The pivotal role of sampling recurrent tumors in the precision care of patients with tumors of the central nervous system

Derek Wong,[1,2] Yaoqing Shen,[3] Adrian B. Levine,[1] Erin Pleasance,[3] Martin Jones,[3] Karen Mungall,[3] Brian Thiessen,[4] Brian Toyota,[5] Janessa Laskin,[4] Steven J.M. Jones,[3] Marco A. Marra,[3] and Stephen Yip[1]

[1]Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia V6T 2B5, Canada; [2]Molecular Oncology, BC Cancer, Vancouver, British Columbia V5Z 1L3, Canada; [3]Canada's Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, British Columbia V5Z 4S6, Canada; [4]Division of Medical Oncology, BC Cancer, Vancouver, British Columbia V5Z 4E6, Canada; [5]Department of Neurosurgery, University of British Columbia, Vancouver, British Columbia V5Z 1M9, Canada

Abstract Effective management of brain and spine tumors relies on a multidisciplinary approach encompassing surgery, radiation, and systemic therapy. In the era of personalized oncology, the latter is complemented by various molecularly targeting agents. Precise identification of cellular targets for these drugs requires comprehensive profiling of the cancer genome coupled with an efficient analytic pipeline, leading to an informed decision on drug selection, prognosis, and confirmation of the original pathological diagnosis. Acquisition of optimal tumor tissue for such analysis is paramount and often presents logistical challenges in neurosurgery. Here, we describe the experience and results of the Personalized OncoGenomics (POG) program with a focus on tumors of the central nervous system (CNS). Patients with recurrent CNS tumors were consented and enrolled into the POG program prior to accrual of tumor and matched blood followed by whole-genome and transcriptome sequencing and processing through the POG bioinformatic pipeline. Sixteen patients were enrolled into POG. In each case, POG analyses identified genomic drivers including novel oncogenic fusions, aberrant pathways, and putative therapeutic targets. POG has highlighted that personalized oncology is truly a multidisciplinary field, one in which neurosurgeons must play a vital role if these programs are to succeed and benefit our patients.

[Supplemental material is available for this article.]

INTRODUCTION

Brain tumor behavior is driven by aberrations in the genome and epigenome. Many, such as IDH mutations in diffuse low-grade glioma (DLGG) and aberrations in neurofibromatosis 2 (*NF2*) in hereditary and sporadic meningioma, are common among the same class of tumor (Parsons et al. 2008; Cancer Genome Atlas Research et al. 2015; Eckel-Passow et al. 2015; Bi et al. 2016a; Louis et al. 2016; Waitkus et al. 2016). Knowledge of these aberrations has propelled the adoption of many into diagnostic neuropathology (Louis et al. 2016). However, a given tumor may have other, less common genomic aberrations that are essential for its biological behavior, and knowledge of these may inform on the underlying biology, aberrant

Corresponding author:
Stephen.Yip@vch.ca

cellular pathways, and, most importantly, potential therapeutic agents (Aparicio and Caldas 2013; Favero et al. 2015; Greaves 2015; Suzuki et al. 2015). This genomics-based approach to the management of cancer patients, known as precision or personalized oncology, has established itself within the practice of oncology and is slowly making its way into neuro-oncology.

The publication of the *Update to the Fourth Edition of the WHO Classification of Tumors of the Central Nervous System* (WHO2016) was the culmination of discoveries made in the past decade (Louis et al. 2016). These not only inform on tumor biology but can also significantly impact the diagnostic accuracy and prognosis and guide the most appropriate therapy. WHO2016 codifies and catalogs molecular biomarkers and integrates them within the traditional confine of "glass-based" diagnosis. Postsurgical management of patients, which may include chemoradiotherapy, relies on accurate diagnosis and grading, whereas genomic and epigenomic drivers have supplanted many of the classical histological descriptors in neuropathology. Importantly, the rapidly evolving genomic landscape leads to clonal heterogeneity and clinical behavior, contributing to treatment resistance (Yip et al. 2009; Johnson et al. 2014a). The dearth of effective systemic therapeutic agents presents a challenge for effective management.

The Personalized OncoGenomics (POG) initiative utilizes whole-genome and transcriptome analysis (WGTA) to identify and characterize these changes within a clinically meaningful time frame (4–6 wk from biopsy) in order to return results that may affect the treatment decision (Laskin et al. 2015). Coordinated effort was needed to develop a pipeline for processing biopsies of systemic metastatic specimens (Laskin et al. 2015), which account for 97.4% of all cases profiled. These challenges span multiple domains including tissue acquisition, pathology, sequencing, bioinformatic and analytic pipelines, and finally the unambiguous communication of actionable findings to the clinicians. However, the acquisition of CNS tumor samples remains challenging because of the location, invasiveness of the procedure, and intrinsic heterogeneity of the tumors. Here, we describe our experience of an "operating suite to sequencer" pipeline in the context of CNS tumors and discuss clinically informative findings from selected cases.

RESULTS

Patient Demographics

In total, 16 adult patients (eight male, eight female) with recurrent CNS tumors were recruited from October 2013 to August 2017, constituting 2.61% (16/612) of all adult POG cases. Data was available for 14/16 cases. Reasons for lack of data include suboptimal tissue for WGTA or patient withdrawal from POG. A summary of patient data can be found in Table 1 and Figure 1. All cases were profiled from tumor recurrences following initial treatments such as surgical resection, chemotherapy, and radiation therapy. WGTA was performed on formalin-fixed paraffin-embedded (FFPE) tumor tissue in the absence of matched snap-frozen tissue (only applicable to ODG1 and ODG2). A summary of genomic findings per case can be found in Table 2 and Figure 2. Data from selected cases will be presented. WGTA was also performed on 18 pediatric CNS tumors (25.7% [18/70] of all pediatric cases) and will be reported in a separate publication.

Extra-Axial Tumors

Meningioma

Meningiomas, the most common CNS neoplasia, are most often benign and treatable with surgical resection alone (Hasseleid et al. 2012; Ostrom et al. 2013). However, ~15%–20%

