CORRESPONDENCE

Next generation sequencing impacts the classification and management of primary brain tumours

Sir,

The 2016 World Health Organization (WHO) classification of central nervous system (CNS) tumours heralded a shift towards an integrated diagnosis for brain tumours, incorporating both histological features and molecular data.¹ This was reiterated in 2018 by the Consortium to Inform Molecular and Practical Approaches to CNS Tumour Taxonomy (c-IMPACT).² It is now recognised that a tumour's molecular phenotype not only assists in classification, but also provides prognostic information and impacts clinical management. Knowledge of the molecular biology of brain tumours is rapidly expanding beyond what immunohistochemistry alone can identify.^{3,4} While there are many techniques available for molecular testing, next generation sequencing (NGS) has been identified as an efficient and cost-effective method allowing for parallel analysis of multiple genetic alterations.^{4,5}

We obtained clinical accreditation from the National Association of Testing Authorities Australia (NATA) in November 2018 to perform NGS on formalin-fixed, paraffin-embedded tissue using a customised glioma gene panel. This uses multiplex polymerase chain reaction (PCR) with ion semiconductor sequencing (AmpliSeq, Ion Torrent S5; ThermoFisher, Australia) to assess a panel of 26 glioma gene mutations and copy number variations (CNV). The customised glioma panel was modified from a previously published panel⁴ and is detailed in Fig. 1.

Between November 2018 and March 2019, 75 gliomas were analysed by our clinical service. These included a mix of second opinion consultation cases which required sequencing after review, direct requests for molecular testing from other hospitals, and cases from our own institution. At our hospital, we routinely perform an immunohistochemical panel (*IDH1* R132H, *ATRX*, *P53*, *EGFR*, *Ki-67*) on all gliomas, with the addition of *H3* K27M immunohistochemistry where clinically relevant. However, this practice is not standardised amongst other referring laboratories. Cases were predominantly from Australia, with a smaller number from New Zealand. There were 34 female and 40 male patients with an age range from 3 to 80 years, including five paediatric cases.

The cohort included 46 high grade gliomas (HGG). Of these, 35 were diagnosed or favoured to be glioblastomas (WHO grade IV), and 11 were anaplastic gliomas (grade III). Twenty-five cases were low grade gliomas (LGG; grade I or II). Four cases were not included in the final data: two had no original diagnoses provided, one did not have sufficient tumour for analysis and one case was tested as part of a validation run.

A total of 15 cases (21%) had the histological diagnosis altered by additional molecular information (Table 1), leading to a change in tumour classification/grading. Of note, 12 cases of grade II or grade III diffuse gliomas demonstrated molecular features of a glioblastoma *IDH*-wildtype (grade IV). One of these had *EGFR* amplification only; eight had a *TERT*

promoter mutation, with one also showing gain of Chr7 and loss of Chr10; three cases had both *EGFR* amplification and a *TERT* promoter mutation, and one of these also demonstrated gain of Chr7 and loss of Chr10. Tumour specific alterations (*H3F3A* and *KIAA1549-BRAF* fusion/duplication) were identified in two additional cases, one of which had a broad range of histological differential diagnoses.

Fourteen cases had the original diagnosis refined (20%; Table 1), encompassing tumour categories that were suspected on morphology but required molecular confirmation. A large number of this group consisted of gliomas that were *IDH1* R132H immuno-negative but harboured an alternate *IDH1/2* mutation.

There were 37 cases (52%) of 'confirmed diagnoses' where NGS played an important role in excluding alternative diagnoses, but the molecular information did not alter tumour classification. These included *IDH*-wildtype glioblastomas, in which NGS identified a supporting molecular signature (*TERT* promoter mutation, *EGFR* amplification), tumours (weakly) immunoreactive for *IDH1* (R132H) confirmed on sequencing, or *IDH1* (R132H) immuno-negative astrocytic tumours with no alternate *IDH1/2* mutation identified.

Five cases yielded non-contributory NGS findings that did not affect final diagnoses.

