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Original Research

Next Generation Sequencing Impacts The Classification And Management Of Primary Brain Tumours- Original Research

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ABSTRACT:
Aim: The purpose of the present study was assess the impact of next generation sequencing on the classification as well as management of primary brain tumors. **Methodology:** A retrospective analysis was conducted amongst 58 neuro-oncology patients who underwent NGS tumor profiling using a single commercially available platform on paraffin-embedded tissue obtained at diagnosis (20 low-grade gliomas, 12 high-grade gliomas, 11 embryonal tumors, four ependymal tumors, three meningeal tumors, and eight other CNS tumors. NGS results were analyzed for actionable mutations, variants of unknown significance and clinical impact. **Results:** Seventy-four percent of patients (43 of 57) had actionable mutations; 26% had only variants of uncertain significance (VUS). NGS findings impacted treatment decisions in 55% of patients; 24% were given a targeted treatment based on NGS findings. Seven of eight patients with low-grade tumors treated with targeted therapy. Turnaround time between sample shipment and report generation averaged 13.4 ± 6.4 days. **Conclusion:** Our experience highlights the feasibility and clinical utility of NGS in the management of neuro-oncology patients. Future prospective clinical trials using NGS are needed to establish efficacy.
Keywords next-generation sequencing, brain tumors, precision medicine, targeted therapy

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INTRODUCTION

According to the 3rd edition of the International Classification of Diseases for Oncology, tumors of the central nervous system (CNS) are those affecting the spinal cord and brain, including the pituitary gland, meninges, pineal gland, and nerves.[1] Brain tumors have been traditionally classified based on the microscopic investigation of hematoxylin and eosin-(H & E) stained tissue sections. The increased comprehensive knowledge in relevant genetic alterations and mutations with clinical outcomes resulted in the incorporation of molecular signatures as a part of the diagnosis, management, and treatment of CNS malignancies.[2] Various brain cancer susceptibility genes are involved in DNA damage response, which strongly indicates that critical DNA repair pathways and checkpoint controls are necessary for preventing tumor malignancy.[3] Since the number of prognostic and predictive neurooncologic genetic markers is steadily increasing, comprehensive analyses of the molecular techniques used to examine neuro-oncology samples are vastly required for the evaluation of brain tumor specimens in a modern pathology setting. Molecular analysis and profiling of brain cancers lead to improved diagnostic accuracy, target identification and predictive prognosis.[4] The recent development of NGS technology and other complementary genomic platforms have transformed our capacity to investigate the molecular landscapes of human cancers, including brain tumors.[5]This integration of NGS has highlighted some important points to consider in the evolving practice of neuropathology. CNS tumours with similar histological features can have different prognostic outcomes depending on their molecular signature. A compelling early

example was the discovery that IDH1/2-mutated gliomas exhibit very different clinical behaviour to their IDH-wildtype counterparts.[6] The cIMPACT-NOW defined a subset of IDH-wildtype grade II/III tumours with specific molecular alterations that predict a clinical course equivalent to grade IV tumours.[7] These alterations include EGFR amplification, TERT promoter mutation and combined gain of Chr7 and loss of Chr10.NGS is also a valuable resource in the setting of small biopsy specimens, as it allows for the simultaneous assessment of multiple genetic alterations from small amounts of DNA (20 ng). Small biopsies present a histological challenge since there is limited material for interpretation.In one paediatric patient, the midbrain location yielded scant biopsy material for examination. The differential diagnoses were broad and encompassed low and high grade tumours. NGS detected an isolated KIAA1549-BRAF fusion/ duplication event, which is typically associated with pilocytic and pilomyxoid astrocytomas.So, it becomes imperative to know more about NGS which can directly help in classifying as well having a targeted treatment strategy for brain tumors.

AIM OF THE PRESENT STUDY

The purpose of the present study was assess the impact of next generation sequencing on the classification as well as management of primary brain tumors.