Table 1. Patient demographics and diagnoses of POG cases

| POG ID	Sex	Age at diagnosis	Diagnosis	WHO grade	Intra-/ Extra-axial	Origin	Tumor side	Location
MGM1	Female	48	Metastatic meningioma	II	Primary	Extra-axial	Left	Frontal lobe with orbital extension
MGM2	Female	50	Left petroclival meningioma	I	Primary	Extra-axial	Left	Petro-clival
MGM3	Female	52	Meningioma	II	Primary	Extra-axial	Right	Sphenoid wing
CHD1	Male	54	Clival chordoma	NA	Metastasis	Extra-axial	Midline	Clivus
CHD2	Male	45	Chordoma	NA	Metastasis	Extra-axial	Midline	Sacral spine
CHD3	Female	18	Chordoma	NA	Primary	Extra-axial	Midline	Clivus
MPE1	Male	29	Anaplastic myxopapillary ependymoma	NA	Primary	Extra-axial	Midline	Thoracic spine
ODG1	Male	27	Oligodendroglioma	III	Primary	Intra-axial	Right	Frontal lobe
ODG2	Female	29	Oligodendroglioma	II	Primary	Intra-axial	Right	Frontal lobe
GBM1	Female	16	Glioblastoma multiforme	IV	Primary	Intra-axial	Left	Parietal lobe
GBM2	Male	64	Glioblastoma multiforme	IV	Primary	Intra-axial	Left	Temporal lobe
PXA1	Female	21	Pleomorphic xanthoastrocytoma	II	Primary	Intra-axial	Right	Frontal lobe
GNG1	Male	57	Ganglioglioma of the left temporal lobe	II	Primary	Intra-axial	Left	Temporal lobe
EPN1	Female	27	Anaplastic ependymoma	III	Primary	Intra-axial	Right	Occipital lobe
EPN2	Male	18	ependymoma	III	Primary	Intra-axial	Right	Cingulate gyrus
EPN3	Male	44	Extramedullary spinal ependymoma	II	Primary	Intra-axial	Midline	Thoracic spine
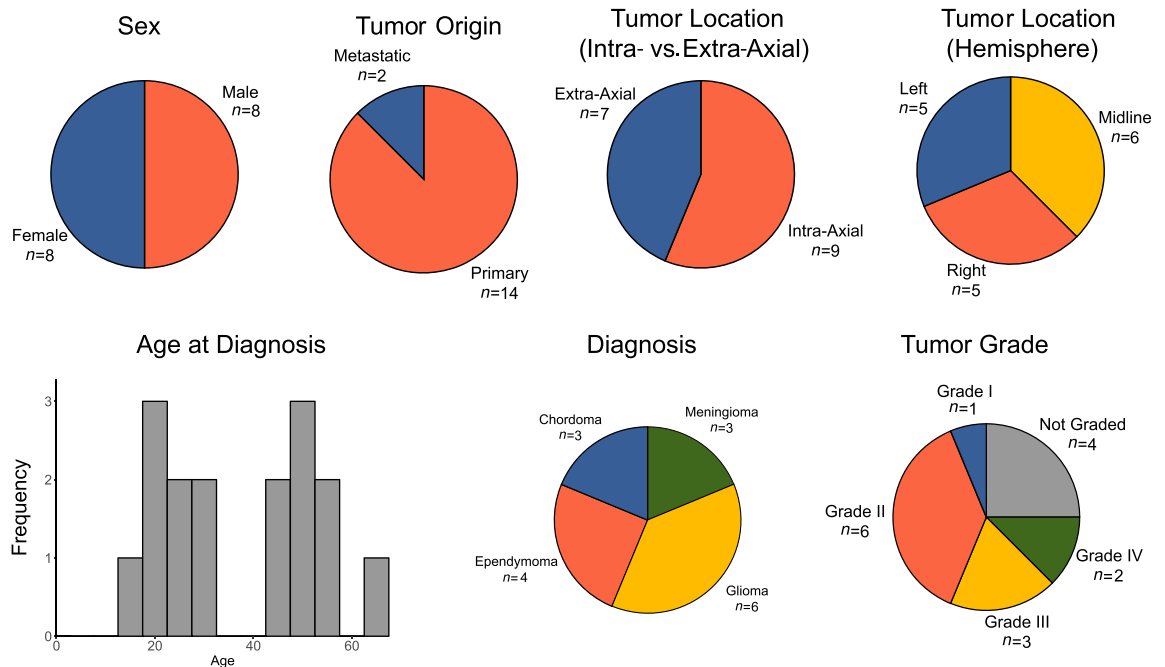
Figure 1. Patient demographics. Pie charts showing the distribution of sex, tumor origin, tumor location (intra- vs. extra-axial), tumor location (brain hemisphere), tumor type, and tumor grade and a bar chart showing the frequency of age at diagnosis for all POG patients.

Table 2. Summary of genomic findings

POG ID	Tumor type	SNVs (truncating)	Indels (frameshift)	Structural variants (fusions expressed)	Mutation burden per Mb genome
MGM1	Meningioma	22 (1)	5 (3)	47 (1)	2.5660
MGM2	Meningioma	22 (4)	1 (1)	55 (5)	2.3237
CHD1	Chordoma	33 (1)	3 (3)	19 (1)	1.5275
CHD2	Chordoma	25 (2)	13 (11)	52 (2)	1.8623
MPE1	Myxopapillary ependymoma	28 (2)	3 (1)	144 (40)	1.1146
ODG1	Oligodendroglioma	17 (1)	4 (3)	12 (2)	2.4312
ODG2	Oligodendroglioma	38 (0)	9 (8)	36 (11)	1.6305
GBM1	Glioblastoma	24 (0)	9 (6)	31 (9)	2.9786
GBM2	Glioblastoma	70 (3)	15 (12)	203 (27)	4.7236
PXA1	Pleomorphic xanthoastrocytoma	97 (5)	10 (7)	74 (9)	2.3147
GNG1	Ganglioglioma	40 (5)	4 (4)	64 (4)	2.4431
EPN1	Ependymoma	25 (1)	4 (3)	31 (2)	1.8239
EPN2	Ependymoma	16 (2)	7 (5)	65 (8)	0.9798
EPN3	Ependymoma	36 (2)	0 (0)	61 (17)	2.4393

present with more aggressive features such as brain invasion and mitotic activity (Riemenschneider et al. 2006). Two recurrent meningiomas with anaplastic histologic features were enrolled following multiple local recurrences (MGM1) or metastasis (MGM2) despite surgical resections and radiation therapy. Transcriptomic analyses of both meningiomas revealed up-regulation of the MAPK and Wnt pathway (reported in up to 50% of meningiomas) as a result of *NF2* loss (Brastianos et al. 2013; Bi et al. 2016a), a genomic feature identified in MGM2 but not MGM1. Interestingly, a fusion event identified in MGM1 involving *MN1* and *CXXC5* (Supplemental Fig. 1A,B) was predicted by POG analyses to mimic *NF2*, resulting in up-regulation of the MAPK and Wnt pathways through FGF up-regulation and β-catenin activation, and derepression of several Wnt factors, respectively (Lee et al. 2015). Fusions involving *MN1*, including an *MN1-CXXC5* fusion, have also been detected in a subset of *MN1*-altered primitive neuroectodermal tumors (PNETs) (Sturm et al. 2016). Interestingly, pathological assessment of MGM1 revealed inconsistent morphology and immunophenotypes across primary and recurrent biopsies. The primary tumor was diagnosed as an atypical meningioma, one recurrence was diagnosed as a PNET, and another recurrence was diagnosed as a malignant neoplasm with neuroendocrine differentiation. However, a unifying diagnosis of atypical meningioma was given because of consistent immunopositivity for SSTR2, a specific marker for meningothelial neoplasms (Menke et al. 2015), across the primary and all recurrent samples. Epigenetic dysregulation (*KDM3B* and *DNMT1* in MGM1; *KMT2D*, *ARID1A*, *ARID1B*, and *EP300* in MGM2) was detected in both tumors, consistent with published findings in anaplastic meningioma (Bi et al. 2016a). Therapeutic targets identified were FGF/FGFR in MGM1 and MEK in MGM2. A summary of pathway analyses and therapeutic targets identified for each case can be found in Table 3.