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

NATA-accredited gene tests:

- *IDH1* (codon 132)
- *IDH2* (codon 172)
- *TERT* promoter (c. 1-124 & c. 1-146)
- *H3F3A* (codons 27 and 34)
- *BRAF* (codon 600)
- Chromosome 1p CNV
- Chromosome 1q CNV
- Chromosome 19q CNV
- Chromosome 19p CNV
- *EGFR* exons 12-28 CNV

Additional test available as 'research use only':

- Additional gene coding regions analysed

▪ <i>ATRX</i>	▪ <i>NF1</i> , <i>NF2</i>
▪ <i>BRAF</i>	▪ <i>NRAS</i>
▪ <i>CDKN2A</i> , <i>CDKN2B</i>	▪ <i>PDGFRA</i>
▪ <i>CIC</i>	▪ <i>PIK3CA</i>
▪ <i>EGFR</i>	▪ <i>PTEN</i>
▪ <i>FUBP1</i>	▪ <i>RB1</i>
▪ <i>HIST1H3B</i> , <i>HIST1H3C</i>	▪ <i>TP53</i>
▪ <i>MYC</i> , <i>MYCN</i>	
- Additional copy number variations (CNVs):
 - Chromosome: 7p, 7q, 10p, 10q
 - Gene: *BRAF* 3', *BRAF* 5', *KIAA1549* 5', *KIAA1549* 3', *CDKN2A*, *CDKN2B*, *EGFR* (vIII), *MYC*, *MYC-N*, *PDGFRA*, *PTEN*

Fig. 1 Next generation sequencing glioma gene panel.

Table 1 Altered and refined diagnoses: original histological diagnoses provided at time of referral and subsequent integrated diagnoses following next generation sequencing (*n*=71)

Histological diagnoses	Molecular diagnoses	Cases <i>n</i> (%)
Altered diagnoses		
LGG, IDH1 (R132H)–	Molecular features of GBM IDH-wildtype	5
Anaplastic astrocytoma, IDH1 (R132H)–	Molecular features of GBM IDH-wildtype	5
Intermediate grade glioma with oligodendroglioma features	Molecular features of GBM IDH-wildtype	2
Ganglioglioma vs high grade glioma vs pilocytic astrocytoma	IDH1 (R132H)+ diffuse glioma (GBM)	1
LGG vs HGG at consult	LGG (<i>KIAA1549</i> - <i>BRAF</i> fusion)	1
Giant cell GBM	H3(K27M)-mutant diffuse midline glioma	1
	Subtotal	15 (21.4)
Refined diagnoses		
LGG NOS	Oligodendroglioma, <i>IDH1</i> -mutant and 1p/19q codeleted	1
LGG, IDH1 (R132H)+	Oligodendroglioma <i>IDH1</i> -mutant and 1p/19q codeleted	1
LGG IDH1 (R132H)–	Diffuse astrocytoma, alternate <i>IDH1</i> mutation	2
LGG, oligodendroglioma morphology, IDH1 (R132H)–	Oligodendroglioma with alternate <i>IDH1/2</i> mutation and 1p/19q codeleted	2
	Diffuse astrocytoma, alternate <i>IDH1</i> mutation	1
Oligoastrocytoma, IDH1 (R132H)–	Diffuse astrocytoma, alternate <i>IDH1</i> mutation	1
LGG, ganglioglioma vs pilocytic astrocytoma	Pilocytic astrocytoma, <i>KIAA1549</i> - <i>BRAF</i> fusion	1
HGG, IDH1 (R132H)–	HGG, alternate <i>IDH1</i> mutation	2
HGG with PNET, IDH1 (R132H)–	IDH1 (R132H)+ HGG, <i>MYC</i> amplification	1
Diffuse high-grade astrocytoma (paediatric case)	H3 (G34R)-mutant high grade paediatric astrocytoma	1
High grade diffuse astrocytoma (midline)	H3(K27M)-mutant diffuse midline glioma	1
	Subtotal	14 (19.7)

GBM, glioblastoma; HGG, high grade glioma; LGG, low grade glioma; NOS, not otherwise specified; PNET, primitive neuroectodermal tumour.