METHODOLOGY

Fifty-eight non-consecutive patients with primary CNS tumors who had surgical resection or biopsy underwent NGS testing using a single commercially available platform. The selection of patients for NGS testing was by the treating neuro-oncologist. Selection for NGS was due to the uncertainty of diagnosis by histology alone, (2) failure of established treatment options and screening for targetable mutations, and (3) atypical tumor behavior, such as an unexpected rate of progression of low-grade tumors. Specimens underwent a pathologic evaluation, and formalin-fixed paraffin-embedded (FFPE) sections were sent for NGS analysis.NGS at high depth (>500x) utilizing the Illumina HiSeq® for uniform sequencing coverage enabled the detection of all classes of genomic alterations including single-base substitutions, small insertions and deletions, rearrangements, and copy number alterations. The resulting report was reviewed by the treating physician. Actionable mutations were defined as those which altered diagnosis, altered treatment, or diagnosed a cancer predisposition syndrome. The timing of initiation of targeted therapy was by treating neuro-oncologist's discretion. The duration of follow-up was until patient death.Patient's NGS results were then binned into categories of clinical actionability: 1. those affecting diagnosis, 2. those in whom a change was made in patient management and 3. those leading to a cancer predisposition syndrome diagnosis.

RESULTS

Our 58-patient cohort was composed of 31 females and 27 males with an average age of 7.4 ± 5.3 years at the time of surgical resection (range: four months to 19 years, median: 6.5 years).

The NGS analysis included 20 low-grade gliomas, 12 high-grade gliomas, 11 embryonal tumors, four ependymal tumors, three meningeal tumors, and eight other CNS tumors.The average time between the date of surgical resection or biopsy and the decision to pursue NGS was one year but varied widely (standard deviation 21 months, median: three months, mode: one month, range: 11 days to 11.5 years). This reflects both clinical heterogeneity and the ability to perform NGS on archived samples. The turnaround time between sample shipment and report generation averaged 13.4 days (standard deviation: 6.4 days). Seventy-four percent (43/57) of samples that completed NGS testing were found to have "actionable" mutations as defined above, whereas the remaining 14 patients (26%) had only variants of uncertain significance (VUS) detected. Patients with actionable variants had an average of 2.8 actionable variants per report (standard deviation: 3.8, range: 1-23, mode: 1). Seventy-three genes were found to be actionable, 19 of which were detected at least twice. The clinical impact of NGS sequencing included refining pathologic diagnosis, guidance in targeted agent choice, guiding use of radiation, and confirming a cancer predisposition syndrome. NGS enabled a more refined diagnosis in 23 (40%) cases where pathologic workup was limited by unclear/mixed histology or quantity of tissue. Fourteen patients (24%) were given targeted therapy based on NGS results. Eighty-eight (7/8) percent of patients with low-grade gliomas who received targeted therapy had either a partial response or stabilization of their disease. Patients receiving a targeted agent for high-grade tumors all experienced progressive disease. Radiation therapy was avoided in 18 cases (32%), where there was a lack of malignant molecular features based on NGS.

Table 1- Patient demographic and treatment characteristics

Variables	Characteristics
Age at time of surgery	4 months to 19 years (Range)
Gender	27 male, 31 female
Time to next-generation sequencing (NGS) testing after surgery	11 days to 11.5 years (Range)
Tumor type	Low grade-20, High grade- 38

Mean number of actionable mutations types of mutation (% total)	2.8 (1-23)
Time to reporting (average)	13.4 days (SD±6.4)
Targeted therapies used	14 (24%)

Table 2- Most frequently observed genetic variants on next-generation sequencing

No	Gene	Variant	Count
1	BRAF	KIAA1549-BRAF fusion	8
2	BRAF	V600E	5
3	CDKN2A/B	loss	4
4	CDK4	amplification	3
5	MUTYH	G382D	2
6	SMARCB1	loss	2
7	KDR	amplification	2
8	KIT	amplification	2
9	PDGFRA	amplification	2
10	MYC	amplification	2