Chordoma

Chordomas are highly recurrent and treatment-resistant bone tumors that are thought to be of notochordal descent (Vujovic et al. 2006; Walcott et al. 2012). POG analyses were
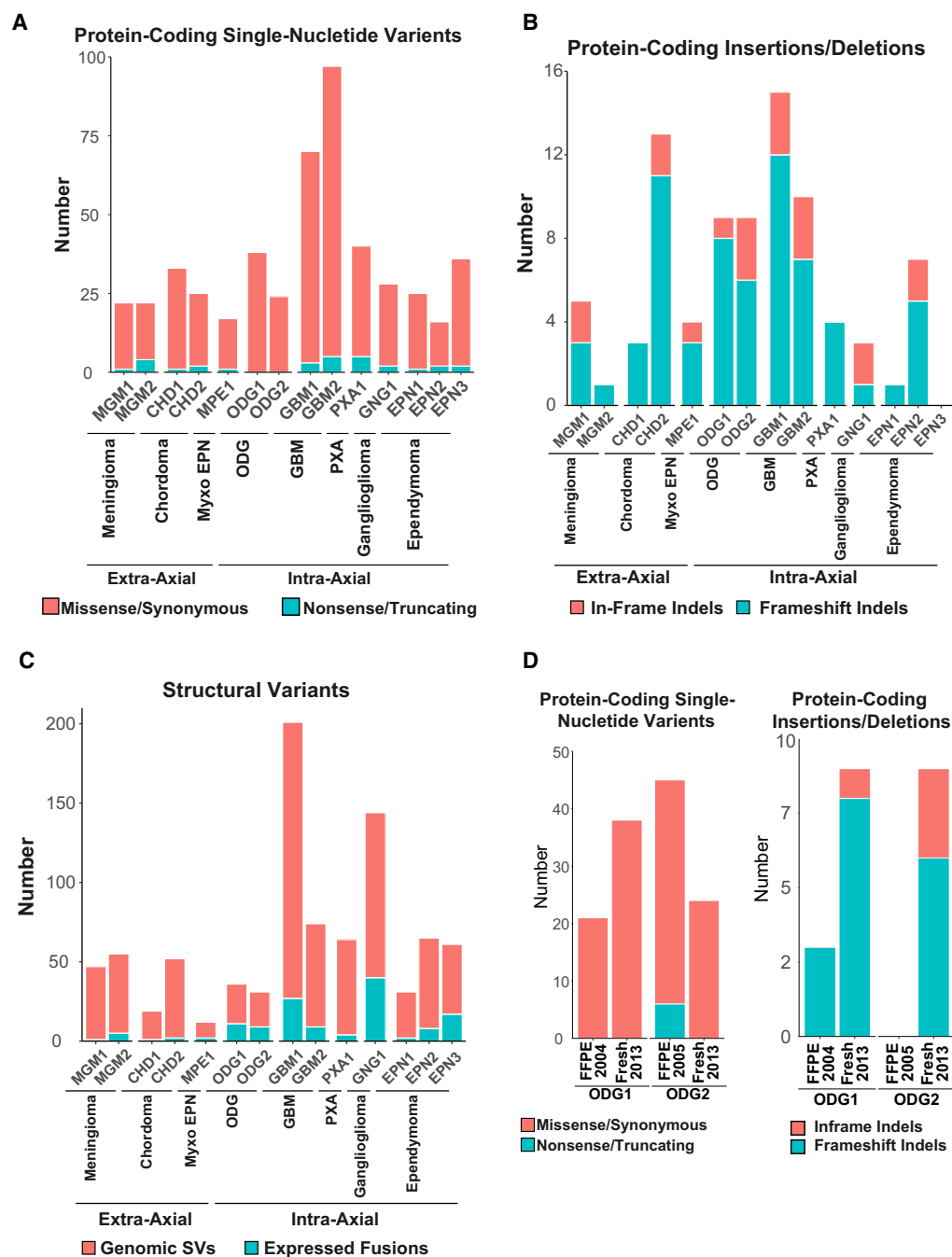
Figure 2. (*A*) Stacked bar chart showing total number of protein-coding single-nucleotide variants and truncating single-nucleotide variants identified in each POG case. (*B*) Stacked bar chart showing total number of insertion/deletion events and frameshift insertion/deletion events identified in each POG case. (*C*) Stacked bar chart showing the total number of structural variants and expressed fusion transcripts identified in each POG case. (*D*) (*Left*) Stacked bar chart comparing the total number of protein-coding single-nucleotide variants and truncating single-nucleotide variants identified in archived FFPE and fresh tumor in ODG1 and ODG2, both oligodendrogliomas. (*Right*) Stacked bar chart comparing the total number of insertion/deletions and frameshift insertion/deletions identified in archived FFPE and fresh tumor in ODG1 and ODG2, both oligodendrogliomas.