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.² These alterations include *EGFR* amplification, *TERT* promoter mutation and combined gain of Chr7 and loss of Chr10. In our early NGS data, this molecular signature was demonstrated in twelve IDH-wildtype grade II/III tumours (17%). Synhaeve *et al.* analysed molecular data on 433 gliomas and demonstrated the value of NGS in identifying glioma subtypes with different prognostic outcomes.⁷ They reported 16.9% of histologically grade II astrocytomas were molecularly grade IV glioblastomas,⁷ corroborating our results. If our results were reclassified to include alteration of prognosis rather than tumour classification alone, then the 'altered diagnoses' group would increase to 30%, including cases with alternate IDH mutations, and tumours that were shown on NGS to be astrocytomas, despite oligodendroglioma-like histological features.

We identified two H3(K27M)-mutant diffuse midline gliomas and a paediatric high grade diffuse astrocytic tumour harbouring a H3 (G34R) mutation. The cIMPACT-NOW has reinforced the 'midline' and 'diffuse' prerequisites in defining this tumour, as H3(K27M) mutations can rarely be observed in non-infiltrative tumours such as pilocytic astrocytomas and gangliogliomas.^{3,8} So despite the growing importance of molecular phenotype, incorporating radiological and histological features remains essential for a final integrated diagnosis.

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.

Our early experience demonstrates the value in integrating comprehensive molecular assessment into brain tumour classification. We found that the NGS panel results changed tumour classification/grading in 21% of the tumours tested, aligning with another recent report of NGS gene panel testing where it changed diagnosis in 28.4% of cases.¹ Integrating NGS into the diagnostic workup of primary brain tumours improves tumour classification with important implications for patient management.

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Mycobacterial spindle cell pseudotumour of the brain in an immunocompetent adult

Sir,

Mycobacterial spindle cell pseudotumour is a rare tumour-like lesion that predominantly occurs in immunocompromised individuals, especially those with HIV infection. Most

commonly described in the lymph nodes, this neoplastic mimic has also been reported in various other anatomical locations like skin, soft tissue, bone marrow, spleen, retroperitoneum and lungs. Brain is a rare site for mycobacterial spindle cell pseudotumour with only five documented cases.^{1–5} Here, we describe the second case of mycobacterial spindle cell pseudotumour involving the brain in an adult lacking any evidence of immunosuppression.

A 53-year-old male presented with evening rise in temperature, night sweats and weight loss (about 10 kg in 2 months) since September 2019. One month later, he developed slurred speech, deviation of angle of mouth to left and right upper limb weakness. For these symptoms he was evaluated elsewhere with magnetic resonance imaging (MRI) which showed multiple intra-axial space occupying lesions, the largest measuring 5.5×2.2 cm in the right occipital lobe. The lesions were hypointense to isointense on T2-weighted and FLAIR images and hyperintense on diffusion weighted images. The lesions showed thick ring and nodular post-contrast enhancement (Fig. 1A–D). He was referred to our institute and treatment with anti-tubercular drugs along with corticosteroid was started. Following this, there was symptomatic improvement. However, after 4 months, he had an episode of seizure. A repeat MRI at this point showed no change in the size of the lesions.

Fig. 1 Imaging and histopathology findings: (A) MRI showing multiple intra-axial lesions that are hypointense to isointense on T2-weighted image and (B) FLAIR sequence. (C) The lesions are hyperintense in diffusion weighted image with (D) thick ring and nodular post-contrast enhancement. (E) Histopathology shows a lesion with fascicles of spindle cells admixed with lymphocytes, plasma cells and (F) few ill-formed granulomata (H&E). (G) The spindle cells are diffusely positive for CD68 (immunoperoxidase). (H and inset) Acid-fast bacilli were detected on Ziehl–Neelsen stain.