DISCUSSION

Human primary or intrinsic brain and CNS neoplasms indicate characteristic molecular signatures consistent with tumor type. Numerous studies have recently focused on analyzing genomic alterations in brain tumors. Detection of alterations or mutations in specific genes of some brain tumors, such as glioma, has revolutionized our understanding of the pathogenesis of many types of glioma.[8]The diagnosis, management, and treatment of patients with intrinsic or primary brain tumors have been previously dependent on a classification system using protein expression levels, microscopic and immunohistochemical examinations. The increased knowledge of relevant genetic alterations or genomic landscape of primary human brain tumors and clinical outcomes led to the incorporation of molecular signatures in the diagnosis, management, and treatment of brain malignancies.[9]Based on the latest WHO classification of the CNS tumors (2016), molecular investigations of primary brain tumors have become an important part of the diagnostic workup of human CNS tumors.[9] The advances in sequencing technologies have recently resulted in the incorporation of NGS assays in many clinical diagnostic laboratories and have increased the demand for identifying molecular profiles of human brain tumors.[4] It has been demonstrated that there is a significant histological overlap between brain tumors, such as astroblastoma with GB, particularly in the absence of characteristic molecular signatures of the tumor.[10]

In total, NGSis an attractive, efficient, and cost-effective technique in detecting a wide variety of molecular alterations, including genomic mutations such as insertions and deletions (indels), CNVs, single-nucleotide variations, and SNPs, which make it as a supplier unimodal molecular platform for the classification of human brain tumors.

Genomic characterization of brain neoplasms has been recently performed using NGS and has resulted in the generation of a large amount of information that can be very usual in the practice of neuropathology.[4] The NGS analysis has shown that the most clinically relevant genes for brain neoplasms are TP53, IDH1, IDH2, PIK3CA, EGFR, BRAF, PDGFRA, and FGFR1, 2 and, 3. According to the 2016 CNS WHO guidelines, molecular testing of IDH1 and 2 genes are critical for the diagnosis and management of diffuse gliomas. As TP53 mutations are rare in neoplasms with 1p/19q co-deletion, TP53 can be helpful to identify DGs that are 1p/19q-intact. The commercial NGS assay serves to detect IDH-wild-type, IDH, and TP53-mutant status in diffuse glioma. The combination of a separate assay to identify 1p/19q status using NGS is also helpful for the molecular classification of most of gliomas such as GBM based on the Latest WHO CNS tumor classification. The expression or genomic profiles of BRAF, PIK3CA, PDGFRA, EGFR, and FGFR1, 2 and 3 can be helpful to choose the most appropriate therapeutic approach.[2,11]It is notable that the routine sequencing of patients with recurrent GBM has not been widely adopted and data utilization for clinical actionability can vary.[12] Additionally, the cost of NGS can be prohibitive, further making widespread adoption difficult.[13] However, more centers are beginning to publish their own experiences with NGS and its implications for therapeutic applicability.[14]

Our experience shows a significant impact of molecular profiling on diagnosis, prognosis, andtreatment and validates its feasibility within clinically meaningful timeframes. In another published single institutional experience, NGS similarly helped refine diagnosis, and 61% of patients in that cohort were found to have potentially targetable variants. NGS clarified the diagnosis in 23 (40%) cases and was especially useful when histology was not definitive or tissue was limited. The clinical impact of NGS in neuro-oncology patients portends a hopeful future of true precision medicine, in which diagnosis is definitive, ineffective or inappropriate therapies are avoided, and

mechanistic treatment plans prolong durable responses.

CONCLUSION

NGS led to a change in diagnosis, the discovery of a cancer predisposition syndrome, and altered the course of treatment in a significant proportion of cases. Future prospective clinical trials using NGS are needed to establish the efficacy of molecular-based targeted therapy in children with primary and relapsed CNS tumors.

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