Table 3. Dysregulated pathways and potential therapeutic targets

POG ID	Tumor type	Pathways dysregulated	Oncogenic drivers	Therapeutic targets	Drug available	Potential caveats
MGM1	Meningioma	MAPK, NOTCH, Wnt, DNA methylation	*FGF4, FGFR3, MN1-CXXC5*	*FGF/FGFR*	Erlotinib, afatinib	NA
MGM2	Meningioma	MAPK, insulin signaling, Wnt, cell cycle, epigenome	*MAP2K2, EEF2*	*MEK2*	Trametinib, cobimetinib	NA
CHD1	Chordoma	MAPK, cell cycle	*Brachyury*	*Brachyury, Fos/Jun*	Tarmogen, nadroparin/ irbesartan	NA
CHD2	Chordoma	MAPK, cell cycle	*Brachyury*	*Brachyury, RTK*	Tarmogen, imatinib/ afatinib	NA
MPE1	Myxopapillary Ependymoma	MAPK, PI3K/AKT/ mTOR, cell cycle, TGF-β, NOTCH	*ABL1, FIP1L1, PDGFRA, HIF1A*	*PDGFRα, ABL1, FIP1L1*	Imatinib	Downstream effects unknown
ODG1	Oligodendroglioma	MAPK, DNA methylation, NOTCH, PI3K/AKT/ mTOR	*IDH1, EGFR, ETV1/4/5, DLL3*	*EGFR/Fyn*	Cetuximab + dasatinib	CIC loss may subvert upstream MAPK inhibition
ODG2	Oligodendroglioma	MAPK, DNA methylation, NOTCH, PI3K/AKT/ mTOR	*IDH1, EGFR, PDGFRA/B, DLL1/3*	*VEGF, PDGFRα/β*	Sorafenib/sunitinib, becaplermin/ imatinib/dasatinib	CIC loss may subvert upstream MAPK inhibition
GBM1	Glioblastoma	MAPK, chromatin remodeling, cell cycle, mismatch repair, MYC, PI3K	*IDH1, PI3K, TP53, CDK4*	*PIK3Cα*	Idelalisib	NA
GBM2	Glioblastoma	MAPK, PI3K, cell cycle	*EGFR, PTEN, CDKN2A*	*EGFR*	Erlotinib, afatinib, dacomitinib, cetuximab	PTEN loss may cause resistance to EGFR Ab
PXA1	Pleomorphic xanthoastrocytoma	MAPK, PI3K/AKT, cell cycle	*BRAF V600E*	*BRAF*	Dabrafenib, trametinib	NA
GNG1	Ganglioglioma	MAPK, DNA damage, Hedgehog	*PTEN, NF1, BRCA1, Brachyury*	*PARP*	Olaparib	NA
EPN1	Ependymoma	MAPK, NOTCH, cell cycle, Hedgehog, mismatch repair	*EWSR1-PATZ1*	*PDGFR/FGFR*	Imatinib, erlotinib, afatinib, cetuximab	NA
EPN2	Ependymoma	MAPK, NF-κB, DNA damage repair, hypoxia, NOTCH, Wnt, cell cycle	*C11orf95-RELA, CCND1/3*	*C11orf95-RELA. PD-L1*	Pembrolizumab	NA
EPN3	Ependymoma	MAPK, Wnt, NOTCH, chromatin remodeling, mismatch repair	No clear driver	*ERBB2, KIT/ ABL1, FGFR*	Imatinib, lapatinib	Lapatinib found to be ineffective in ependymoma

performed on one primary clival chordoma (CHD1) and one sacral metastasis of a clival chordoma (CHD2). Transcriptomic and pathway analyses identified overexpression of *Brachyury*, a hallmark of chordoma (Vujovic et al. 2006), and activation of the MAPK pathway through *EGFR* amplification in both cases. Interestingly, up-regulation of the oncogenic transcription

factors *FOS* and *JUN* was identified in CHD1, downstream from MAPK. Both cases also harbored dysregulation of cell cycle genes including homozygous loss of the cell cycle regulators *CDKN2A/B* in CHD1 and *RB1* in CHD2. Copy-number gain of *ETV1* and up-regulation of *SNAI1* was also identified in CHD2, which may explain the increased metastatic potential and invasiveness (Li et al. 2013). Although each chordoma harbored unique genomic and transcriptomic aberrations, therapeutic options for both cases were to target *Brachyury* directly and indirectly through *FGFR*.

Myxopapillary Ependymoma

Myxopapillary ependymomas are rare, slow-growing tumors associated with the conus medullaris, cauda equina, and filum terminale of the spinal cord and rarely show anaplastic features (Louis et al. 2007). Our patient MPE1 presented histologically with a recurrent disseminated anaplastic myxopapillary ependymoma (Awaya et al. 2003; Huynh et al. 2018; Lee et al. 2019) following multiple surgical resections and radiation therapy. WGTA revealed copy-number losses of *NF2*, *MSH3*, *PTEN*, *RB1*, and *CDKN1B*. Transcriptomic analyses showed up-regulation of several RTKs including *NTRK2*, *NRG2*, *ABL1*, *PDGFRA*, and *FIP1L1*. Other dysregulated pathways included Notch and Wnt activation, most likely because of the *NF2* loss (Bi et al. 2016a). Top therapeutic targets included the RTKs *ABL1*, *PDGFRA*, and *FIP1L1*. However, the downstream effects were unclear from our transcriptomic and pathway analyses. POG analyses of rare tumors such as this case not only inform of potentially actionable targets, they also provide insight into the intrinsic genomic and transcriptomic landscape of these tumors.

   Surgical resection remains one of the best predictors of outcome in many extra-axial tumors, in part because of a lack of durable and effective systemic or targeted therapies and the benign nature of many extra-axial tumors (Walcott et al. 2012; Bi et al. 2016b; Paldor et al. 2016). However, targeting of specific members of the MAPK and RTK signaling cascade may be an effective strategy moving forward, as evidenced by our cohort of extra-axial POG cases. Representative histologic, radiologic, and select genomic findings for select extra-axial cases can be found in Figure 3.

