. 2017;5(1):78. doi: 10.1186/s40478-017-0479-8

20. Wang L, Shao L, Li H, et al. Histone H3.3 G34-mutant Diffuse Gliomas in Adults. Am J Surg Pathol. 2022;46:249-257. doi: 10.1097/PAS.0000000000001781

21. Haque, F, Varlet, P, Puntonet, J, et al. Evaluation of a novel antibody to define histone 3.3 G34R mutant brain tumours. Acta Neuropathol Commun. 2017; 5, 45. doi.org/10.1186/s40478-017-0449-1

22. Onishi S, Amatya VJ, Karlowee V, et al. Radiological and Immunostaining Characteristics of H3.3 G34R-Mutant Glioma: A Report of 3 Cases and Review of the Literature. Pediatr Neurosurg. 2020;55(5):319-325. doi: 10.1159/000511672.

23. Cheng Y, Bao W, Wu Q. Cerebral hemispheric glioblastoma with PNET-like morphology and histone H3.3 G34 mutation in younger patients: Report of three rare cases and diagnostic pitfalls. Indian J Pathol Microbiol. 2020;63 (2):262-266. doi: 10.4103/IJPM.IJPM_544_19

24. Gianno F, Antonelli M, Di Dio T, et al. Correlation Between Immunohistochemistry and Sequencing in H3G34-

Citation: Thammachantha S, Teerapakpinyo C. Evaluation Of Surrogate Markers to Define *H3 G34R/V*-Mutant Gliomas. *SVOA Neurology* 2024, 5:1, 46-52.