Intra-Axial Tumors

Ependymoma

Ependymomas are glial tumors that occur along the neuraxis. Treatment success is largely affected by tumor location (Ostrom et al. 2013), as surgery remains the most effective treatment (Merchant et al. 2009). Three recurrent adult ependymomas, two originating from the brain (EPN1, EPN2) and one originating from the spine (EPN3), were enrolled. WGTA revealed all three to be heavily driven by recurrent copy-number loss or down-regulation in cell cycle regulators/DNA repair genes and copy-number gains or high-expression outliers of several RTKs. A *C1orf53/C11orf95-RELA* fusion, an oncogenic driver fusion reported in up to 70% supratentorial ependymomas (Parker et al. 2014), was detected in EPN2. Interestingly, a novel *EWSR1-PATZ1* fusion was identified and validated in EPN1 (Supplemental Fig. 1C,D), which was predicted, using pathway analyses, to behave biologically similar to *RELA* fusion through activation of the mTOR and MAPK pathways. Activation of NF-κB and subsequent cell cycle up-regulation, downstream hallmarks of *RELA* fusions, were also identified in EPN1 and EPN2. Potential therapeutic targets for all three cases involved targeting the RTK and MAPK signaling cascades, which have been previously reported using imatinib (Fakhrai et al. 2004) and lapatinib (Fouladi et al. 2013).
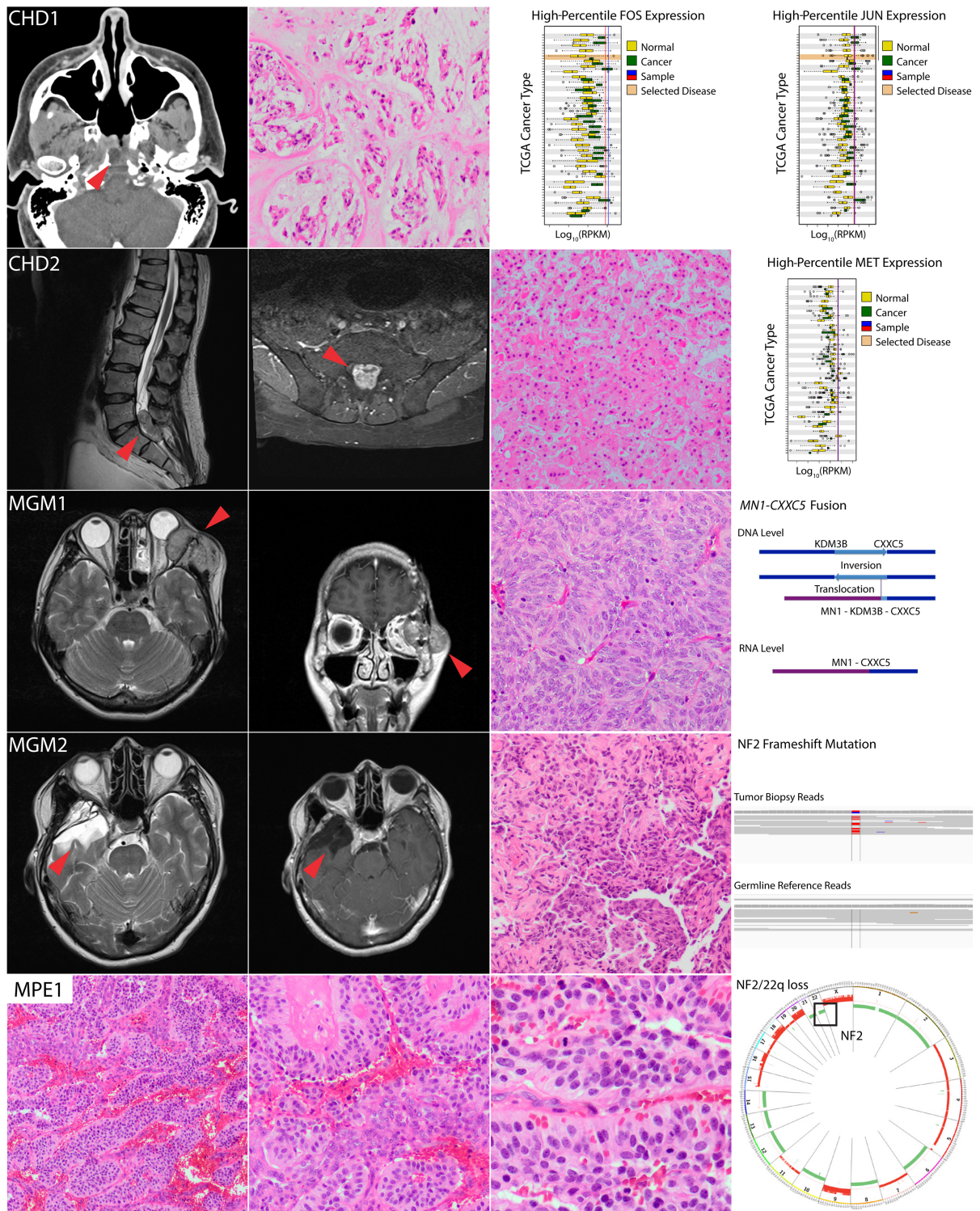
Figure 3. Extra-axial collage showing MRI scans, histology, and a genomic finding of interest for select cases. Cases are presented from *top* to *bottom* as follows: (CHD1) chordoma (high *FOS*/*JUN* expression), (CHD2) chordoma (high *T* and *MET* expression), (MGM1) orbital meningioma (*MN1-CXXC5* fusion), (MGM2) meningioma (*NF2* loss), (MPE1) anaplastic myxopapillary ependymoma (*NF2* loss).

Oligodendroglioma

Oligodendrogliomas (ODGs) are DLGGs molecularly defined by IDH mutation and 1p19q-co-deletion, and associated with favorable prognosis (Huse et al. 2014; Cancer Genome Atlas Research et al. 2015; Suzuki et al. 2015; Louis et al. 2016). Two patients (ODG1, ODG2) were enrolled following multiple resections and systemic/radiation therapy. For these two cases, we were also able to investigate tumor evolution by performing WGTA using fresh tumor from the latest resection and archival FFPE tissue. Comparison of fresh and FFPE ODG1 samples identified several shared molecular characteristics including 1p19q-co-deletion, *IDH1* R132H, and *TET2* mutations. Fresh ODG1 also harbored a *CIC* R215W mutation, a more recent molecular marker of ODG (Bettegowda et al. 2011; Yip et al. 2012), suggesting tumor evolution toward a more aggressive phenotype (Chan et al. 2014; Gleize et al. 2015; Padul et al. 2015; LeBlanc et al. 2017; Wong et al. 2018). Conversely, comparison of fresh and FFPE tissue from ODG2 revealed *IDH1* R132H as the only shared mutation, suggesting dramatic tumor evolution. Archival ODG2 tissue harbored missense mutations in *TP53*, *ATRX*, and *PI3CK* and lacked detectable 1p deletion; whereas 1p19q-co-deletion and a *CIC* frameshift mutation were detected in the fresh ODG2. Analyses of both cases revealed up-regulation of *SOX2*, and *OLIG2*, indicative of an oligo-dendroglial fate, and up-regulation of the MAPK signaling pathway and the ETS transcription factors (*ETV1/4/5*), likely a result of CIC inactivation (Jimenez et al. 2012; Okimoto et al. 2016; LeBlanc et al. 2017; Wong et al. 2018). Although both ODG cases only shared mutations in *IDH1*, *CIC*, 1p19q-co-deletion, the transcriptomic and pathway analyses revealed striking similarities, suggesting that the biology of ODG is driven heavily by these three genomic aberrations. A summary of clinically informative or useful molecular findings in our two primary and recurrent ODG samples is summarized in Table 4. Top therapeutic candidates included EGFR, MEK, and PI3K. However, inhibition of upstream RTK components may be precluded by CIC inactivation (LeBlanc et al. 2017; Wang et al. 2017), making systemic therapies particularly vexing once the tumor develops resistance to standard chemotherapeutic agents such as temozolomide.

Glioblastoma

GBM1 (<20 yr of age) was positive for *IDH1* R132H and *ATRX/TP53* loss-of-function mutations—genomic characteristics suggestive of an astrocytic precursor lesion (Parsons et al. 2008; Brennan et al. 2013). Conversely, GBM2 (>60 yr of age) presented with *EGFR* amplification, a genomic characteristic of primary GBM in the elderly (Hegi et al. 2005; Aldape et al. 2015). Copy-number losses in the cell cycle genes (*PTEN* – GBM1, *PTEN/*

Table 4. Molecular findings of primary and recurrent ODG samples

Molecular finding	Detection method	ODG1		ODG2	
		2004 FFPE	2013 fresh frozen	2005 FFPE	2013 fresh frozen
IDH1	WGS	R132H (38%)	R132H (45%)	R132H (25%)	R132H (42%)
TERT promoter	WGS	WT	C250T (43%)	WT	C228T (38%)
1p19q	LOH PCR	LOH	–	LOH	–
1p19q	FISH	–	LOH	–	LOH
1p19q	WGS	LOH	LOH	ROH	LOH
CIC	WGS	WT	R215W	WT	P97*fs

CDKN2A – GBM2) and copy-number gains of cell cycle/signaling genes (*ACVR1B* [39 copy gains], *CCND1* – GBM1; *EGFR* [103 copy gains], *BRAF* – GBM2) were identified in both cases. Low-percentile expression (0%–22%) of DNA mismatch repair genes (*MSH2/6*, *MLH1/3*, *PMS1/2*) and *MGMT* were identified in GBM1, suggesting impaired DNA damage repair and predicted response to TMZ (Hegi et al. 2005; Brennan et al. 2013). Although GBM1 originated from DLGG, the oncogenic drivers identified in both cases were similar, which may suggest convergent evolution regardless of the precursor lesion. GBM remains a difficult disease to treat because of a lack of efficient and effective drug delivery.

Intra-axial tumors are often particularly challenging to treat because of their location and infiltrative nature. Many tumors that initially respond ultimately develop treatment resistance as a result of tumor heterogeneity and clonal evolution (Yip et al. 2009; Sottoriva et al. 2013; Johnson et al. 2014a; Suzuki et al. 2015). Similar to extra-axial tumors, analyses of intra-axial tumors revealed that the majority of tumors, regardless of pathology, are driven by activation of the RTK/MAPK pathways and inactivation of cell cycle regulators (TP53/CDKN family). Targeting specific components of the MAPK pathway may be a viable therapeutic option in the future, as new treatment delivery methods are developed to circumvent the blood–brain barrier. Representative histologic, radiologic, and select genomic findings for select extra-axial cases can be found in Figure 4. Genomic variants for each case discussed can be found in Table 5.

DISCUSSION

Genomic Fusions and Transcriptomic Implications

Genomic instability, a hallmark of cancer (Hanahan and Weinberg 2011), can result in structural aberrations such as fusions, which can drive cancers. With the increasing accessibility of genomic studies, testing of prognostically informative fusions such as *FGFR3-TACC1* and *NTRK* fusions in GBM is becoming more available (Stransky et al. 2014; Granberg et al. 2017). It is also becoming more evident that fusion partners behave much more promiscuously than previously appreciated and have the potential to drive oncogenesis in several cancer types, such as the *EWSR1-PATZ1* fusion identified in EPN1, which has been previously reported in small round cell sarcoma (Cantile et al. 2013) and ganglioglioma (Qaddoumi et al. 2016). Fusions involving *EWSR1* have also been identified in CNS PNETs (Sturm et al. 2016)

In our cohort, genomic fusions and fusion transcripts were detected in all cases. The challenge lies in properly identifying those that are clinically and/or biologically informative. The usage of whole-transcriptome data, in POG, has been essential to the discovery of suspected driver fusions. For example, the *MN1-CXXC5* fusion identified in MGM1 was first identified in the transcriptome and was not detected in the genome because of a complicated set of inversions and translocations involving *MN1*, *CXXC5*, and *KDM3B*. Within the POG analytic pipeline, fusion transcripts are evaluated for significance by integrating pathway analyses, transcriptome data, predicted protein structure, and published literature. Without the addition of RNA-seq to POG, oncogenic drivers and potential therapeutic targets would not have been identified in many cases.

The Role of the Neurosurgeon in Personalized Oncology

From the neurosurgeon's perspective, the most immediate effect on practice is likely the increased demand for tissue sampling, particularly in poorly accessible and recurrent tumors that can harbor significant progression in the molecular features (Johnson et al. 2014b).
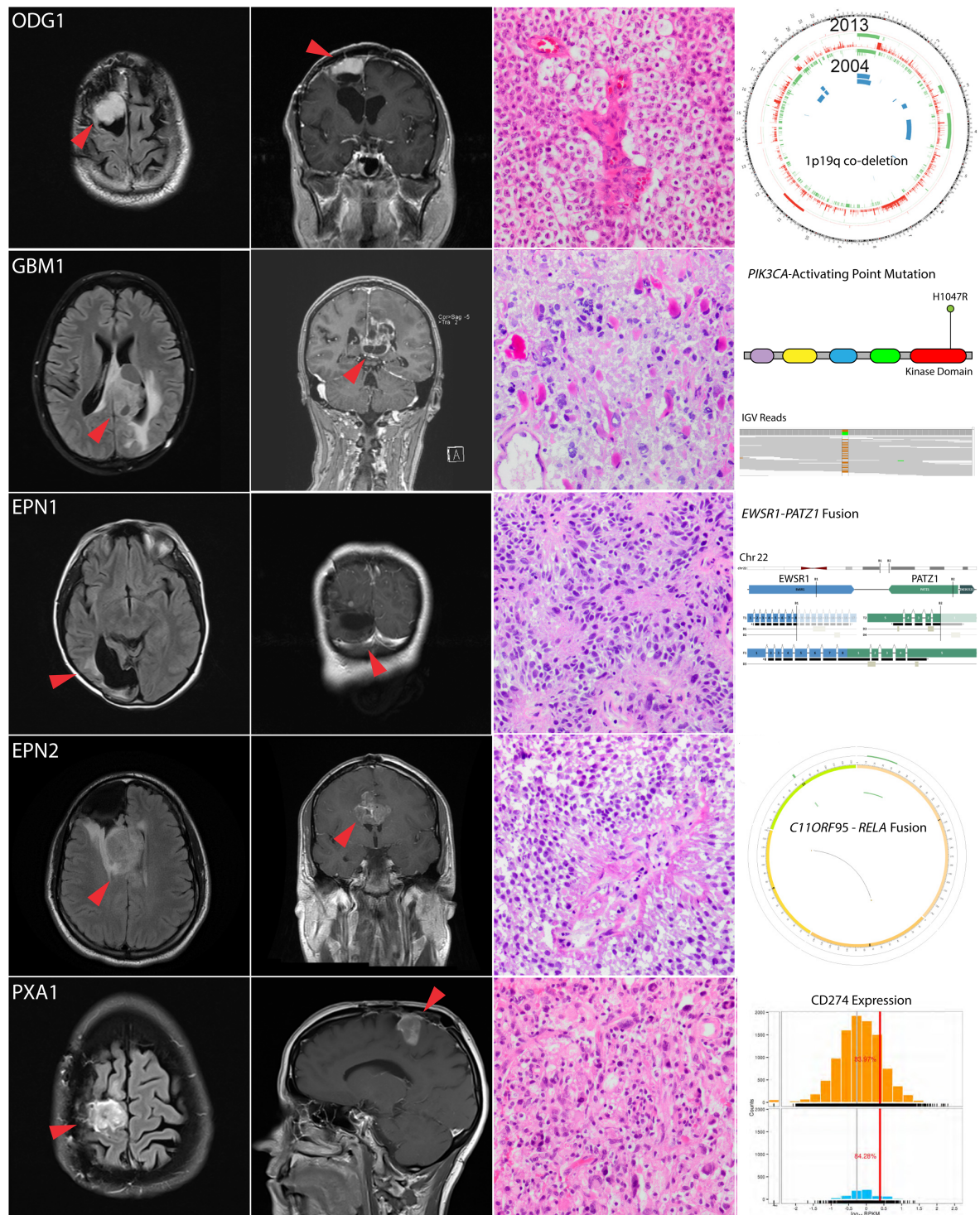
Figure 4. Intra-axial collage showing MRI scans, histology, and a genomic finding of interest for select cases. Cases are presented from *top* to *bottom* as follows: (ODG1) oligodendroglioma (1p19q co-deletion in primary and recurrent tissue), (GBM1) IDH mutated recurrent glioblastoma (*PI3KCA*-activating missense mutation), (EPN1) supratentorial ependymoma (*EWSR1-PATZ1* fusion), (EPN2) ependymoma (*RELA* fusion), (PXA1) pleomorphic xanthoastrocytoma (high *PDL1* expression).

Table 5. Variant table

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect (substitution, deletion, etc.)	dbSNP/ dbVar ID	ClinVar ID	Genotype (heterozygous/ homozygous)	Parent of origin (optional)
MN1;; CXXC5	t(5;22)(q31.2; q12.1)	22:28192751| 5:139060788	–	SV	Translocation		SCV000920879	NA	MGM1
DNMT1	19	19:10265450 C>A	p.Xnspl	SNV	Splice site		SCV000924575	Heterozygous	MGM1
KMT2D	12	12:49427441 G>A c.11047C>T	p.Q3683*	SNV	Stop		SCV000924576	Heterozygous	MGM2
MSH3	5	5:79950724 G>C c.178G>C	p.A60P	SNV	Substitution		SCV000924577	Heterozygous	MGM2
MN1	22	c.785delG	p.G262fs	Indel	frameshift		SCV000924571	Subclone	CHD1
MN1	22	c.772_776delCATTA	p.H258fs	Indel	Frameshift		SCV000924572	Subclone	CHD1
EWSR1:: PATZ1	inv(22)(q12.2)	22:2968471 6 EWSR1-001 (ENST00000397938) 22:31740655 PATZ1-001 (ENST00000266269)	–	SV	Inversion		SCV000920877	NA	EPN1
C11orf95:: RELA	dup(11)(q13.1)	11:63533279| 11:65429676	–	SV	Duplication		SCV000920878	NA	EPN2
CHD6	6	c.3296G>A	p.R1099Q	SNV	Substitution		SCV000924579	Heterozygous	EPN3
CIC	19	c.643C>T	p.R215W	SNV	Substitution		SCV000924567	Heterozygous	ODG1
IDH1	2	c.395G>A	p.R132H	SNV	Substitution	rs121913500		Heterozygous	ODG1
IDH1	2	c.395G>A	p.R132H	SNV	Substitution	rs121913500		Heterozygous	ODG2
CIC	19	c.289delC	p.P98fs	Indel	Frameshift		SCV000924568	Heterozygous	ODG2
TP53	17	c.319T>G	p.Y107D	SNV	Substitution	rs368771578		Homozygous	ODG2
ATRX	X	c.4213A>G	p.R1405G	SNV	Substitution		SCV000924569	Heterozygous	ODG2
IDH1	2	c.395G>A	p.R132H	SNV	Substitution	rs121913500		Heterozygous	GBM1
TP53	17	c.725G>A	p.C242Y	SNV	Substitution	rs121912655		Heterozygous	GBM1
TP53	17	c.524G>A	p.R175H	SNV	Substitution	rs28934578		Heterozygous	GBM1
ATRX	X	c.6440G>T	p.S2147I	SNV	Substitution		SCV000924570	Heterozygous	GBM1
PTEN	10	c.697C>T	p.R233*	SNV	Stop	rs121909219		Homozygous	GBM2
EGFR	6	c.3056C>A	p.P1019Q	SNV	Substitution		SCV000924573	Heterozygous	GBM2
EGFR	6	c.3055C>A	p.P1019T	SNV	Substitution		SCV000924574	Heterozygous	GBM2
NF1	17	c.6852_6855delTTAC	p.Y2264Tfs	Indel	Frameshift		SCV000924578	Homozygous	GNG1
BRAF	7	c.1799T>A	p.V600E	SNV	Substitution	rs113488022		Heterozygous	PXA1

From this there are two important conclusions—first, that repeat sampling of recurrent tumors is likely necessary to guide targeted therapy and, second, that sampling from a single region in tumors, such as diffuse glioma, is unlikely to fully represent tumor biology. The role of surgery in the management of recurrent glioma is controversial given that these tumors tend to recur in deeper or distant locations, reducing the feasibility of significant debulking (Wick et al. 2008; Dardis et al. 2015).

The greater challenge, now, is developing tissue sampling strategies that adequately represent the heterogeneity of a given tumor and provide sufficient material for genomic analyses. This is already recognized as an issue in tumor grading, whereby sampling of a peripheral region of lower grade can lead to misdiagnosis (Jackson et al. 2001). Neuroimaging in conjunction with machine learning analysis of radiological images may be an important tool in optimizing tissue acquisition (Liu et al. 2017) as will MRI-guided open biopsy to ensure representative sampling (Gill et al. 2014). Stereotactic and intraoperative MRI-guided biopsies have also similar diagnostic yield and safety (Lu et al. 2015). Regardless of the approach, minimally invasive biopsies have an intrinsic barrier to reaching sufficiently representative regions of the tumor. For many tumors, a single-needle trajectory likely will not suffice, necessitating multiple passes and increased risk of injury. With the rapid growth of next-generation sequencing, future analyses may require less tissue but may not recapitulate the heterogeneous nature of tumors, especially diffusely infiltrative tumors such as GBM. Therefore, surgical expertise is still required to accrue sufficient and representative tissue for molecular analyses.

Tissue Banking Moving Forward

With the emergence of single-cell genomic and transcriptomic profiling, the methods of banking tumor tissue have become more pertinent in determining the feasibility and quality of these studies. Previous studies investigating the single-cell heterogeneity and clonal hierarchy of gliomas have utilized fresh tissue to minimize the effects of storage and preservation on cell viability and the transcriptome (Patel et al. 2014; Tirosh et al. 2016; Filbin et al. 2018). However, many groups have also started to investigate methods of preservation that allow for molecular analyses at a single-cell resolution without compromising the quality of the data such as cryopreservation (Guillaumet-Adkins et al. 2017), fixation (Attar et al. 2018), and even archival FFPE tissue (Habib et al. 2017). Therefore, going forward, the methods used to bank surgical tissues should be revisited to accommodate studies that aim to utilize single-cell technologies to further study tumor heterogeneity and evolution in a clinical setting as precision medicine moves toward assessing the drivers of each individual tumor at a greater resolution.

Precision Medicine in Neuro-Oncology

Genomic drivers and prognostic markers have been integrated into routine clinical workups for several CNS tumors such as IDH mutation in DLGG. However, the genomic and transcriptomic events that drive tumor recurrence, treatment resistance, and metastatic potential can be unique for each individual tumor. The POG program is able to characterize these genomic and transcriptomic drivers within a clinically meaningful time frame of 4–6 wk to determine the best therapeutic course for the patient. That said, the bioinformatic expertise and manpower required to analyze and present the data in a practical and clinically useful format remains a bottleneck for POG. Although POG analyses often find potentially actionable therapeutic targets, in CNS cases, implementation of these targeted therapies into the clinic has been hindered by the lack of available clinical trials and the lack of drugs capable of penetrating the blood–brain barrier. None of the CNS-POG cases included in this report received POG-guided therapies. In contrast, for non-CNS patients, the POG program has

been regarded as a success, with ∼70% of patients receiving a POG-informed therapy and half of those treated achieving some disease control (Laskin et al. 2015). These results clearly emphasize the need for developing targeted therapies or delivery methods that are able to effectively cross the blood–brain barrier and access CNS tumors. Regardless, the WGTA generated from our small cohort of CNS POG cases has provided an opportunity to further study the genomic and transcriptomic landscape of these recurrent tumors.

Our program only enrolled patients with recurrent tumors, but as the benefits of personalized oncology continue to be demonstrated and, importantly, as more effective targeted therapies are developed, this approach will likely make its way into the treatment of primary tumors, necessitating a shift in surgical strategy, to, at the very least, differentially label tissue samples from different regions of the tumor in order to recapitulate tumor heterogeneity. The need for correlation of imaging, intraoperative findings, and molecular analysis underscores the importance of close collaboration between radiologists, surgeons, and pathologists. Further down the pipeline, the process of analyzing and formulating an actionable treatment plan requires clear communication between pathologists, molecular biologists, bioinformaticians, and oncologists. Personalized oncology is thus truly a multidisciplinary field, one in which neurosurgeons must play a vital role if these programs are to succeed and benefit our patients.

METHODS

Patient Recruitment and Tissue Accrual

The study was approved by and adhered to the University of British Columbia Research Ethics Committee (REB# H12-00137 and H14-00681-A019) as part of the Personalized OncoGenomics program of British Columbia (NCT02155621). Written informed patient consent was obtained by a participating oncologist prior to comprehensive genomic profiling. Cases were selected based on factors including exhaustion of treatment options and progressive disease despite multiple conventional treatments. Recruitment of brain tumor patients must also take into consideration the ease of tissue access. Patients were recruited with the understanding that information and results from these analyses are experimental and may not result in actionable treatments. All tissue acquisition procedures were conducted during scheduled surgeries, whereas a small number of cases utilized banked tissue from previous neurosurgical procedures. All tissue specimens were rushed to pathology from the operating room, where the on-call neuropathologist assessed the tissue for sufficient viable tumor (∼55% tumor), quality, and adequacy for standard clinical diagnostic workup. Materials were sent directly for DNA/RNA extraction or snap frozen for storage.

POG Sequencing and Analytic Pipeline

DNA and RNA were extracted from tumor tissue and matched whole blood, used as a germline reference. Whole-genome (∼40-fold sequence coverage for germline and >80-fold coverage for tumor; see Supplemental Table S1) and transcriptome (tumor, ∼200 million reads) sequencing analysis were performed on the Illumina HiSeq platform (Laskin et al. 2015). Somatic mutations, small insertions or deletions, copy-number alterations, and structural variants were detected using a bioinformatic pipeline as previously described (Jones et al. 2016).

Identification of Potential Therapeutic Targets

To identify candidate therapeutic targets, an initial automated matching of genes and variants to a database of known drug–gene and drug–variant associations was performed.

Relevant gene alterations include small mutations, copy changes, structural variants, and expression alterations. Genes with expression alterations are identified by comparison of gene expression to reference sets, including normal expression values from the Illumina Human Body Map 2.0 project (www.illumina.com; ArrayExpress ID: E-MTAB-513) (Asmann et al. 2012), and cancer expression values from The Cancer Genome Atlas (https://tcga-data.nci .nih.gov/tcga/) (Hoadley et al. 2018). Genes with expression in a matched cancer type greater than the 70th percentile compared to the matched cancer type and at least twofold increased expression relative to normal tissue, or with expression less than the 30th percentile and at least twofold reduced relative to normal tissue, are considered potentially over- or underexpressed; those with >90th- or <10th-percentile expression are considered candidate low- or high-expression outliers. The database of known drug–gene and drug–variant associations is an expert-curated in-house knowledgebase describing therapeutic, diagnostic, prognostic, and biological associations from the literature, from experience with previous patients within the POG program (Laskin et al. 2015), curated from automated natural-language processing through CIVICmine (http://bionlp.bcgsc.ca/civicmine/; https://www.biorxiv.org/content/10.1101/500686v1), and drawing on other curated sources including OncoKB (Chakravarty et al. 2017), CIViC (Griffith et al. 2017), and the MD Anderson Knowledgebase for Precision Oncology (Dumbrava and Meric-Bernstam 2018).

Genes are also associated with pathways in cancer using ConsensusPathDB (Kamburov et al. 2013) and the COSMIC cancer gene census (Sondka et al. 2018), and additional gene–drug associations are identified using DGIdb (Cotto et al. 2018). The automated matching of genes and variants to potential therapeutics is then reviewed and extended by expert manual review, including review of novel mutations and fusions for likely functional effect, identification of dysregulated pathways based on multiple alterations or alterations that propagate to pathway outputs, examination for highly unusual or targetable extreme expression outliers, identification of drugs that may indirectly impact driver alterations, and considerations of levels of evidence such as clinical trial or preclinical data and tumor type context. Analyzed data including genomic aberrations and expression profiles were then compiled into a standardized report including any informative and potentially actionable features (Laskin et al. 2015).

ADDITIONAL INFORMATION

Data Deposition and Access
The whole-genome and transcriptome sequencing data for these cases are available as .bam files from the European Genome-phenome Archive (EGA; www.ebi.ac.uk/ega/home) as part of the study EGAS00001001159. Interpreted variants including fusions have been deposited into ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under the accession numbers listed in Table 5.

Ethics Statement
The study was approved by and adhered to the University of British Columbia Research Ethics Committee (REB# H12-00137 and H14-00681-A019) as part of the Personalized OncoGenomics program of British Columbia (NCT02155621). Written informed patient consent was obtained by a participating oncologist prior to comprehensive genomic profiling.

Acknowledgments

Competing Interest Statement

Author Contributions

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